Understanding How Madecassic Acid Affects Glioblastoma Cells: Its Antioxidant and Anti-cancer Properties

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Received: August 26, 2023 **Accepted:** August 28, 2023 **Published:** 29 August, 2023

To cite this article: Okkay et al. (2023). Understanding How Madecassic Acid Affects Glioblastoma Cells: Its Antioxidant and Anti-cancer Properties. Recent Trends in Pharmacology, vol 1, issue 2: 63-71.

Abstract

Glioblastoma, a malignant brain tumor, poses significant therapeutic challenges due to its aggressive nature and limited treatment options. The search for novel compounds with potential therapeutic efficacy against this devastating disease remains imperative. Madecassic acid (MA), a triterpenoid derived from *Centella asiatica*, has emerged as a promising candidate with multifaceted pharmacological activities, including antioxidant and antitumor effects. However, its specific impact on glioblastoma cells necessitates further exploration to fully comprehend its therapeutic potential. In this investigation, we examined the effects of MA on a human glioblastoma cell line, with a primary focus on its antioxidant and anti-proliferative properties. Utilizing a comprehensive range of cellular and molecular assays, we unraveled the potential of MA in scavenging free radicals, mitigating oxidative stress, and inhibiting glioblastoma cell proliferation. The study revealed that MA significantly inhibited glioblastoma cell growth. Furthermore, MA-treated cells exhibited higher antioxidant enzyme activity and lower oxidative stress levels compared to untreated cells. These findings demonstrate the potential of MA as an effective therapeutic agent against glioblastoma. By elucidating its antioxidant and anti-proliferative effects, this research opens new avenues for innovative treatment strategies to combat this relentless brain tumor. The insights gained from this study may contribute to improved patient outcomes and revolutionize the landscape of glioblastoma therapy.

Keywords: cell viability, glioblastoma cell line, GBM, madecassic acid, oxidative stress.

1. Introduction

Glioblastoma (GBM), a formidable and malignant brain tumor, presents significant therapeutic challenges due to its aggressive nature and limited treatment options (Shergalis, Bankhead, Luesakul, Muangsin, & Neamati, 2018; Ulasov, Singh, Laevskaya, Timashev, & Kharwar, 2023). Despite considerable advances in cancer research, there remains an urgent need to explore novel compounds with potential therapeutic efficacy against this devastating disease.

Madecassic acid (MA), a triterpenoid derived from *Centella asiatica*, has emerged as a promising candidate with multifaceted pharmacological activities, including notable antioxidant and antitumor effects (Ferah Okkay, Okkay, Aydin, et al., 2022; Sun et al., 2020; Valdeira et al., 2019; Yang et al., 2016). However, its specific impact on GBM cells necessitates further exploration to fully comprehend its therapeutic potential. In this investigation, we endeavor to delve into the effects of MA on a human GBM cell line, with a primary focus on elucidating its antioxidant and anti-proliferative properties. Through a comprehensive range of cellular and molecular assays, we seek to unravel the potential of MA in scavenging free radicals, mitigating oxidative stress, and inhibiting GBM cell proliferation.

The findings of this study hold great promise in unveiling novel therapeutic agents that could effectively target GBM. By comprehensively deciphering the antioxidant and anti-proliferative effects of MA in GBM cells, this research may open new avenues for the development of innovative treatment strategies to combat this relentless brain tumor. Ultimately, these profound insights hold the potential to lead to enhanced patient outcomes and fundamentally reshape the paradigm of therapy for GBM.

2. Materials and Methods

2.1.Cell Culture

To investigate how MA affects cancer cells, we used GBM cells in our experiments. These cells were grown in RPMI 1640 medium containing 100 units/mL of penicillin, 100 µg/mL of streptomycin, 10% fetal bovine serum, and 2.5 mM L-glutamine (Gibco®, Invitrogen™ GmbH, Karlsruhe, Germany). The cells were kept in a controlled environment at 37 °C with 5% CO2 and 95% air, providing the necessary humidity. The culture medium was changed three times a week to maintain cell health and growth (Ferah Okkay et al., 2021).

