Title

The Effects of Natural Medicine as Possible Treatment for Glioblastoma

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Publication Date

2024-10-01

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Abstract

The goal of this literature review is to analyze different herbal medicine treatment options for Glioblastoma, a highly malignant cancer that starts as a growth of cells in the brain or spinal cord. The four different herbal medicine treatments that are studied in the paper are Curcumin, Flavonoids, Quercetin, and Terpenoids. Curcumin is used as a key inhibitor in various pathways in the cell proliferation and apoptosis of GBM cells. Flavonoids regulate tumor cell glucose metabolism by preventing cancer cells from uptaking glucose and cannot harness energy in the form of ATP. Quercetin is targeted at glioma cancer growth without negatively impacting normal CNS cells. Terpenoids are used to inhibit the transfer of P-glycoprotein, which can inhibit glioblastoma formation. All four herbal treatments have been shown to slow the progression of Glioblastoma by inhibiting the proliferation of Glioblastoma cells. This can be seen through the decrease in the tumor size of Glioblastoma patients.

Introduction

Glioblastoma [glioblastoma multiforme (GBM)] is a highly malignant cancer that starts as a growth of cells in the brain or spinal cord. It grows quickly and can invade and destroy healthy tissue. The cancer can spread into other areas of the brain as well. Rarely the cancer spreads outside the brain to other parts of the body. Glioma tumors like GBM start in glial cells which are vital to nerve cell function. GBMs specifically form in glial cells called astrocytes. GBMs are the fastest-growing astrocytoma (a tumor that forms in astrocytes)[1]. Approximately 15% of all primary brain tumors are GBM. GBM may arise de novo or, occasionally, from a low-grade astrocytoma. Median survival with treatment is 15 months, with a two-year survival rate of less than 25%, and survival without treatment is usually only a few months. Frequent presenting signs include headaches, nausea, seizures, blurred vision, vomiting, and personality changes.

The common treatment for GBM is a combination of surgery, radiotherapy, and chemotherapy. The effectiveness of surgery is limited by the difficulty of complete tumor resection and the presence of residual tumor cells. If surgery is not an option due to tumor size, tumor location, or very poor patient performance status, a combination of radiation and chemotherapy is used. Usually used alongside radiation, temozolomide (TMZ) treatment is also an option. TMZ is an oral alkylating agent that can cross the blood-brain barrier and is often utilized as a first-line treatment for GBM. However, like all drugs, it does not come without its drawbacks. The limits on treating glioblastoma can be summarized into two main points. Firstly, the blood-brain barrier (BBB) blocks the medication from directly reaching the cancer. This can thus result in recurrence without total recovery, and the cancer stem cells (CSCs) that manage to

[1]Cleveland Clinic. (2021b, March 9). *Glioblastoma: Symptoms, causes, treatment & prognosis*. Cleveland Clinic. https://my.clevelandclinic.org/health/diseases/17032-glioblastoma

evade cytotoxicity can develop therapeutic resistance to said medication. Secondly, conventional chemotherapy medications, such as TMZ, can result in a myriad of side effects, such as myelosuppression and cerebral edema.

Glioblastoma can happen at any age. However, it tends to occur more often in older adults and more often in men. GBM commonly affects people aged 45 to 70. The average age at diagnosis is 64. Also, as already mentioned, even though men have a slightly higher risk, the disease affects all ages and genders. More than 13,000 Americans are diagnosed with GBM every year. GBM accounts for almost half of all cancerous brain tumors.[2]It is not specifically known why some people develop cancerous brain tumors, including GBM. Factors that increase the risk of GBM are exposure to chemicals, such as pesticides, petroleum, synthetic rubber, and vinyl chloride; genetic tumor-causing conditions, such as neurofibromatosis, Li- Fraumeni syndrome, and Turcot syndrome, and previous radiation therapy to the head.

While there is no current cure for GBM, some research has been turning towards natural medicine derived from plants for its potential in combating the disease. This use of natural medicinal products for therapy – called phytotherapy - has emerged within the past two decades, becoming a popular potential alternative due to both its effectiveness and being less toxic (and thus fewer side effects) when compared to other treatments. These herbal remedies have been shown to inhibit the growth of glioblastoma and, when used alongside other traditional methods such as radiotherapy, could amplify the results and potentially prevent the recurrence of the tumor. Recent studies have shown that through phytotherapy, patients observed a reduction in tumor size and symptoms. Their life expectancy was also prolonged to years longer than with traditional treatments, even with primary tumor conditions. Other studies have shown patients with no progression of the tumor after months and an increase in survival for all trials. There

[2] Cleveland Clinic. (2021b, March 9). *Glioblastoma: Symptoms, causes, treatment & prognosis*. Cleveland Clinic. https://my.clevelandclinic.org/health/diseases/17032-glioblastoma

were also cases with mice where natural remedies slowed the growth of the tumor, which could have implications for its use in humans as well. Some of these natural substances include specific terpenes, carotenoids, flavonoids, steroids, and natural ingredients like curcumin (to name a few) that inhibit tumor growth (measured through tumor area, perimeter, weight, and volume) as seen in past studies.

Methodology

Due to the relatively new idea of the use of natural remedies for brain tumors, there is limited clinical research on this approach. For this treatment to be used in future clinical cases, significant research and experiments on certain remedies would need to be conducted. Therefore, this research paper will examine a multitude of natural resources that were tested on glioblastoma from credible sources to analyze the results of survival time, rate, and measure of tumor size after treatment and potentially suggest the best combinations or uses for each. We will take into account the study demographic, size, number of trials conducted, the period of the study, the publication date, and the reproducibility of the methods used in their research.

