

The Effect of Amygdalin on Glioblastoma: Focus on Oxidant Capacity and Antioxidant Status

ABSTRACT

Objective: Amygdalin (Vitamin B-17) is a type of vitamin, naturally found in many fruits and plants. The aim of the study was the evaluation of Amygdalin effect on the oxidant capacity and oxidant status of the T98G cancer cells. A T98G cell line was used in the study. Cell viability and oxidative stress evaluation were done.

Methods: Amygdalin was used at 1, 4, and 8 µg/mL doses. TAC and TOS values were measured.

Results: According to the result, amygdalin 8 µg/mL shows the highest anticancer effects. TAC level was 3.2 Trolox Equiv/L and TOS was 3.6 H₂O₂ Equiv/L.

Conclusion: Vit B17 can increase oxidative stress in T98G cells and decrease cell viability.

Keywords: Amygdalin, MTT, TAC, TOS, T98G

Sıdıka GENÇ¹
Kübra KARABULUT¹
Esmanur NİĞDE¹
Yunus Emre AYDIN¹
Beyzanur AYDIN¹
Alperen Enes AYDIN¹
Ali TAGHIZADEHGHLEHJOUGHİ¹

¹Şeyh Edebalı University, Faculty of Medicine, Department of Medical Pharmacology, Bilecik, Türkiye



Introduction

Glioblastoma multiforme (GBM) is one of the aggressive types of brain cancer. It is also the most common malignant primary tumor of the brain and central nervous system (Altinoz et al., 2022; Yeni et al., 2023). It accounts for 14.5% of all central nervous system tumors and 48.6% of malignant central nervous system tumors. Cancer treatment is traditionally done with chemotherapy, surgery, or a combination of these. Despite medical advances today, treatment options and success rates are low. Therefore, it is essential to develop or search new substances for brain tumors (Kafagi et al., 2024; Loginova et al., 2024). Our study aims to evaluate the effect of Amygdalin on the glioblastoma line and oxidative stress parameters. Amygdalin or vitamin B17 is naturally found in many plants, fruits, and seeds.

Vit B17 has many effects. Some of them are; antitussive, antiasthmatic, fibrosis prevention, anticancer, anti-inflammatory, and anti-ulcer activities (El-Desouky et al., 2020; Tousson et al., 2020). In our study, TAC and TOS tests as well as viability rate will be evaluated to determine anticancer activity.

Method

Chemicals and reagents

All of the reagents were of analytical grade and used without further purification. Amygdalin (Vit B17, Gloden Pharm, Kyiv, Ukraine). Dulbecco-modified eagle medium (DMEM), fetal bovine serum (FBS), Antibiotic, and dimethyl sulfoxide (DMSO) were purchased from Sigma Aldrich (St. Louis, MO, USA).

T98G cell culture

The T98G cell line was obtained from Bilecik University's Department of Medical Pharmacology. Cells were centrifuged at 1200 rpm for 5 minutes. They were suspended in fresh Dulbecco-modified eagle medium-F12 (DMEM), fetal bovine serum (FBS) 10%, and antibiotics 1% (penicillin, streptomycin, and amphotericin B). 48-well plates (5% CO₂; 37 °C)



Received 29.07.2024
Accepted 26.08.2024
Publication Date 31.08.2024

Corresponding author:

Ali Taghizadehghalehjoughi
E-mail: ali.tgzd@bilecik.edu.tr
Cite this article: Genç, S., Karabulut, K., Niğde, E., Aydın, Y.E., Aydın, B., Aydın, A.E., & Taghizadehghalehjoughi, A. (2024). The Effect of Amygdalin on Glioblastoma: Focus on Oxidant Capacity and Antioxidant Status. *Recent Trends in Pharmacology*, 2(2), 75-78.



Content of this journal is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.

were used for seeding.

MTT assay

Briefly, cells were resuspended in fresh DMEM medium, 10% FBS, and 1% antibiotic (penicillin, streptomycin, and amphotericin B). Amygdalin 1 µg/mL, Amygdalin 4 µg/mL, and Amygdalin 8 µg/mL were administered for 24 hours. The optical density of the solutions was read at 570 nm using a Multiskan™ GO microplate spectrophotometer (Thermo Fisher, Porto Salvo, Portugal).

TAC (total antioxidant capacity) and TOS (total oxidant status)

TAC level was measured by using the Rel Assay Total Antioxidant Capacity (Rel Assay Diagnostics, Gaziantep, Türkiye) commercial kit. Briefly, the supernatant was used and the reagent was added according to the manufacturing protocol. The color change was evaluated by measuring at a wavelength of 660 nm wavelength for TAC and 530 nm wavelength for TOS. TAC Results were expressed per µmol Trolox Equiv/L. TOS results are expressed per µmol H₂O₂ Equiv/mg protein.