2.2. MTT Assay

To understand how MA affects GBM cells, we used a test called the MTT assay. We performed this test three times to get

accurate results. First, we put GBM cells in 96-well plates, with 5,000 cells in each well. Then, we treated the cells with different concentrations of MA: 1 µM, 3 µM, and 10 µM. The cells were exposed to MA for 24 hours. After the incubation period, we added a special solution called MTT to each well. We covered the plate with foil and kept it at a temperature of 37°C for 2 hours. Later, we removed the MTT solution and found that a blue-violet substance called formazan had formed in the wells. To measure this substance, we added 150 µL of another chemical called dimethylsulfoxide (DMSO) to dissolve it. We kept the plate in the dark for 30 minutes. To see the results, we used an Elisa Reader to measure the absorbance values at a wavelength of 570 nm (Ferah Okkay et al., 2021). By doing this, we could understand how much mitochondrial activity was present in the cells after the treatment with MA. To compare, we also had some cells that were not treated with MA, and they acted as a control to show us the normal level of mitochondrial activity (100%) in the cells.

2.3. Measurement of Oxidative Stress

To understand the antioxidant abilities of the cell culture supernatants, we conducted specific tests. These tests include Superoxide Dismutase (SOD), Catalase (CAT), and Malondialdehyde (MDA) assays (Ahiskali et al., 2021; Ferah Okkay et al., 2023; Ferah Okkay, Okkay, Gundogdu, et al., 2022; Okkay, Ferah Okkay, Aydin, et al., 2021; Okkay, Ferah Okkay, Cicek, et al., 2021; Okkay, Ferah Okkay, Cicek, Aydin, & Ozkaraca, 2022). We used commercial kits from Elabscience® to perform these tests. In the SOD assay, we measured the activity of Superoxide Dismutase, an important antioxidant enzyme that helps neutralize harmful free radicals in the cells. The CAT assay allowed us to assess the activity of CAT, another vital enzyme that defends the cells against oxidative damage by breaking down hydrogen peroxide. Lastly, the MDA assay helped us determine the levels of MDA, a marker of oxidative stress and lipid peroxidation. By conducting these tests, we gained valuable insights into the antioxidant potential of the cell culture supernatants and how well they can protect the cells from oxidative burden. Understanding these aspects is crucial in evaluating the effects of our experiment and its implications for future research in this area.

3. RESULTS

3.1.Cell viability

The MTT test, a colorimetric assay, was used to measure the percentage of viable GBM cells. We wanted to see how effective MA is, so we treated the GBM cells with different amounts of MA for 24 hours. The results of this experiment are

shown in Figure 1. After analyzing the data, we found that the best concentration and duration of MA treatment was 10 μM for 24 hours (Fig. 1). At this concentration, MA significantly inhibited cell growth. This effect was statistically significant when compared to the control group, with a p-value less than 0.05. These findings suggest that MA has the potential to be an effective treatment for inhibiting the growth of GBM cells.

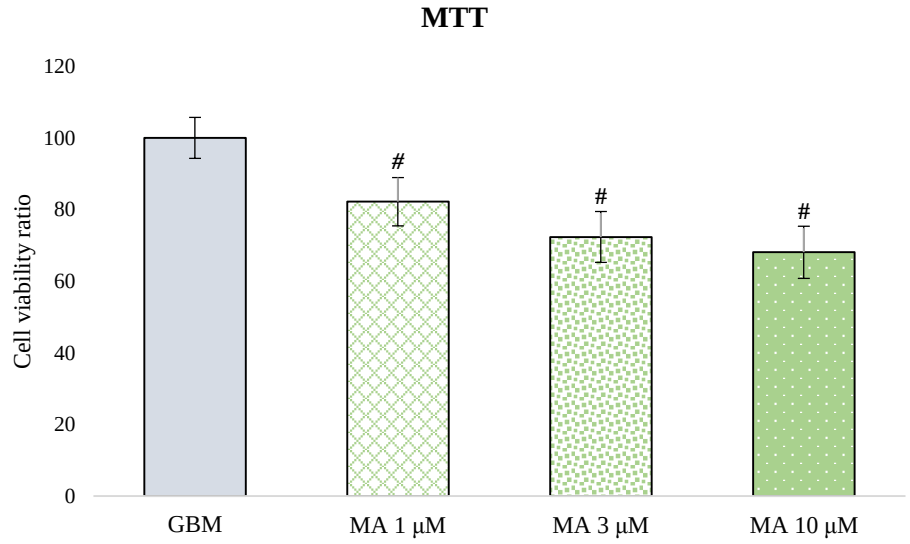

Figure 1. Effects of MA on the cell viability. Data are expressed as the means ± SD. # p<0,05 vs GBM group.