Our paper will include previous research papers from databases such as NIH, ScienceDirect, MDPI, PubMed, and Karger. The National Library of Medicine (NIH) is the world's largest biomedical library, supporting the research and development in biomedical research and health information technology. One study focused on identifying compounds that can control the blood-brain barrier, inhibit tumor growth, and prevent the development of tumors already formed.[3] Another study explores the effect of curcumin on dysregulated pathways common in cells affected by glioblastoma. These sources provide a summary of the research done on natural medicine and its effects on glioblastoma.

[3] Galati, G., & O'Brien, P. J. (2004). Potential toxicity of flavonoids and other dietary phenolics: Significance for their chemopreventive and anticancer properties. Free Radical Biology and Medicine, 37(3), 287–303. https://doi.org/10.1016/j.freeradbiomed.2004.04.034

ScienceDirect, one of the largest peer-reviewed journals, gathered research focusing on the negative side effects of traditional treatment of glioblastoma. Natural medicine provides a better approach to combat the low survival rate from traditional medical approaches, such as surgery or chemotherapy. Another study examined preclinical experiments of curcumin on cancer cells in rats to evaluate the effects it had on tumor growth.[4]

The Multidisciplinary Digital Publishing Institute (MDPI) is an open-access peer-reviewed journal site that publishes research on the mechanistic pathways involved in treating glioblastoma with natural medicine. Multiple pathways such as apoptosis, cell growth, Akt/mTOR signaling,[5] and more are examined and compared with and without the use of natural medicine.

Karger is a peer-reviewed medical publishing journal that was used to examine how the flavonoid quercetin inhibits glioblastoma growth. To do so, researchers conducted CCK-8 and clone-formation assays.[6] Mice models were then used to evaluate the condition of the GBM tumors when they were treated with quercetin.

For this paper, we used the following key terms in our searches: “glioblastoma,” “phytotherapy,” “medicinal plants,” “curcumin,” “natural remedies,” and “herbal.” From these initial searches, the abstract and results were screened for results that include both in-vitro and in-vivo data from either human or non-human processes that share similar pathways as humans. All results, including negative or side effects, are included. The paper will only use data from credible sources, including primary sources as well as secondary literature reviews.

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[4] Zanotto-Filho, A., Braganhol, E., Edelweiss, M. I., Behr, G. A., Zanin, R., Schröder, R., Simões-Pires, A., Battastini, A. M., & Moreira, J. C. (2012). The curry spice curcumin selectively inhibits cancer cells growth in vitro and in preclinical model of glioblastoma. The Journal of Nutritional Biochemistry, 23(6), 591–601. https://doi.org/10.1016/j.jnutbio.2011.02.015

[5] Zhai, K., Siddiqui, M., Abdellatif, B., Liskova, A., Kubatka, P., & Büsselberg, D. (2021). Natural compounds in glioblastoma therapy: Preclinical insights, mechanistic pathways, and outlook. Cancers, 13(10), 2317. https://doi.org/10.3390/cancers13102317

[6] Wang, L., Ji, S., Liu, Z., & Zhao, J. (2022). Quercetin inhibits glioblastoma growth and prolongs survival rate through inhibiting glycolytic metabolism. *Chemotherapy*, *67*(3), 132–141. https://doi.org/10.1159/000523905

Results

Curcumin

One type of phytochemical that has been studied for its potential anticancer properties is curcumin. Curcumin is a common Indian spice found in fruits and vegetables that has many health benefits as it is an antioxidant, anti-inflammatory, antiviral, antimutagenic and holds anti-carcinogenic properties. Nowadays, curcumin is widely used due to its multiple biological activities, encompassing anti-inflammatory, anti-oxidant, scavenging reactive oxidative species (ROS), regulation of mitochondrial homeostasis, stem cell modulation, and neurogenesis.[7] Hence, curcumin may be useful in a wide range of human diseases, encompassing cardiovascular[8], inflammatory[9], and metabolic disorders.[10] Curcumin is of interest to researchers for its beneficial effects on the central nervous system (CNS). It is a useful agent for adjunct therapy in a variety of neurodegenerative disorders (NDDs), too. Numerous studies demonstrated the chemo- and radio-sensitizing effects of curcumin in various cancers, including glioblastoma multiforme (GBM).[11]

As a result, curcumin has also been shown to play a key role in regulating various pathways involved in the cell proliferation and apoptosis of GBM cells. The two most supported

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[7] Limanaqi, F., Biagioni, F., Busceti, C. L., Ryskalin, L., Polzella, M., Frati, A., & Fornai, F. (2019). Phytochemicals bridging autophagy induction and alpha-synuclein degradation in parkinsonism. *International Journal of Molecular Sciences*, *20*(13), 3274. doi:10.3390/ijms20133274

[8] Miriyala, S., Panchatcharam, M., & Rengarajulu, P. (2007). Cardioprotective effects of Curcumin. *In Advances of Experimental Medicine and Biology* (pp. 359–377). doi:10.1007/978-0-387-46401-5_16

[9] Funk, J. L., Oyarzo, J. N., Frye, J. B., Chen, G., Lantz, R. C., Jolad, S. D., … Timmermann, B. N. (2006). Turmeric extracts containing curcuminoids prevent experimental rheumatoid arthritis. *Journal of Natural Products*, *69*(3), 351–355. doi:10.1021/np050327j

