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The effects of sertraline on breast cancer (MDAMB-231) cells viability *in vitro*

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Abstract

Antidepressant drugs have been reported to affect the viability of cancer cells; however, the effects of the antidepressant sertraline on breast cancer cells is still unclear. The present study investigated the cytotoxic effects of sertraline on breast cancer cell viability *in vitro*. In this experimental-laboratory study, MDAMB-231 breast cancer cells were divided into control (untreated) and groups treated with 1.56, 3.12, 6.25, 12.5, 25, 50 and 100 µg / ml of sertraline. Cell viability was measured 24 hours after treatment using MTT assay method. Data were analyzed using one-way analysis of variance. The results showed that treatment with 1.56 µg / ml of sertraline had no significant effect on the viability of MDAMB-231 cells, however, sertraline with concentrations ≥ 3.12 µg / ml significantly reduced the MDAMB-231 cell viability after 24 and 48 hours. In conclusion, the findings of this study indicated that sertraline has cytotoxic effects on breast cancer cells in a dose dependent manner.

Keywords: Sertraline, MDAMB-231, Viability

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1 Introduction

Breast cancer is the most common cancer among women in the world, which is characterized by the abnormal growth of abnormal cells in the milk-producing ducts in the breast (Sun et al., 2017). According to available forecasts and statistics, the prevalence and mortality of breast cancer is increasing. As a result, 1.7 million people are diagnosed with this cancer annually and it covers 25% of all types of cancers (Azubuike et al., 2018). Studies show that by 2030, the global incidence of this cancer in women will reach 3.2 million new cases per year (Momenimovahed and Salehiniya, 2019; Lince-Deroche et al., 2017). The importance of this disease with high prevalence and mortality rates in developing countries is very important (Azubuike et al., 2018). Breast cancer can be influenced by genetic, physiological and lifestyle factors in individuals (Caldon, 2014; Duffy et al., 2018; Ikhuoria and Bach, 2018). One of the first obvious signs of breast cancer is a new mass in the breast tissue that is usually painless, stiff, and has rough edges. Other symptoms can include swelling in the breast, pain in the nipple, indentation of the nipple, redness and thickening of the breast skin, and abnormal discharge from the nipple (Ikhuoria and Bach, 2018). Studies show that some antidepressants, such as sertraline, can affect the proliferation of cancer cells (Geeraerts et al., 2021). Sertraline, under the brand name Zoloft, is an antidepressant of the selective serotonin reuptake inhibitor (Li et al., 2020). Sertraline with the chemical formula C17H17Cl2N has a half-life of 13 to 45 hours (Pinheiro et al., 2015). It acts on chemicals in the brain and is used to treat depressive, panic, anxiety, or symptoms of obsessive-compulsive disorder (Sanchez et al., 2014). Sertraline also has anti-cancer effects (Chinnapaka et al., 2020). Because people with breast cancer experience stress, insomnia, and depression during chemotherapy, specialists help them with sedatives (Moore et al., 2011). Research has shown that sedatives can have inhibitory or stimulatory effects on the development of some cancers (Jiao et al., 2017). In this regard, research findings indicate the effects of sedatives on cancers of the reproductive system (Li et al., 2021). Recently, significant effects of sedatives on the growth and proliferation of breast cancer cells have been reported (Guo et al., 2021). Antidepressants are one of the most important sedatives that can significantly affect the growth and proliferation of cancer cells (Ahmadian et al., 2017). The results of studies show that there is a significant relationship between antidepressants and the development of reproductive cancers (Lee et al., 2020). In this regard, studies indicate that antidepressants can reduce the proliferation of breast cancer cells (Cho et al., 2019). Sertraline is one of the most important antidepressants that can have significant inhibitory effects on cancer cell proliferation (Kuwahara et al., 2015). Laboratory studies have shown that sertraline can reduce the proliferation of cancer cells in the reproductive system (Fayyaz et al., 2021; Mørch et al., 2017). Although research findings confirm the cytotoxic effects of sertraline on breast cancer cell proliferation, some research has shown that antidepressants not only do not have anti-cancer effects but can also play a stimulating role in cancer progression. In this regard, research on selective serotonin reuptake inhibitors and breast cancer mortality in women has shown that the use of antidepressants is associated with an increased risk of death from breast cancer (Kelly et al., 2010). On the other hand, in studies on the effects of sertraline on ovarian cancer tumors, the results showed that