Statistical analysis

The results were analyzed with SPSS 20.0 (IBM SPSS Corp., Armonk, NY, USA) Windows program and given as mean and standard error. Statistical comparison tests were performed between groups using the One Way Anova test, and data with a significance of $p < .05$ were considered statistically significant.

Results

MTT assay

MTT results regarding cell viability are shown in Figure 1. In this study, the 24-hour exposure results of the groups Control, Amygdalin 1 µg/mL, Amygdalin 4 µg/mL, and Amygdalin 8 µg/mL were evaluated. The control group was rated as 100 and the viability rate of the other groups was proportioned and compared to the control group. Amygdalin 1 µg/mL, Amygdalin 4 µg/mL, and Amygdalin 8 µg/mL cell viability ratios were 89, 83, and 75 respectively. It was determined that Amygdalin 8 µg/mL was significantly different from the control at a rate of $p < .05$.

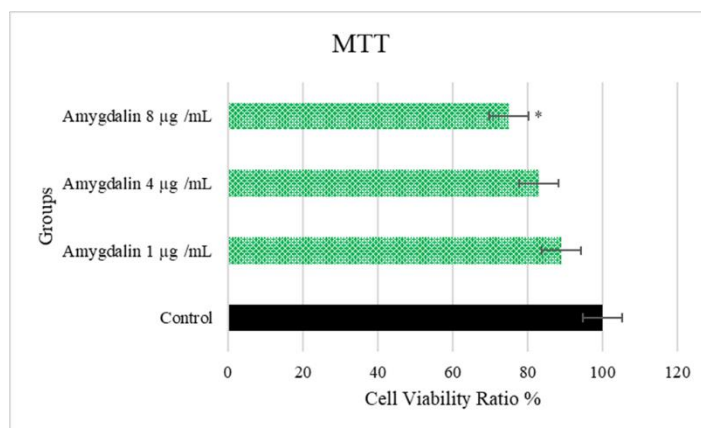


Figure 1. The cell viability ratio of T98G 24 hours after treatment. The experimental groups contain the Control group, Amygdalin 1 µg/mL, Amygdalin 4 µg/mL, and Amygdalin 8 µg/mL. Statistical significance: * $p < .05$ compared to the control group.

TAC and TOS assay

AK Evaluation results regarding TAC and TOS results are shown in Figure 2. TAC and TOS evaluations resulting from 24-hour exposure to Amygdalin 1 µg/mL, Amygdalin 4 µg/mL, and Amygdalin 8 µg/mL were determined.

TAC: It is observed that the use of Amygdalin 1 µg/mL, Amygdalin 4 µg/mL, and Amygdalin 8 µg/mL decreased TAC values. Amygdalin 4 µg/mL, and Amygdalin 8 µg/mL show statistically differences of 3.5 and 3.2 respectively ($p < .001$).

TOS: Amygdalin increased TOS value. At doses of Amygdalin 4 µg/mL $p < .05$, and Amygdalin 8 µg/mL $p < .001$ statistically significant differences were obtained.

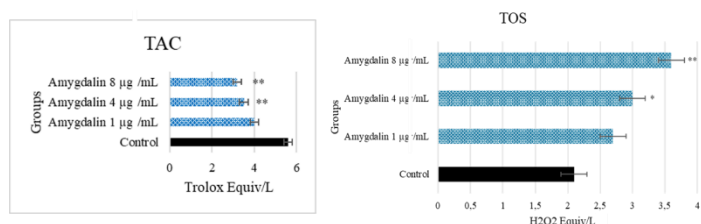


Figure 2. The Total Antioxidant Capacity and Total Oxidant Capacity of T98G cell lines 24 hours after treatment. The experimental groups contain the Control group, Amygdalin 1 µg/mL, Amygdalin 4 µg/mL, and Amygdalin 8 µg/mL. Statistical significance: * $p < .05$, ** $p < .001$ compared to the control group.

Discussion

In recent years, scientific research has been conducted

to investigate the effectiveness and safety of Amygdalin (Vit B17) against many types of cancer. Although in vitro studies are widely conducted on prostate, digestive system, and breast cancer, glioblastoma studies are limited. Cell cycle disruption, apoptosis, and toxicity due to oxidative stress have been shown in some studies (Jaszczak-Wilke et al., 2021; Kolesarova et al., 2021). However, the study on the level of TAC and TOS levels on the T98G cell line is presented for the first time with this publication.