[10] Alappat, L., & Awad, A. B. (2010). Curcumin and obesity: evidence and mechanisms. *Nutrition Reviews*, *68*(12), 729–738. doi:10.1111/j.1753-4887.2010.00341.x

[11] Aggarwal, B. B., Shishodia, S., Takada, Y., Banerjee, S., Newman, R. A., Bueso-Ramos, C. E., & Price, J. E. (2005). Curcumin suppresses the paclitaxel-induced nuclear factor-kappaB pathway in breast cancer cells and inhibits lung metastasis of human breast cancer in nude mice. *Clinical Cancer Research: An Of icial Journal of the American Association for Cancer Research*, *11*(20), 7490–7498. doi:10.1158/1078-0432.CCR-05-1192; Li, M., Zhang, Z., Hill, D. L., Wang, H., & Zhang, R. (2007). Curcumin, a dietary component, has anticancer, chemosensitization, and radiosensitization effects by down-regulating the MDM2 oncogene through the PI3K/mTOR/ETS2 pathway. *Cancer Research*, *67*(5), 1988–1996. doi:10.1158/0008-5472.CAN-06-3066 ; Kunnumakkara, A. B., Guha, S., Krishnan, S., Diagaradjane, P., Gelovani, J., & Aggarwal, B. B. (2007).

pathways include the Retinoblastoma (Rb)[12] and Sonic hedgehog (Shh) pathways[13]. Both of these pathways are dysregulated in GBM cells, causing the cell proliferation, growth, and migration of these tumor cells.

The Retinoblastoma (RB) pathway functions to regulate the cell cycle as cyclin D1 activates CDK4, phosphorylating the RB protein and progressing the transcription from the G1 phase to the S phase, where DNA replication occurs.[8] There is a negative regulator called CDKN2A/p16, which can inhibit CDK4 when bound, keep the RB protein unphosphorylated, and stop the cell cycle process. In GBM cells, this pathway is altered with mutations or deletions of the negative regulator, CDKN2A/p16, and an increase in the production of CDK4 and cyclin D. The use of curcumin has been shown to counter these effects by increasing the CDKN2A/p16 to compete with the cyclin D1, and as a result, downregulate the amount of phosphorylated RB protein. In addition, as curcumin downregulates cyclin D1, it also inhibits P-glycoprotein on the blood-brain barrier that protects substances and drugs from entering. One of the many concerns with traditional therapeutics for GBM is the inability of anticancer drugs to enter the blood-brain barrier, and with the assistance of curcumin it could help overcome such chemoresistance and amplify the effect of these drugs.

The Sonic Hedgehog (Shh) pathway plays a role in cell signaling and controlling cell division. In normal conditions, Shh ligands bind to its receptor, Patched (PTCH), which then cannot inhibit another protein called Smoothened (SMO).[8] The Smo then signals for the negative regulator of glioma-associated oncogene homologue (GLI) to release GLI, which translocates

[12] Ryskalin, L., Biagioni, F., Busceti, C. L., Lazzeri, G., Frati, A., & Fornai, F. (2020). The multi-faceted effect of curcumin in glioblastoma from rescuing cell clearance to autophagy-independent effects. Molecules, 25(20), 4839. https://doi.org/10.3390/molecules25204839

[13] Wong, S. C., Kamarudin, M. N., & Naidu, R. (2021). Anticancer mechanism of curcumin on human glioblastoma. Nutrients, 13(3), 950. https://doi.org/10.3390/nu13030950

into the nucleus and promotes gene expression. The deregulation of the Shh pathway, increase in Shh ligands and receptors, and overexpression of Smo are notable causes for the poor prognosis of GBM patients. The intake of curcumin can inhibit these key components of the pathway, such as the Shh, Smo, and Gli proteins. Thus decreasing cell proliferation and migration and increasing the apoptosis of these tumor cells. Studies have shown that the inhibition of GLI can also work together with chemotherapy, as it decreases the metabolic activity of the GBM cells and impacts cells that have grown chemoresistant.

Although there is much research supporting the use of curcumin as a treatment option for GBM patients, there has been very little in regard to pre-clinical or clinical studies. One study was conducted both in vitro in rat and human GBM cells and in vivo in rats. A panel of GBM cell lines (C6- rat, U138MG- human) were treated with different concentrations of curcumin. Short-term incubation (24 h) with curcumin treatment (0μM, 15μM, 30μM) reduced the clonogenic potential of both GBM cells. With long-term exposure (72 h), the treatment groups showed that with increasing concentration of curcumin, there was a significant decrease in the proliferation by 73%±12%. Subtoxic curcumin (10μM) also decreased the migration of both the GBM cells. Even with six days of washout, there was still significant inhibition of GBM cells– indicating a long-term effect after drug withdrawal (Figure 1). Results were analyzed to quantify the tumor growth by removing the brain and quantifying the tumor volume with three hematoxylin and eosin coronal sections.[14] The area of the tumor was obtained using Image Tool Software, and the volume was found by computing the slice sections and area. Statistical analysis was assessed following Tukey's test, and statistical significance between the treatment and non-treatment groups was assessed using the t-test. Significance was calculated at $P < 0.05$. For

[14] Zanotto-Filho, A., Braganhol, E., Edelweiss, M. I., Behr, G. A., Zanin, R., Schröder, R., Simões-Pires, A., Battastini, A. M., & Moreira, J. C. (2012). The curry spice curcumin selectively inhibits cancer cells growth in vitro and in preclinical model of glioblastoma. The Journal of Nutritional Biochemistry, 23(6), 591–601. https://doi.org/10.1016/j.jnutbio.2011.02.015

their in vivo experiment, 7/11 curcumin-treated rats developed detectable tumors with decreases in tumor volume in five of the seven rats, while 11/11 control rats developed tumors. The number of apoptotic cells in the tumors of the curcumin-treated group was also significantly higher than the control group.