3.2.Measurement of Oxidative Stress

In this part of the study, we wanted to understand the impact of MA on oxidative burden in GBM cells. To do this, we measured the activity of two important antioxidant enzymes, SOD and CAT, in both the MA group and the GBM group. The results showed that the MA group had significantly higher SOD and CAT activity compared to the GBM group. This means that the MA group had a stronger overall antioxidant capacity, which suggests it could better protect the cells from harmful free radicals and oxidative damage. Moreover, we also measured the levels of MDA, which is the end product of lipid peroxidation. The MDA values in the MA group were significantly lower compared to the GBM group. This indicates that the MA group had a lower overall level of oxidative stress. To better understand these findings, the study included figures to visually represent the SOD and CAT activity and MDA levels for both groups (Fig. 2, 3, 4). In conclusion, these results suggest that MA may have a beneficial effect on oxidative burden in GBM cells by enhancing antioxidant defenses and reducing oxidative stress.

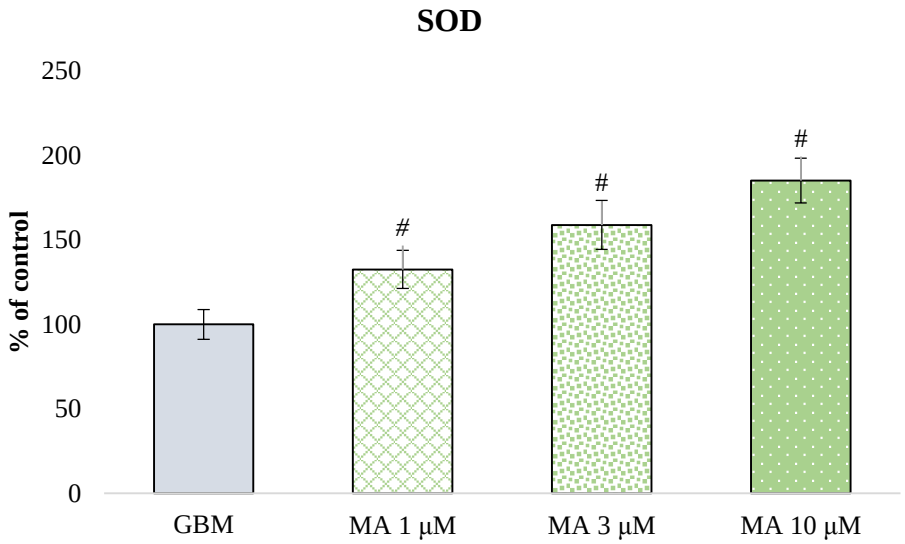

Figure 2. Effects of MA on the SOD levels. Data are expressed as the means ± SD. # p<0,05 vs GBM group.