the injection of this drug doubled the average tumor weight in mice with cancer (Christensen et al., 2016). Another study also found that sertraline in particular was associated with an increased risk of breast cancer (Steingart et al., 2003). Due to the widespread prevalence of breast cancer in the world (Azubuike et al., 2018) and Iran (Rafiemanesh et al., 2016) and mortality due to this cancer (Azubuike et al., 2018) as well as the increasing use of sertraline in many communities (Cipriani et al., 2010) and also due to the widespread clinical complications , Socio-economic status of breast cancer (Dean et al., 2019) and also that most previous studies on the effects of sertraline on cancers have focused on the effects of this drug on clinical trials and limited research at the cellular level, especially on cells. Breast cancer has been performed and also considering that the results of previous studies have been significantly contradictory (Kuwahara et al., 2015; Kelly et al., 2010), the present study investigates the cytotoxic effects of sertraline on the proliferation of breast cancer cells in vitro and The results of this study are of particular importance in the study of the effects of sertraline on breast cancer and can be used to manage the use of this drug in cases of breast cancer.

2 Materials and Methods

During this experimental-laboratory study, sertraline was prepared from Iran Drug Pharmaceutical Company in pure powder and stored at normal laboratory temperature (25 ° C) until the test. Also, MDAMB-231 breast cancer cells were prepared from the cell bank of Pasteur Institute of Iran and transferred to the laboratory in a nitrogen tank and stored according to standard conditions. First, by initial examination and determination of lethal and non-lethal doses, the range of drug doses was determined and finally the doses were selected by serial and logarithmic methods. To prepare these concentrations, 1 gram of sertraline powder was first measured with a digital scale and then 100 µl of sodium hydroxide solution was added to it. To increase the solubility by 1000 µl of phosphate buffer solution (PBS) was added to the solution. The resulting solution was then sterilized and filtered by syringe filter. Following the process, 9 ml of DMEM culture medium containing fetal bovine serum was added to the prepared solution to reach a volume of 10 ml. Subsequently, the desired concentrations were prepared from the resulting solution during the study.

MDAMB-231 cells were divided into control and sertraline groups with concentrations of 1.56, 3.12, 6.25, 12.5, 25, 50 and 100 µg / ml sertraline. 10 mg of sertraline, which was prepared as a pure powder, was dissolved in 200 µl of DMSO solvent and then sterilized using a 0.2 µm syringe filter. The concentration of sertraline in this solution was one mg / ml, which after filtering the solution, the desired concentrations were obtained.

MDAMB-231 cell line was first defreezed and then the contents were transferred to cell culture flasks with DMEM culture medium and bovine embryo serum (FBS) (Sigma) for cell culture. After cell counting by Invert light microscope (Euromex), 10^5 cells from the cultured cells were poured into each well of the microplates and the culture medium was added to them. The microplate was then incubated overnight at 37 ° C, suitable humidity and 5% carbon dioxide. In order to evaluate the cytotoxic effects of sertraline on the target cells, MTT assay was used. In this regard, different doses of sertraline were added to a row of wells containing

cultured cells and repeated 3 times for each concentration. Also, a row of wells was considered as a control group (without drug exposure). The plates were then kept in an incubator for 24 hours (at 37 ° C and 5% CO2). After this time, the fluid was drained from the plates (which contained the culture medium and sertraline). In the next step, MTT dye was prepared. According to Sigma protocol, stock 10% MTT was prepared and 5mg/ml of MTT was added to each well. After 4-6 hours, the MTT solution was drained and DMSO (Sigma Company) was added to each well and after its complete dissolution, the insoluble purple crystals of formazan were removed. Finally, absorbance measured at 570 nm wavelength with the use of an ELISA plate reader (Bio-Rad, Hercules, CA, USA). The concentration of semi-maximal inhibitor (IC50) of sertraline was also calculated by plotting the curve.