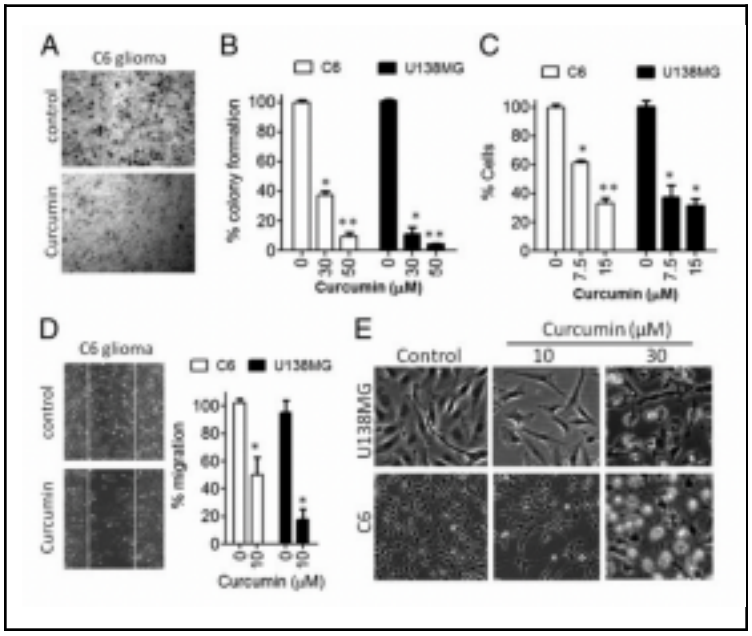

Figure 1: C6 and U138MG cell growth in various curcumin concentrations.

Additionally, Byeong Hyeok Choi and his team conducted a study where they assessed the mechanism by which curcumin stimulates transcriptional activation of p21 in U-87MG cells. The results of the experiment determined that curcumin arrests the cell cycle at G_1 in U-87MG glioblastoma cells and induces expression of the cyclin-dependent kinase inhibitor p21. p21 plays an important role in cell cycle arrest, senescence, differentiation, and apoptosis, and its expression is modulated by a wide variety of external stimuli, including radiation, DNA damage,

and growth factors.[15] It has become apparent that at low concentrations, p21 promotes the association and activation of D-type cyclin kinase, whereas at higher concentrations, it inhibits Cdk activity, resulting in the suppression of cell cycle progression. Curcumin stimulates p21 promoter activity through a p53-independent mechanism. Egr-1 is required for curcumin-induced activation of the p21 promoter, and curcumin upregulates the Egr-1 expression at the transcription level through extracellular signal-regulated kinase (ERK) and c-Jun NH_2-terminal kinase (JNK), but not the p38, mitogen-activated protein kinase (MAPK) pathways, which mediate the transactivation of Elk-1. To understand the mechanism by which curcumin inhibits cell growth, the antiproliferative effect of it was tested on U-87MG cells using a Cell Counting kit-8. Exponentially growing cells were exposed to various concentrations of curcumin for 12, 24, and 48 h, and their proliferation was monitored. A significant decrease in proliferation was observed in cells treated for 48 h with 20 μmol/L curcumin compared to untreated control cells (Figure 1). Exposure to higher concentrations of curcumin produced an even stronger effect by probably promoting cell death. Thus, curcumin has an antiproliferative effect on U87MG glioma cells. Additionally, time-response analysis showed that curcumin treatment increased the abundance of the p21 protein in a concentration-dependent manner independent of the p53 mechanism (Figure 2). P53 is a tumor suppressor, which is the major transcriptional activator of p21, which binds specific sites within the p21 promoter. It did so by increasing the transcriptional activity of the full-length promoter by 1.8-fold, which was statistically significant ($P < 0.05$; $n = 9$). As the accumulation of p21 increased, there was also a corresponding increase in Egr-1, a transcription factor that regulates cell growth, differentiation, and development, which was also tested as being curcumin induced, that effect being concentration-dependent, with a near maximum at 15 μmol/L (Figure 3)

[15] Xiong, Y., Hannon, G. J., Zhang, H., Casso, D., Kobayashi, R., & Beach, D. (1993). P21 is a universal inhibitor of cyclin kinases. *Nature*, *366*(6456), 701–704. doi:10.1038/366701a0