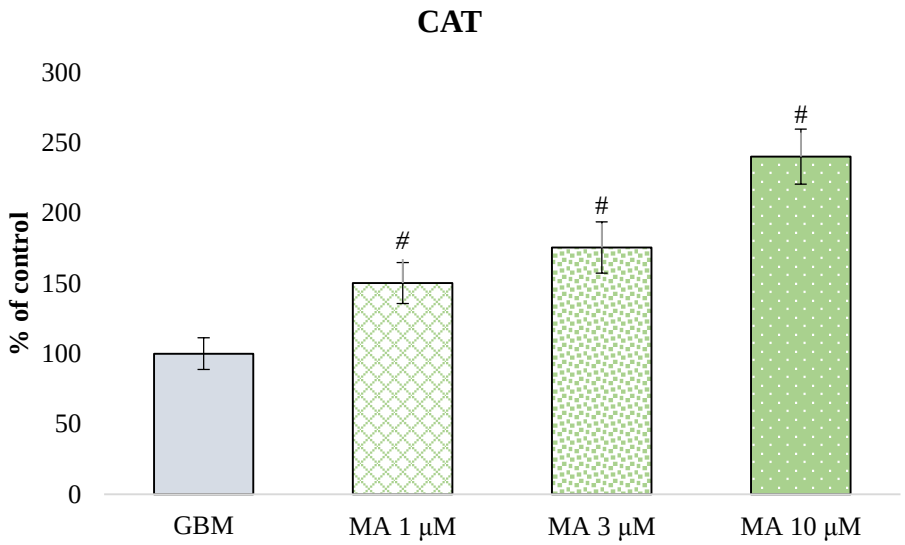

Figure 3. Effects of MA on the CAT levels. Data are expressed as the means ± SD. # p<0,05 vs GBM group.

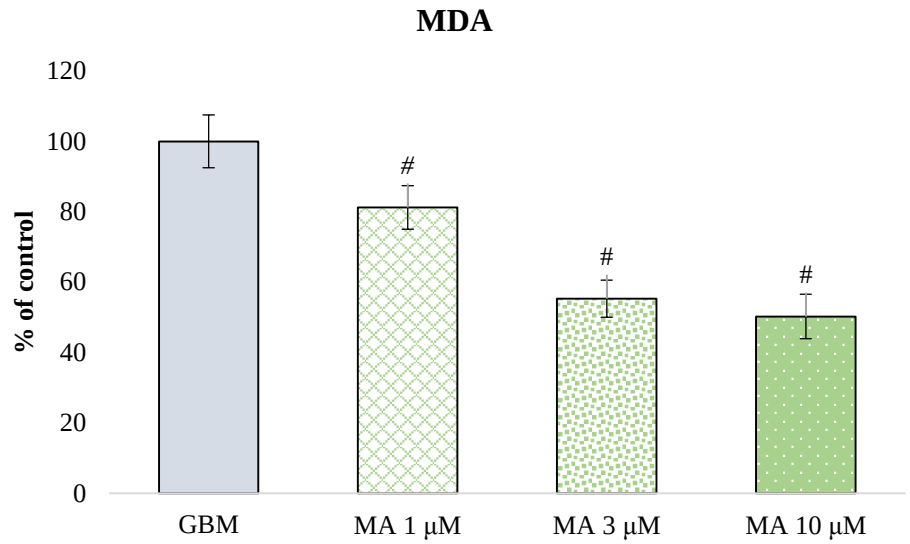

Figure 4. Effects of MA on the MDA levels. Data are expressed as the means ± SD. # p<0,05 vs GBM group.

3.3.Statistical Analysis

To determine the significance of our findings, we used a statistical method called one-way analysis of variance (ANOVA). After running the ANOVA, we conducted a post hoc test called Tukey's test to make more specific comparisons between the groups. We used IBM SPSS version 22.0 software to perform these statistical analyses. In our results, a p-value less than 0.05 was considered statistically significant. To present the data in a clear manner, we used mean (average) values ± SD (standard deviation).

4. Discussion

In this study, we aimed to understand how MA, a compound derived from *Centella asiatica*, affects GBM cells, a highly aggressive and challenging brain tumor (Wu et al., 2021). GBM currently lacks effective treatment options, making it crucial to explore new therapeutic compounds (Liu et al., 2022). The research on MA has shown promising results, indicating that it possesses various pharmacological activities, including antioxidant and antitumor effects. However, its specific impact on GBM cells required further investigation to fully grasp its potential as a therapeutic agent. Our study focused on elucidating the antioxidant and anti-proliferative properties of MA in a human GBM cell line. We conducted a series of cellular and molecular assays to explore its ability to scavenge free radicals, reduce oxidative stress, and inhibit GBM cell growth. The results from the MTT assay demonstrated that MA effectively inhibited the growth of GBM cells. These findings suggest that MA has potential as an effective treatment to hinder the proliferation of GBM cells. Oxidative stress