The data were analyzed using SPSS21 software (IBM company, USA) and one-way analysis of variance followed by Tukey's post hoc test. The difference between the groups at the level of $P < 0.01$ was considered as a significant difference.

3 Results and Discussion

The results of MTT showed that 1.56 µg / ml sertraline had no significant effect on the viability of MDAMB-231 cells, however, sertraline with concentrations ≥ 3.12 µg / ml significantly reduced the MDAMB-231 cell viability after 24 and 48 hours (Figure 1 and 2).

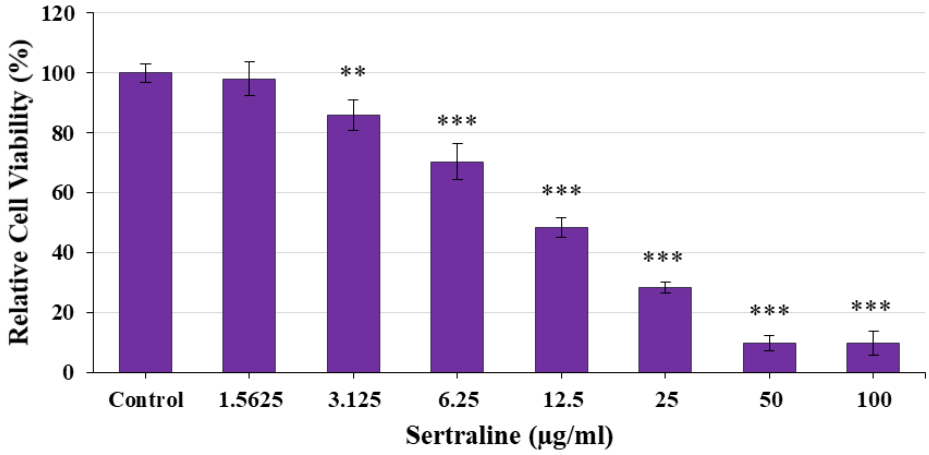

Figure 1. Viability of MDAMB-231 cells treated with different concentrations of sertraline 24 hours after treatment. * indicates a significant difference compared to the control group (**:p<0.01, ***:p <0.001).

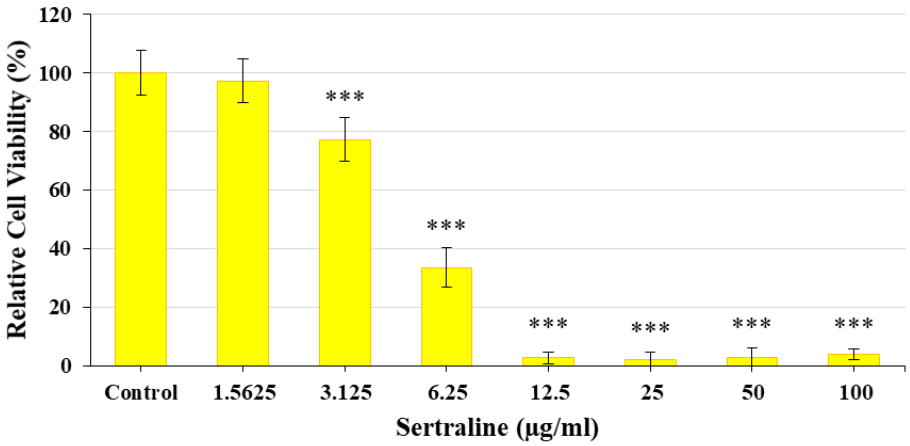

Figure 2. Viability of MDAMB-231 cells treated with different concentrations of sertraline 24 hours after treatment. * indicates a significant difference compared to the control group (***: p<0.001).