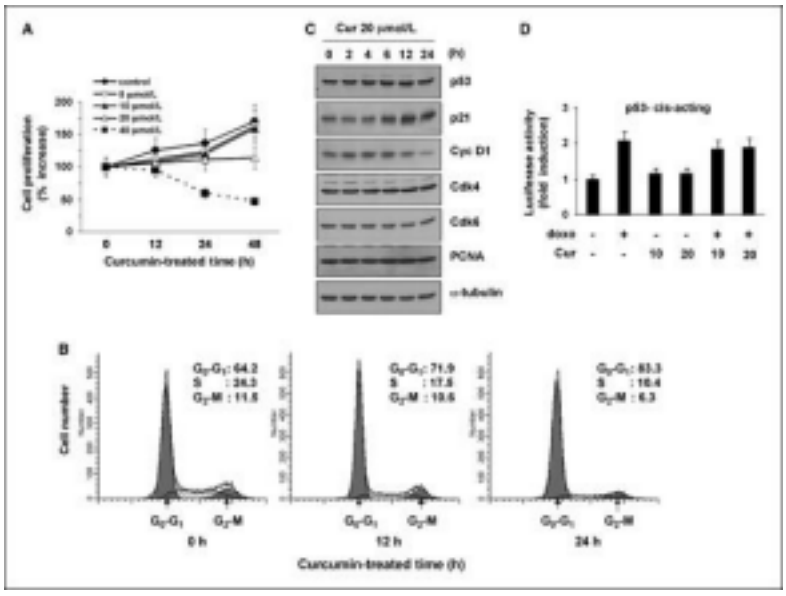

Figure 2: Concentrations of p53 with and without curcumin

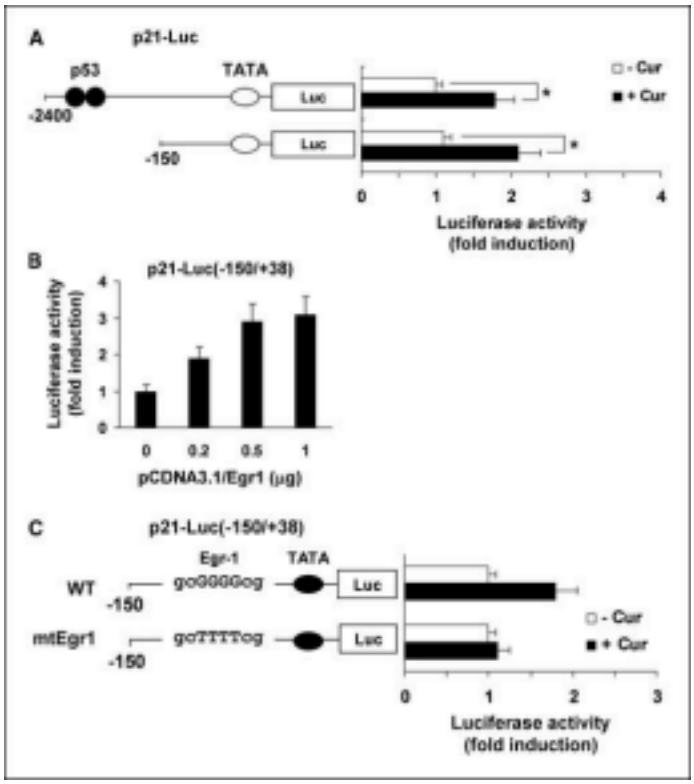

Figure 3: Concentration of Egr21 with and without curcumin

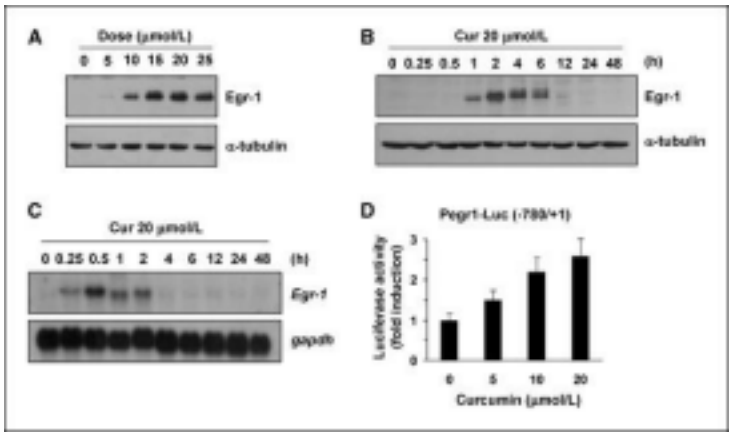

Figure 4: Curcumin effect on cellular detachment and nuclear condensation

A similar study was done by Dhandapani KM and his team where they investigated curcumin as a potential therapeutic for GBM and the effect of curcumin on glioma survival on humans and rat glioma cell lines. Results showed that curcumin reduced the viability of glioma cell lines over time from concentrations as low as 15 μmol/L, as it increased cellular detachment and increased nuclear condensation (Figure 4). It did so by reducing NFKB-DNA binding. Additionally, in the presence of the mutated p53 gene in the U87MG cells and the T98 G cells, curcumin was successfully able to induce cell death due to reducing the binding of NF KB and AP-1, the nature of curcumin in downregulating the expression of several anti-apoptotic genes bcl-xL, bfl-1/A1, HSP70 was discovered. Interestingly, the presence of curcumin over time didn't affect the regulation of pro-apoptotic genes in the same bcl family (Figure 5).