plays a crucial role in cancer development, and we wanted to assess how MA affects this burden in GBM cells. Our measurement of antioxidant enzymes, SOD and CAT, revealed that the MA-treated group had significantly higher activity levels compared to the untreated GBM group. This indicates that MA enhances the antioxidant capacity of the cells, potentially protecting them from harmful free radicals and oxidative damage in line with the previous studies (Arancibia-Radich et al., 2019; Valdeira et al., 2018; Won et al., 2010; Xia et al., 2015; Yang et al., 2016; Zhang, Zhang, Tao, Wang, & Xia, 2014). Furthermore, the MDA assay demonstrated that the MA-treated group exhibited lower levels of oxidative stress compared to the GBM group. This suggests that MA could help reduce oxidative damage in GBM cells. Overall, this study sheds light on the potential of MA as a promising therapeutic agent for GBM. By unraveling its antioxidant and anti-proliferative effects, this research paves the way for the development of innovative treatment strategies against this aggressive brain tumor. Further investigations and clinical trials are warranted to validate the therapeutic efficacy of MA and its potential to improve patient outcomes in GBM therapy.

References

Ahiskali, I., Ferah Okkay, I., Mammadov, R., Okkay, U., Keskin Cimen, F., Kurt, N., & Suleyman, H. (2021). Effect of taxifolin on cisplatin-associated oxidative optic nerve damage in rats. *Cutan Ocul Toxicol, 40*(1), 1-6. doi:10.1080/15569527.2020.1844726

Arancibia-Radich, J., Gonzalez-Blazquez, R., Alcala, M., Martin-Ramos, M., Viana, M., Arribas, S., . . . Gil-Ortega, M. (2019). Beneficial effects of murtilla extract and madecassic acid on insulin sensitivity and endothelial function in a model of diet-induced obesity. *Sci Rep, 9*(1), 599. doi:10.1038/s41598-018-36555-1

Ferah Okkay, I., Okkay, U., Aydin, I. C., Bayram, C., Ertugrul, M. S., Mendil, A. S., & Hacimuftuoglu, A. (2022). Centella asiatica extract protects against cisplatin-induced hepatotoxicity via targeting oxidative stress, inflammation, and apoptosis. *Environ Sci Pollut Res Int, 29*(22), 33774-33784. doi:10.1007/s11356-022-18626-z

Ferah Okkay, I., Okkay, U., Bayram, C., Cicek, B., Sezen, S., Aydin, I. C., . . . Hacimuftuoglu, A. (2023). Bromelain protects against cisplatin-induced ocular toxicity through mitigating oxidative stress and inflammation. *Drug Chem Toxicol, 46*(1), 69-76. doi:10.1080/01480545.2021.2011308

Ferah Okkay, I., Okkay, U., Cicek, B., Yilmaz, A., Yesilyurt, F., Mendil, A. S., & Hacimuftuoglu, A. (2021). Neuroprotective effect of bromelain in 6-hydroxydopamine induced in vitro model of Parkinson's disease. *Mol Biol Rep,*

48(12), 7711-7717. doi:10.1007/s11033-021-06779-y

Ferah Okkay, I., Okkay, U., Gundogdu, O. L., Bayram, C., Mendil, A. S., Ertugrul, M. S., & Hacimuftuoglu, A. (2022). Syringic acid protects against thioacetamide-induced hepatic encephalopathy: Behavioral, biochemical, and molecular evidence. *Neurosci Lett, 769*, 136385. doi:10.1016/j.neulet.2021.136385

Liu, H., Qiu, W., Sun, T., Wang, L., Du, C., Hu, Y., . . . Sun, H. (2022). Therapeutic strategies of glioblastoma (GBM): The current advances in the molecular targets and bioactive small molecule compounds. *Acta Pharm Sin B, 12*(4), 1781-1804. doi:10.1016/j.apsb.2021.12.019

Okkay, U., Ferah Okkay, I., Aydin, I. C., Bayram, C., Ertugrul, M. S., Gezer, A., & Hacimuftuoglu, A. (2021). Effects of Achillea millefolium on cisplatin induced ocular toxicity: an experimental study. *Cutan Ocul Toxicol, 40*(3), 214-220. doi:10.1080/15569527.2021.1919137