The results of MTT test showed that there was not significant difference between viability of MDAMB-231 between groups treated with 1.5625 µg/ml of sertraline for 24 and 48 hours, however, MDAMB-231 cells viability significantly decreased in groups treated with 3.125, 6.25, 12.5, 25, 50 and 100 µg/ml of sertraline for 48 hours compared with MDAMB-231 cells treated with the same concentrations for 48 hours (p<0.05, p<0.001, p<0.01p<0.001, p<0.01, and p<0.05, respectively) (Figure 3).

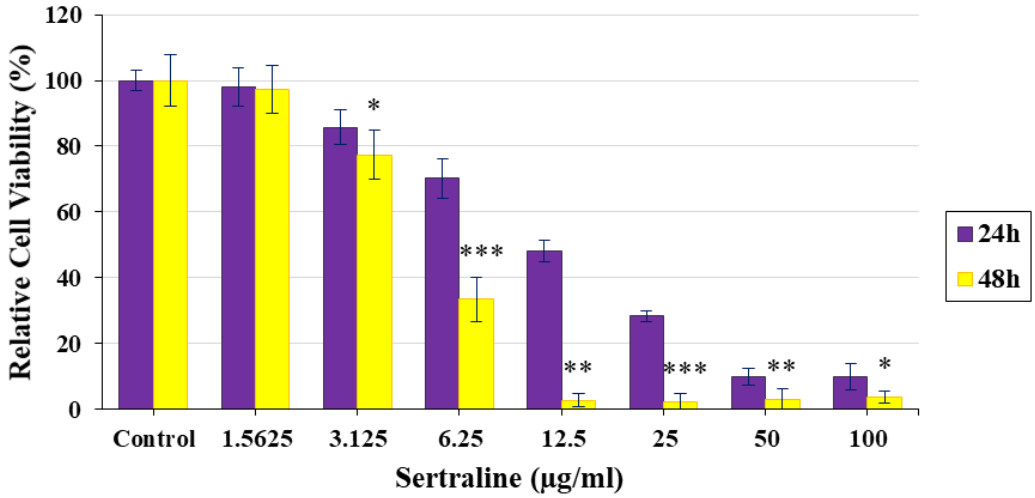

Figure 3. Viability of MDAMB-231 cells treated with different concentrations of sertraline for 24 hours and 48 hours. (***:p<0.001). * indicates significant difference compared with 24 hours (*:p<0.05, **:p<0.01, ***:p<0.001).

The half-maximal inhibitory concentration (IC50) of sertraline was calculated by linear approximation regression of the percentage survival versus the sertraline concentration. The results showed that the IC50 value was 12.6 µg/ml for sertraline 24 hours after treatment (Figure 4).