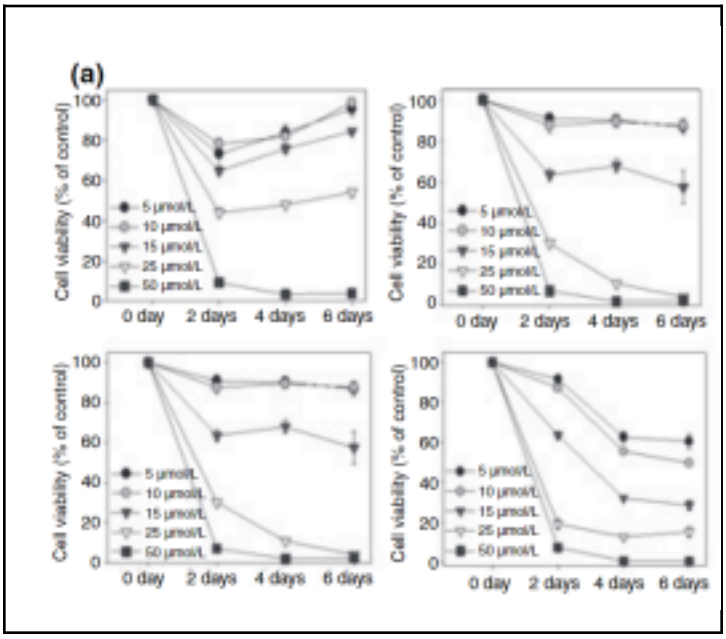

Figure 5: Effect of curcumin overtime on the regulation of BC1 family

Although curcumin lacks clinical research, in the future, it could solve many of the problems associated with current traditional therapeutics such as chemotherapy, radiation, and anticancer drugs. There are various harmful complications of chemo-radiation and other therapeutic drugs, such as pancytopenia, organ failure, healing complications, or even death. Since curcumin is a natural product, its side effects are drastically less severe and toxic. Curcumin can also work alongside other standard therapeutics,[16] as it has shown great promise in being able to enter the blood-brain barrier that some anticancer drugs cannot. It can thus help overcome such chemo or radioresistant characteristics of the GBM cells.

While curcumin can be a good alternative for traditional medicine, it also poses some concerns based on its low bioavailability. Due to the chemical structure of curcumin that results in its low solubility in certain pH levels, poor absorption, and rapid metabolism,[17]the amount that actually enters the blood circulation and tumor is very low. However, studies have shown that

[16] Luís, Â., Amaral, L., Domingues, F., Pereira, L., & Cascalheira, J. F. (2024). Action of curcumin on glioblastoma growth: A systematic review with meta-analysis of Animal Model Studies. Biomedicines, 12(2), 268. https://doi.org/10.3390/biomedicines12020268

[17] Dei Cas, M., & Ghidoni, R. (2019). Dietary curcumin: Correlation between bioavailability and health potential. Nutrients, 11(9), 2147. https://doi.org/10.3390/nu11092147

incorporating curcumin in micelles, nanoparticles, and liposomes can help in increasing curcumin absorption of the GBM cells, and has decreased the size of tumors in rats as compared to the injection of curcumin alone.

Flavonoids

Flavonoids are a group of natural substances with different phenol structures commonly found in fruits, vegetables, grains, barks, roots, stems, flowers, tea, and wine.[18] These compounds have antitumor effects through the upregulation of apoptosis and disrupt the migration, invasion, and metastasis of the tumor due to the fifteen-carbon backbone arranged in a three-ring structure. [19] Their anti-oxidative, anti-inflammatory, anti-mutagenic, and anti-carcinogenic properties are associated with a low cardiovascular mortality rate and the prevention of congenital heart defects.[18] Flavonoids also regulate tumor cell glucose metabolism, preventing the rate of glucose uptake from increasing and the production of lactate so that cancer cells cannot harness energy in the form of ATP.[20] In addition, many flavonoids with anti-GBM, such as epigallocatechin-3-gallate are found in green tea or quercetin, a flavonol found in oak, onions, and kale, involves deregulation or upregulation of GBM mechanisms, leading to apoptosis or the inhibition of cancer cell growth.[21]

Figure 4 shows the process of GBM replication and progression, with the mechanisms contributing to proliferation, chemoresistance, metabolism, apoptosis, and migration.[19] Normally, in GBM, the cell cycle checkpoints, autophagy, and apoptosis are inhibited, but as seen in Figure

[18] Panche, A. N., Diwan, A. D., & Chandra, S. (2016). Flavonoids: an overview. Journal of Nutritional Science, 5. https://doi.org/10.1017/jns.2016.41

[19] Zhai, K., Siddiqui, M., Abdellatif, B., Líšková, A., Kubatka, P., & Büsselberg, D. (2021). Natural Compounds in Glioblastoma Therapy: Preclinical Insights, Mechanistic Pathways, and Outlook. Cancers, 13(10), 2317. https://doi.org/10.3390/cancers13102317

[20] Liberti, M. V., & Locasale, J. W. (2016). The Warburg Effect: How Does it Benefit Cancer Cells? Trends in Biochemical Sciences, 41(3), 211–218. https://doi.org/10.1016/j.tibs.2015.12.001

[21] Alshweiat, A., Jaber, M., Abuawad, A., Athamneh, T., & Oqal, M. (2024). Recent insights into nanoformulation delivery systems of flavonoids against glioblastoma. Journal of Drug Delivery Science and Technology, 91, 105271. https://doi.org/10.1016/j.jddst.2023.105271

4, flavonoids ECCG, formononetin, hispidulin, icariin, and rutin combined with TMZ exhibit anti-GBM effects.[19] We see that formononetin, hispidulin, and icariin enhance TMZ-mediated apoptosis, activating caspases.[19]In addition, ECCG downregulates P-gp, which increases the sensitivity of GBM cells to TMZ, preventing cancer cells from evading therapeutic treatments.[19] Finally, we observe that rutin inhibits TMZ-induced autophagy, promoting apoptotic cell death of GBM cells, necessary to prevent the proliferation of GBM.[19]