A study has shown that Amygdalin can induce apoptosis in the human promyelocytic leukemia (HL-60) cell line (Kwon HeeYoung et al., 2003). Another study with SNU-C4 cells has shown that cell cycle and proliferation are suppressed with amygdalin (Kwon HeeYoung et al., 2003; Lin et al., 2022). In the current study, it has been shown that the use of Amygdalin reduces the viability of cancer cells depending on the dose. In addition, the most effective dose was determined as 8 µg/mL. Although this effect is consistent with the literature, the point that should be noted is the amygdalin concentration. Although the effect starts with a dose of 4 µg/mL, significant change and death occur when this dose is doubled. When the total antioxidant and oxidant levels are examined in the continuation of the mechanism review, it is understood that the decrease in TOC value means that the protective mechanism of cancer is disabled and oxidants could have damaged mitochondrial organelles (Makarević et al., 2016). Although amygdalin is only related to the rhodenase enzyme, which causes death in cancer cells as a mechanism, studies (Systemic-Review 2015) have shown that amygdalin is beneficial in cancer patients (Milazzo et al., 2006; Song & Xu, 2014). In addition, studies conducted with HeLa, DU145, and LNCaP cells have shown that amygdalin is effective on Bax and Bcl-2 gene expressions, as well as an increase in cellular stress (Milazzo et al., 2006; Park et al., 2005; Seyhan et al., 2023). In vitro experiments have shown that Amygdalin induces apoptosis by increasing the expression of Bax protein and caspase-3 and decreasing the expression of the antiapoptotic Bcl-2 protein (Fernald & Kurokawa, 2013; Carter et al., 1980; Lee & Moon, 2016). Amygdalin also reduces the expression of integrins. Accordingly, it reduces catenin levels and consequently inhibits the metastasis of cancer cells. In addition, studies have shown that it inhibits the adhesion of breast cancer cells, lung cancer cells, and bladder cancer cells by inhibiting the Akt-mTOR pathway. Amygdalin increased the expression of the p19 protein in kidney cancer cells. As a result, it led to the inhibition of cell transfer from the G1 phase to the S phase and thus inhibited cell proliferation (Gogolin et al., 2013; Makarević et al., 2014; Krebs, 1970). Amygdalin also inhibited the NF-κβ signaling pathway and showed anti-

inflammatory activity by affecting the release of proinflammatory cytokines (Krebs, 1970; Bauernfeind et al., 2009). In our study, it was determined that oxidative stress increased in the cancer line after cancer cell exposure to amygdalin and, conversely, antioxidant capacity decreased.

Conclusion

In conclusion, our study showed that Amygdalin has a cytotoxic effect on glioblastoma cancer cells by causing oxidative stress. Based on the information in the literature, this effect is likely to occur through an apoptotic mechanism. Our results suggest that Amygdalin can be considered as an adjuvant treatment in GBM cancer patients. However, further studies are needed to investigate the exact mechanism of the effect.

Ethics Committee Approval: Since it is a cell culture study, ethics committee approval is not required.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – S.G.; Design - S.G.; Supervision – S.G., A.T.; Resources – K.K.; Materials – E.N., S.G.; DA.T.a Collection and/or Processing – YEM, A.T.; Analysis and/or Interpretation – A.T., B.A.; Literature Search – S.G., A.E.A.; Writing Manuscript – K.K., S.G.; Critical Review – A.T., E.N.; Other – A.T..

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

References

- Altinoz, M. A., Yilmaz, A., Taghizadehghalehjoughi, A., Genc, S., Yeni, Y., Gecili, I., & Hacimuftuoglu, A. (2022). Ulipristal-temozolomide-hydroxyurea combination for glioblastoma: In-vitro studies. *Journal of Neurosurgical Sciences*, 68(4):468-481
- Bauernfeind, F. G., Horvath, G., Stutz, A., Alnemri, E. S., MacDonald, K., Speert, D., ... & Latz, E. (2009). Cutting edge: NF-κB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression. *The Journal of Immunology*, 183(2), 787–791.
- Carter, J. H., McLafferty, M. A., & Goldman, P. (1980). Role of the gastrointestinal microflora in amygdalin (laetrile)-induced cyanide toxicity. *Biochemical Pharmacology*, 29(3), 301–304.
- El-Desouky, M. A., Fahmi, A. A., Abdelkader, I. Y., & Nasraddin, K. M. (2020). Anticancer effect of amygdalin (vitamin B-17) on hepatocellular carcinoma cell line (HepG2) in the presence and absence of zinc. *Anti-Cancer Agents in Medicinal Chemistry*, 20(4), 486–494.
- Fernald, K., & Kurokawa, M. (2013). Evading apoptosis in cancer. *Trends in Cell Biology*, 23(12), 620–633.