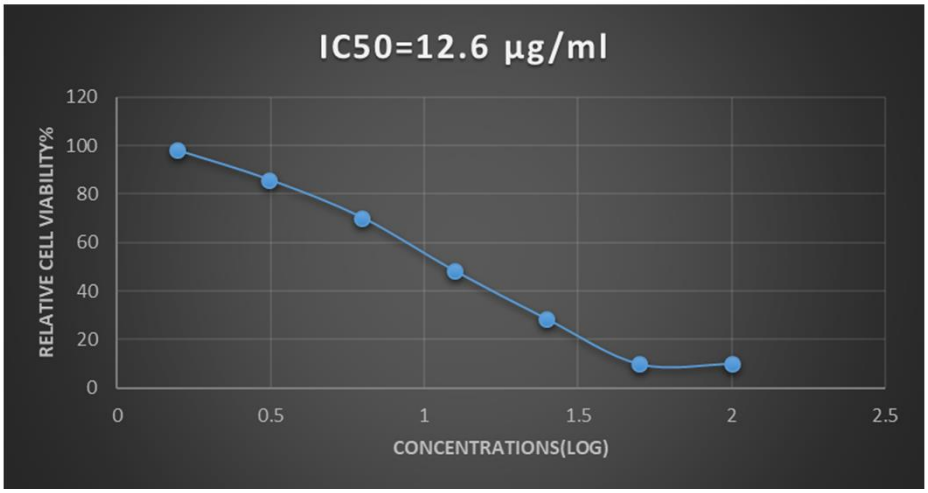

Figure 4. IC50 of sertraline calculated from curve constructed by plotting MDAMB-231 cell viability (%) versus sertraline 24 hours after treatment.

The IC50 value was 5.2 µg/ml for sertraline 48 hours after treatment showing a significant decrease in IC50 value compared to sertraline IC50 during 24 hours. The significant decrease in IC50 of sertraline indicated that the drug cytotoxic effect on MDAMB-231 cell viability significantly increases in response to duration of treatment (Figure 5).

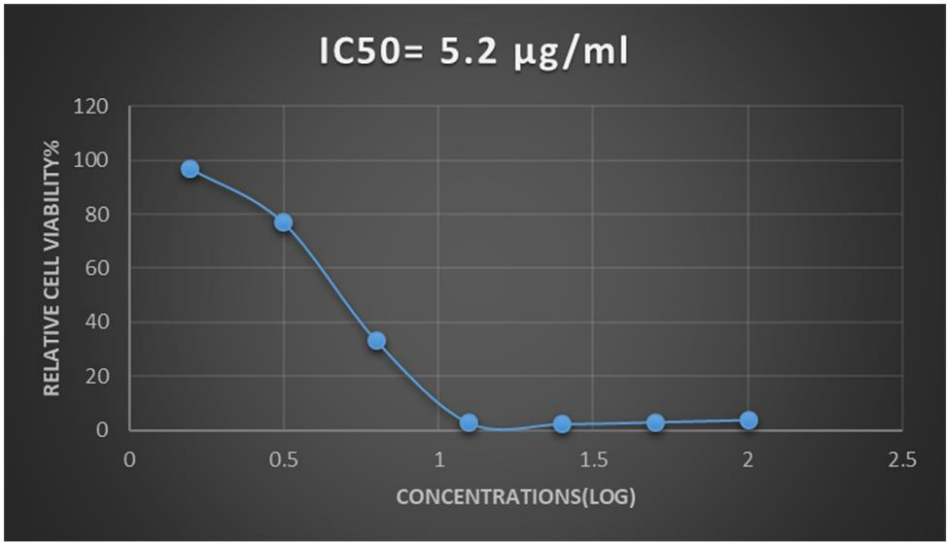

Figure 5. Calculated IC50 value of sertraline from curve constructed by plotting MDAMB-231 cell viability (%) versus sertraline 24 hours after treatment.

The results of this study showed that sertraline can reduce the viability of breast cancer cells in culture medium. Consistent with this finding, the findings have shown that sertraline have cytotoxic effects on breast cancer cells (Geeraerts et al., 2021). The results of a study on serotonin transporter antagonists in mice with breast cancer showed that the onset and progression of breast tumors is stimulated by tumor-initiating cells called BTICs, which are resistant to radiation and chemotherapy. This study suggests that sertraline irreversibly prevents the formation of breast tumor cells in mice by targeting BTIC (Hallett et al., 2016). Research findings