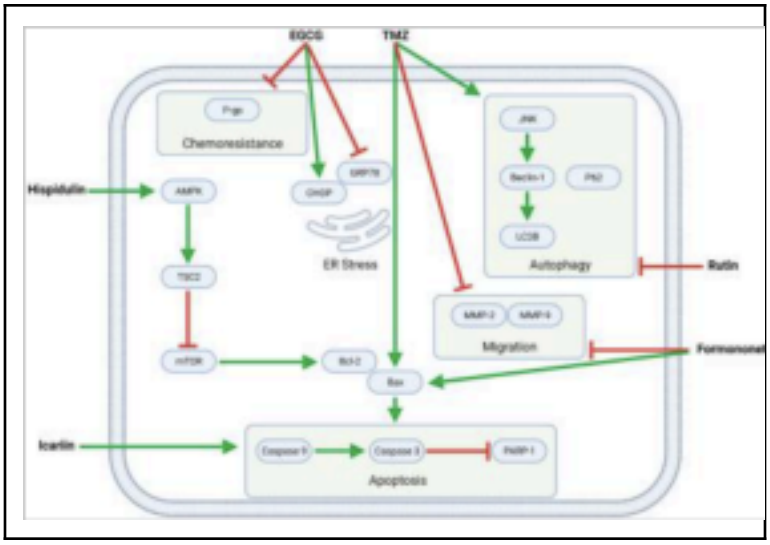

Figure 6: GBM replication and progression process

While flavonoid is generally considered safe, there have been reports of toxic interactions with other drugs, liver failure, dermatitis, hemolytic anemia, estrogenic concerns with reproductive health and breast cancer, and more.[22] Flavonoids have received a lot of attention over the past ten years due to the potential beneficial effects that have been studied.[18] Therefore, further research is needed to examine the usefulness of flavonoids in human diets, as there is potential for improving human health.[18] However, this research is limited due to the need for

[22] Galati, G., & O'Brien, P. J. (2004). Potential toxicity of flavonoids and other dietary phenolics: Significance for their chemopreventive and anticancer properties. Free Radical Biology and Medicine, 37(3), 287–303. https://doi.org/10.1016/j.freeradbiomed.2004.04.034

improvement in analytic techniques to allow for the collection of more data on the measurement of oxidative damage in vivo. Data on long-term consequences are still being researched as there is little data on the ingestion of chronic flavonoids.[18] Currently, the consumption of fruits, vegetables, and beverages containing flavonoids is recommended, but there isn't proven research that recommends daily flavonoid intake.[18] Flavonoids are a form of natural medicine that has the potential to aid in the prevention of chronic diseases such as glioblastoma, but further research is still required.

Quercetin

Phytochemicals are active compounds found in plants that are deemed safe for consumption and have antioxidative properties with beneficial effects on cancer. One of the most diverse groups of phytochemicals would be polyphenols, which are secondary metabolites in plants that have four subdivisions: flavonoids, phenolic acids, lignans, and stilbenes. Flavonoids, in particular, are phenolic compounds that control plant color, flavor, and medicinal properties.[23] These compounds have two phenyl rings linked by a heterocyclic pyrene ring and can be further classified into flavones, flavonols, flavanones, isoflavones, flavanols, and anthocyanidins (Figure 8). Research has indicated that the quantity and position of substituents within the fundamental structure of flavonoids play a key role in their ability to treat different diseases.

[23] Zhai, K., Mazurakova, A., Koklesova, L., Kubatka, P., & Büsselberg, D. (2021). Flavonoids Synergistically Enhance the Anti-Glioblastoma Effects of Chemotherapeutic Drugs. *Biomolecules*, *11*(12), 1841. https://doi.org/10.3390/biom11121841

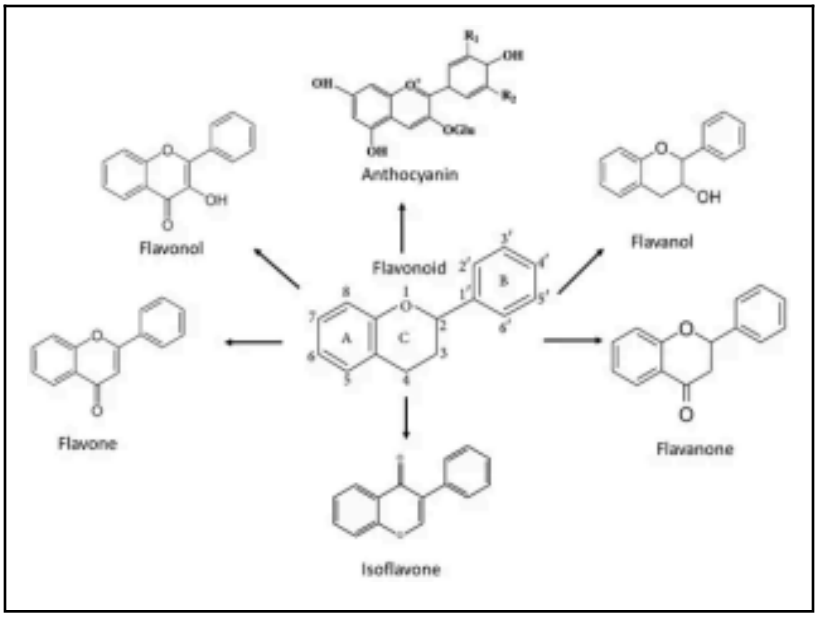

Figure 7: Diagram exhibiting the various subcategories of flavonoids.