Okkay, U., Ferah Okkay, I., Cicek, B., Aydin, I. C., Ertugrul, M. S., Bayram, C., . . . Hacimuftuoglu, A. (2021). Achillea millefolium alleviates testicular damage in paclitaxel-intoxicated rats via attenuation of testicular oxido-inflammatory stress and apoptotic responses. *Andrologia, 53*(5), e14028. doi:10.1111/and.14028

Okkay, U., Ferah Okkay, I., Cicek, B., Aydin, I. C., & Ozkaraca, M. (2022). Hepatoprotective and neuroprotective effect of taxifolin on hepatic encephalopathy in rats. *Metab Brain Dis, 37*(5), 1541-1556. doi:10.1007/s11011-022-00952-3

Shergalis, A., Bankhead, A., 3rd, Luesakul, U., Muangsin, N., & Neamati, N. (2018). Current Challenges and Opportunities in Treating Glioblastoma. *Pharmacol Rev, 70*(3), 412-445. doi:10.1124/pr.117.014944

Sun, B., Wu, L., Wu, Y., Zhang, C., Qin, L., Hayashi, M., . . . Liu, T. (2020). Therapeutic Potential of Centella asiatica and Its Triterpenes: A Review. *Front Pharmacol, 11*, 568032. doi:10.3389/fphar.2020.568032

Ulasov, I., Singh, V., Laevskaya, A., Timashev, P., & Kharwar, R. K. (2023). Inflammatory Mediators and GBM Malignancy: Current Scenario and Future Prospective. *Discov Med, 35*(177), 458-475. doi:10.24976/Discov.Med.202335177.47

Valdeira, A. S. C., Darvishi, E., Woldemichael, G. M., Beutler, J. A., Gustafson, K. R., & Salvador, J. A. R. (2019). Madecassic Acid Derivatives as Potential Anticancer Agents: Synthesis and Cytotoxic Evaluation. *J Nat Prod, 82*(8), 2094-2105. doi:10.1021/acs.jnatprod.8b00864

Valdeira, A. S. C., Ritt, D. A., Morrison, D. K., McMahon, J. B., Gustafson, K. R., & Salvador, J. A. R. (2018). Synthesis and Biological Evaluation of New Madecassic Acid Derivatives Targeting ERK Cascade Signaling. *Front Chem, 6*, 434. doi:10.3389/fchem.2018.00434

Won, J. H., Shin, J. S., Park, H. J., Jung, H. J., Koh, D. J., Jo, B. G., . . . Lee, K. T. (2010). Anti-inflammatory effects of madecassic acid via the suppression of NF-kappaB pathway in LPS-induced RAW 264.7 macrophage cells. *Planta Med, 76*(3), 251-257. doi:10.1055/s-0029-1186142

Wu, W., Klockow, J. L., Zhang, M., Lafortune, F., Chang, E., Jin, L., . . . Daldrup-Link, H. E. (2021). Glioblastoma multiforme (GBM): An overview of current therapies and mechanisms of resistance. *Pharmacol Res, 171*, 105780. doi:10.1016/j.phrs.2021.105780

Xia, B., Bai, L., Li, X., Xiong, J., Xu, P., & Xue, M. (2015). Structural analysis of metabolites of asiatic acid and its analogue madecassic acid in zebrafish using LC/IT-MSn. *Molecules, 20*(2), 3001-3019. doi:10.3390/molecules20023001

Yang, B., Xu, Y., Hu, Y., Luo, Y., Lu, X., Tsui, C. K., . . . Liang, X. (2016). Madecassic Acid protects against hypoxia-induced oxidative stress in retinal microvascular endothelial cells via ROS-mediated endoplasmic reticulum stress. *Biomed Pharmacother, 84*, 845-852. doi:10.1016/j.biopha.2016.10.015

Zhang, H., Zhang, M., Tao, Y., Wang, G., & Xia, B. (2014). Madecassic acid inhibits the mouse colon cancer growth by inducing apoptosis and immunomodulation. *J BUON, 19*(2), 372-376. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/24965394