on the potential use of sertraline as an antitumor drug suggest that this antidepressant drug is able to inhibit tumor-specific proteins (Baú-Carneiro et al., 2022). The results of another study show that sertraline hydrochloride can cause cell death in AU565 breast cancer cells (Fayyaz et al., 2021). On the other hand, treatment of cancer cells with sertraline during the first 72 hours reduces the survival of some cancer cells of the reproductive system (Stapel et al., 2021). In contrast, some research findings have shown that antidepressants do not have a significant effect on breast cancer cells, and in this regard, the results of a study on the use of antidepressants and the risk of breast cancer indicate that continued use of antidepressants such as sertraline can increase the risk of cancer progress in people with breast cancer (Steingart et al., 2003). The findings on the relationship between antidepressants and mortality rate in the people with breast cancer have shown that the risk of death from breast cancer increases in the patients with breast cancer taking antidepressant drugs (Kelly et al., 2010). Possible mechanism of action of sertraline on MDAMB-231 cancer cells is mediated by stopping the cell cycle (Geeraerts et al., 2021). Sertraline is also able to inhibit tumor-specific proteins (TCTP) and therefore possibly exerts its anti-cancer effects by inhibiting the expression of TCTPs (Baú-Carneiro et al., 2022). Further research are required to reveal the molecular mechanism of action behind the anticancer effects of sertraline on breast cancer cells.

4 Conclusion

Overall, the results of this study showed that sertraline can inhibit the proliferation of breast cancer cells, however, *in vivo* research are required to demonstrate anticancer effects of sertraline on breast tumors.

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Conflict of interests

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

References

Ahmadian, E., Eftekhari, A., Babaei, H., Nayebi, A. M., & Eghbal, M. A. (2017). Anti-cancer effects of citalopram on hepatocellular carcinoma cells occur via cytochrome C release and the activation of NF-kB. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)*, *17*(11), 1570-1577. https://doi.org/10.

2174/1871520617666170327155930

Azubuike, S. O., Muirhead, C., Hayes, L., & McNally, R. (2018). Rising global burden of breast cancer: the case of sub-Saharan Africa (with emphasis on Nigeria) and implications for regional development: a review. *World Journal of Surgical Oncology, 16*(1), 1-13. https://doi.org/10.1186/s12957-018-1345-2

Baú-Carneiro, J. L., Sumida, I. A. G., Gallon, M., Zaleski, T., Boia-Ferreira, M., & Cavassin, F. B. (2022). Sertraline repositioning: an overview of its potential use as a chemotherapeutic agent after four decades of tumor reversal studies. *Translational Oncology*, *16*, 101303. https://doi.org/10.1016/j.tranon.2021.101303

Caldon, C. E. (2014). Estrogen signaling and the DNA damage response in hormone dependent breast cancers. *Frontiers in Oncology, 4*, 106. https://doi.org/10.3389/fonc.2014.00106

Chinnapaka, S., Bakthavachalam, V., & Munirathinam, G. (2020). Repurposing antidepressant sertraline as a pharmacological drug to target prostate cancer stem cells: dual activation of apoptosis and autophagy signaling by deregulating redox balance. *American Journal of Cancer Research*, *10*(7), 2043-2065. PMID: 32775000

Cho, Y.W., Kim, E.J., Nyiramana, M.M., Shin, E.J., Jin, H., Ryu, J.H., Kang, K.R., Lee, G.W., Kim, H.J., Han, J., & Kang, D. (2019). Paroxetine induces apoptosis of human breast cancer MCF-7 cells through Ca2+-and p38 MAP kinase-dependent ROS generation. *Cancers*, *11*(1), 64. https://doi.org/10.3390/ cancers11010064

Christensen, D.K., Armaiz-Pena, G.N., Ramirez, E., Matsuo, K., Zimmerman, B., Zand, B., Shinn, E., Goodheart, M.J., Bender, D., Thaker, P.H., & Lutgendorf, S. K. (2016). SSRI use and clinical outcomes in epithelial ovarian cancer. *Oncotarget*, *7*(22), 33179. doi: 10.18632/oncotarget.8891

Cipriani, A., La Ferla, T., Furukawa, T.A., Signoretti, A., Nakagawa, A., Churchill, R., McGuire, H., & Barbui, C. (2010). Sertraline versus other antidepressive agents for depression. *Cochrane Database of Systematic Reviews*, (1). https://doi.org/10.1002/14651858.CD006117.pub3