- Gogolin, S., Ehemann, V., Becker, G., Brueckner, L. M., Drexler, D., Bannert, S., ... & Westermann, F. (2013). CDK4 inhibition restores G₁-S arrest in MYCN-amplified neuroblastoma cells in the context of doxorubicin-induced DNA damage. *Cell Cycle*, *12*(7), 1091–1104.
- Jaszczak-Wilke, E., Polkowska, Ż., Koprowski, M., Owsianik, K., Mitchell, A. E., & Bałczewski, P. (2021). Amygdalin: Toxicity, anticancer activity and analytical procedures for its determination in plant seeds. *Molecules*, *26*(8), 2253.
- Kafagi, A. H., Li, A. S., Jayne, D., & Brix, S. R. (2024). Anti-GBM disease in pregnancy. *BMJ Case Reports CP*, *17*(4), e257767.
- Kolesarova, A., Baldovska, S., & Roychoudhury, S. (2021). The multiple actions of amygdalin on cellular processes with an emphasis on female reproduction. *Pharmaceuticals*, *14*(9), 881.
- Krebs, E. T. (1970). Nitrilosides (vitamin B-17), their nature, occurrence and metabolic significance. *Journal of Applied Nutrition*.
- Lee, H. M., & Moon, A. (2016). Amygdalin regulates apoptosis and adhesion in Hs578T triple-negative breast cancer cells. *Biomolecules & Therapeutics*, *24*(1), 62–70.
- Lin, S., Wen, J., Xu, X., Shi, J., Zhang, W., Zheng, T., ... & Fan, L. (2022). Amygdalin induced mitochondria-mediated apoptosis of lung cancer cells via regulating NF-κB signaling cascade in vitro and in vivo. *The American Journal of Chinese Medicine*, *50*(5), 1361–1386.
- Loginova, N., Aniskin, D., Timashev, P., Ulasov, I., & Kharwar, R. K. (2024). GBM immunotherapy: Macrophage impacts. *Immunological Investigations*, 1–22.
- Makarević, J., Rutz, J., Juengel, E., Kaulfuss, S., Reiter, M., Tsaur, I., ... & Blaheta, R. A. (2014). Amygdalin blocks bladder cancer cell growth in vitro by diminishing cyclin A and cdk2. *PLoS ONE*, *9*(8), e105590.
- Makarević, J., Tsaur, I., Juengel, E., Borgmann, H., Nelson, K., Thomas, C., ... & Blaheta, R. A. (2016). Amygdalin delays cell cycle progression and blocks growth of prostate cancer cells in vitro. *Life Sciences*, *147*, 137–142.
- Milazzo, S., Ernst, E., Lejeune, S., & Boehm, K. (2006). Laetrile treatment for cancer. *Cochrane Database of Systematic Reviews*, *2006*(2).
- Park, H. J., Yoon, S. H., Han, L. S., Zheng, L. T., Jung, K. H., Uhm, Y. K., ... & Hong, S. P. (2005). Amygdalin inhibits genes related to cell cycle in SNU-C4 human colon cancer cells. *World Journal of Gastroenterology*, *11*(33), 5156–5161.
- Seyhan, S. A., Alkaya, D. B., Cesur, S., & Sahin, A. (2023). Investigation of the antitumor effect on breast cancer cells of the electrospun amygdalin-loaded poly(l-lactic acid)/poly(ethylene glycol) nanofibers. *International Journal of Biological Macromolecules*, *239*, 124201.
- Song, Z., & Xu, X. (2014). Advanced research on anti-tumor effects of amygdalin. *Journal of Cancer Research and Therapeutics*, *10*(Suppl 1), C3–C7.
- Tousson, E., Hafez, E., Abo Gazia, M. M., Salem, S. B., & Mutar, T. F. (2020). Hepatic ameliorative role of vitamin B17 against Ehrlich ascites carcinoma-induced liver toxicity. *Environmental Science and Pollution Research*, *27*, 9236–9246.
- Yeni, Y., Taghizadehghalehjoughi, A., Genc, S., Hacimuftuoglu, A., Yildirim, S., & Bolat, I. (2023). Glioblastoma cell-derived exosomes induce cell death and oxidative stress in primary cultures of olfactory neurons. Role of redox stress. *Molecular Biology Reports*, *50*(5), 3999–4009.
- Zhang, H., Chen, F., Wang, X., & Yao, H.-Y. (2006). Evaluation of antioxidant activity of parsley (*Petroselinum crispum*) essential oil and identification of its antioxidant constituents. *Food Research International*, *39*(7), 833–839.