Dean, L.T., Moss, S.L., Ransome, Y., Frasso-Jaramillo, L., Zhang, Y., Visvanathan, K., Nicholas, L.H., & Schmitz, K. H. (2019). "It still affects our economic situation": long-term economic burden of breast cancer and lymphedema. *Supportive Care in Cancer*, *27*(5), 1697-1708. https://doi.org/10.1007/s00520-018-4418-4

Duffy, S.W., Morrish, O.W., Allgood, P.C., Black, R., Gillan, M.G., Willsher, P., Cooke, J., Duncan, K.A., Michell, M.J., Dobson, H.M., & Gilbert, F. J. (2018). Mammographic density and breast cancer risk in breast screening assessment cases and women with a family history of breast cancer. *European Journal of Cancer, 88*, 48-56. https://doi.org/10.1016/j.ejca.2017.10.022

Fayyaz, S., Irshad, R., Siddiqui, R. A., & Choudhary, M. I. (2021). Antidepressant Sertraline Hydrochloride Inhibits the Growth of HER2+ AU565 Breast Cancer Cell Line through Induction of Apoptosis, and Arrest of Cell Cycle. *BioRxiv*. https://doi.org/10.1101/2021.

09.07.459321

Geeraerts, S.L., Kampen, K.R., Rinaldi, G., Gupta, P., Planque, M., Louros, N., Heylen, E., De Cremer, K., De Brucker, K., Vereecke, S., & De Keersmaecker, K. (2021). Repurposing the Antidepressant Sertraline as SHMT Inhibitor to Suppress Serine/Glycine Synthesis–Addicted Breast Tumor Growth. *Molecular Cancer Therapeutics*, *20*(1), 50-63. https://doi.org/10.1158/1535-7163.MCT-20-0480

Guo, L., Liang, Y., Wang, S., Li, L., Cai, L., Heng, Y., Yang, J., Jin, X., Zhang, J., Yuan, S., & Jia, L. (2021). Jujuboside B Inhibits the Proliferation of Breast Cancer Cell Lines by Inducing Apoptosis and Autophagy. *Frontiers in Pharmacology*, *12*, 668887. doi: 10.3389/fphar.2021.668887

Hallett, R.M., Girgis-Gabardo, A., Gwynne, W.D., Giacomelli, A.O., Bisson, J.N., Jensen, J.E., Dvorkin-Gheva, A., & Hassell, J. A. (2016). Serotonin transporter antagonists target tumor-initiating cells in a transgenic mouse model of breast cancer. *Oncotarget*, *7*(33), 53137-53152. doi: 10.18632/oncotarget.10614

Ikhuoria, E. B., & Bach, C. (2018). Introduction to breast carcinogenesis–symptoms, risks factors, treatment and management. *European Journal of Engineering and Technology Research, 3*(7), 58-66. https://doi.org/10.24018/ejeng.2018.3.7.745

Jiao, J., Wang, Y., Sun, X., & Jiang, X. (2017). Insights into the roles of midazolam in cancer therapy. *Evidence-Based Complementary and Alternative Medicine*, *2017*. https://doi.org/10.1155/2017/3826506

Kelly, C. M., Juurlink, D. N., Gomes, T., Duong-Hua, M., Pritchard, K. I., Austin, P. C., & Paszat, L. F. (2010). Selective serotonin reuptake inhibitors and breast cancer mortality in women receiving tamoxifen: a population based cohort study. *Bmj*, *340*. https://doi.org/10.1136/bmj.c693

Kuwahara, J., Yamada, T., Egashira, N., Ueda, M., Zukeyama, N., Ushio, S., & Masuda, S. (2015). Comparison of the anti-tumor effects of selective serotonin reuptake inhibitors as well as serotonin and norepinephrine reuptake inhibitors in human hepatocellular carcinoma cells. *Biological and Pharmaceutical Bulletin*, *38*(9), 1410-1414. https://doi.org/10.1248/bpb.b15-00128

Lee, J. Y., Yang, C., Lim, W., & Song, G. (2020). Methiothepin suppresses human ovarian cancer cell growth by repressing mitochondrion-mediated metabolism and inhibiting angiogenesis in vivo. *Pharmaceutics*, *12*(7), 686. https://doi.org/10.3390/pharmaceutics12070686

Li, W. H., Wei, Z. W., & Liu, X. F. (2020). Clinical efficacy of sertraline in the treatment of depression caused by Alzheimer disease: A protocol of systematic review. *Medicine*, *99*(45), e23076. doi: 10.1097/MD.0000000000023076

Li, T., Yang, J., Yang, B., Zhao, G., Lin, H. and Liu, Q., & Jiang, H. (2021). Ketamine Inhibits Ovarian Cancer Cell Growth by Regulating the lncRNA-PVT1/EZH2/p57 Axis. *Frontiers in Genetics*, 1486. https://doi.org/10.3389/fgene.2020.597467

Lince-Deroche, N., Rayne, S., Van Rensburg, C., Benn, C., Masuku, S., & Holele, P. (2017). Breast cancer in South Africa: developing an affordable and achievable plan to improve detection and survival. *South African Health Review, 2017*(1), 181-188. https://journals.co.za/doi/abs/10.10520/EJC-c8497946c

Momenimovahed, Z., & Salehiniya, H. (2019). Epidemiological characteristics of and risk factors for breast cancer in the world. *Breast Cancer: Targets and Therapy*, *11*, 151-164. doi: 10.2147/BCTT.S176070

Moore, T. A., Berger, A. M., & Dizona, P. (2011). Sleep aid use during and following breast cancer adjuvant chemotherapy. *Psycho-Oncology*, *20*(3), 321-325. https://doi.org/10.1002/pon.1756

Mørch, L. S., Dehlendorff, C., Baandrup, L., Friis, S., & Kjær, S. K. (2017). Use of antidepressants and risk of epithelial ovarian cancer. *International Journal of Cancer*, *141*(11), 2197-2203. https://doi.org/10.1002/ijc.30919

Pinheiro, E., Bogen, D. L., Hoxha, D., Ciolino, J. D., & Wisner, K. L. (2015). Sertraline and breastfeeding: review and meta-analysis. *Archives of Women's Mental Health*, *18*(2), 139-146. https://doi.org/10.1007/s00737-015-0499-y

Rafiemanesh, H., Rajaei-Behbahani, N., Khani, Y., & Hosseini, S. (2016). Incidence trend and epidemiology of common cancers in the center of Iran. *Global Journal of Health Science*, *8*(3), 146. doi: 10.5539/gjhs.v8n3p146

Sanchez, C., Reines, E. H., & Montgomery, S. A. (2014). A comparative review of escitalopram, paroxetine, and sertraline: are they all alike? *International Clinical Psychopharmacology*, *29*(4), 185. doi: 10.1097/YIC.0000000000000023

Stapel, B., Melzer, C., von der Ohe, J., Hillemanns, P., Bleich, S., Kahl, K. G., & Hass, R. (2021). Effect of SSRI exposure on the proliferation rate and glucose uptake in breast and ovary cancer cell lines. *Scientific Reports*, *11*(1), 1-14. https://doi.org/10.1038/s41598-020-80850-9

Steingart, A., Cotterchio, M., Kreiger, N., & Sloan, M. (2003). Antidepressant medication use and breast cancer risk: a case-control study. *International Journal of Epidemiology*, *32*(6), 961-966. https://doi.org/10.1093/ije/dyg155

Sun, Y.S., Zhao, Z., Yang, Z.N., Xu, F., Lu, H.J., Zhu, Z.Y., Shi, W., Jiang, J., Yao, P.P., & Zhu, H. P. (2017). Risk factors and preventions of breast cancer. *International Journal of Biological Sciences, 13*(11), 1387-1397. doi: 10.7150/ijbs.21635