Quercetin, a type of flavonoid, was found to be selective towards high-grade adult-type diffuse glioma cells when it spared 85% of normal astrocytes. It also shows no obvious adverse effects when normal cells were exposed to reasonable dosages. When used in conjunction with TMZ and nano-drug delivery systems, quercetin exhibited significantly higher anti-inflammatory properties than when it was just used by itself.[24]

Wanyu Wang and his team conducted a study to analyze how quercetin affects MGMT glioblastoma multiforme cells. The cell inhibition rate was measured via a CCK8 assay, and apoptosis was measured through Annexin V-FITC/PI double staining. MGMT ("06-methylguanine-DNA-methyltransferase") is an enzyme that repairs DNA damaged by alkylating agents such as chemotherapy drugs (e.g., temozolomide, also known as TMZ). However, because MGMT works to prevent cell mutations and death, it helps glioblastoma (GBM) build up chemotherapy resistance. The MGMT gene promoter's methylation status is usually used as a biomarker to check patient survival; methylated promoter regions result in

[24] Wong, S. C., Kamarudin, M. N., & Naidu, R. (2023). Anticancer Mechanism of Flavonoids on High-grade Adult-type Diffuse Gliomas. *Nutrients*, *15*(4), 797. https://doi.org/10.3390/nu15040797

lower amounts of MGMT expression and an increase in chemotherapy sensitivity. Thus, seeing methylation present correlates to a more favorable patient prognosis. It should be noted, however, that MGMT expression is not always congruent with MGMT promoter methylation. Wang's team analyzed the promoter by using a methylation-specific polymerase chain reaction (MS-PCR). Glioma stem cells (GSCs) act as another factor that decreases the effectiveness of TMZ.[25]

Another instance of quercetin's antitumor ability is seen in an experiment that tested cell viability through CCK-8 and clone-formation assays. U87 cells, a cell line of malignant gliomas, were surgically injected into the mice models to determine how their survival rates and locomotor activity were affected. Upon completion of the experiment, it was found that the mice exposed to quercetin had increased amounts of cell apoptosis in vitro and decreased glioblastoma cell proliferation. Quercetin was also seen to have suppressed glycolytic metabolism in the tumor.[26]

Studies have shown that quercetin displays preferential cytotoxic activity against GBM cells A172 and U138 MG. In other words, quercetin is able to target specific glioma cancer growths without negatively impacting normal CNS cells such as astrocytes. This, paired with the flavonoid's ability to penetrate the blood-brain barrier (BBB), makes it a strong candidate for GBM therapy usage. Wang and his team concluded that quercetin was able to significantly suppress human GBM T98G cells and induce apoptosis through the dual inhibition of the Wnt3a/β-Catenin pathway and the Akt/NF-κB signaling pathway.[22] Quercetin has shown promising results due to its ability to pass through the blood-brain barrier (BBB) and target cytotoxicity towards GBM cells. Thus, this makes quercetin a viable alternative in combating GBM and cells that have developed a resistance towards TMZ.

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[25] Wang, W., Yuan, X., Mu, J., Zou, Y., Xu, L., Chen, J., Zhu, X., Li, B., Zeng, Z., Wu, X., Yin, Z., & Wang, Q. (2023). Quercetin Induces MGMT+ Glioblastoma Cells Apoptosis via Dual Inhibition of WNT3A/β-catenin and AKT/NF-KB Signaling Pathways. *Phytomedicine*, *118*, 154933. https://doi.org/10.1016/j.phymed.2023.154933

[26] Wang, L., Ji, S., Liu, Z., & Zhao, J. (2022). Quercetin Inhibits Glioblastoma Growth and Prolongs Survival Rate through Inhibiting Glycolytic Metabolism. *Chemotherapy*, *67*(3), 132–141. https://doi.org/10.1159/000523905

Terpenoids

Current limitations to Glioblastoma (GBM) treatments include temozolomide (TMZ) resistance and toxicity, blood-brain barrier (BBB) impermeability, and metastatic development of refractory tumors. When examining the issue with BBB permeability specifically, it has been shown that limited cerebral drug efflux is largely attributed to the P-glycoprotein in the cancer stem cells (CSCs) of GBM.[27]

P-glycoprotein, part of the family of ATP-binding cassette transporters, is found in many bodily tissues, including the BBB. Its role includes preventing the intake of xenobiotics into the human body, which reduces the bioavailability of chemotherapeutic drugs and decreases their affectivity. However, the overexpression of P-gp in GBM leads to the issue of TMZ resistance, which causes GBM's characteristically low survival rates.[28] Thus, a potential method to overcome this problem is through the inhibition of P-gp, which can effectively increase the influx of drugs into the brain by modulating the BBB. While there are synthetic inhibitors, their high toxicity causes a turn towards more natural inhibitors of P-gp, such as terpenes, which are more effective and less toxic to the body.[29]

Terpenoids, including (R)-(+)-citronellal, abietic acid, and glycyrrhizic acid, have been shown to inhibit P-gp-mediated transport significantly.[30] Figure 9 below shows basic terpenoid structures that can inhibit P-gp.

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[27] Raghupathy Vengoji et al., "Novel Therapies Hijack the Blood–Brain Barrier to Eradicate Glioblastoma Cancer Stem Cells," OUP Academic, November 24, 2018, https://academic.oup.com/carcin/article/40/1/2/5204255?login=true.

[28] Leonardo Delello Di Filippo et al., "A Receptor-Mediated Landscape of Druggable and Targeted Nanomaterials for Gliomas," Materials today. Bio, May 19, 2023, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10238751/.

[29] Saikat Dewanjee et al., "Natural Products as Alternative Choices for P-Glycoprotein (P-GP) Inhibition," Molecules (Basel, Switzerland), May 25, 2017, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6152721/.

[30] Yu, Jun, Peng Zhou, James Asenso, Xiao-Dan Yang, Chun Wang, and Wei Wei. "Advances in Plant-Based Inhibitors of P-Glycoprotein." Journal of Enzyme Inhibition and Medicinal Chemistry 31, no. 6 (2016): 867–81. doi:10.3109/14756366.2016.1149476.

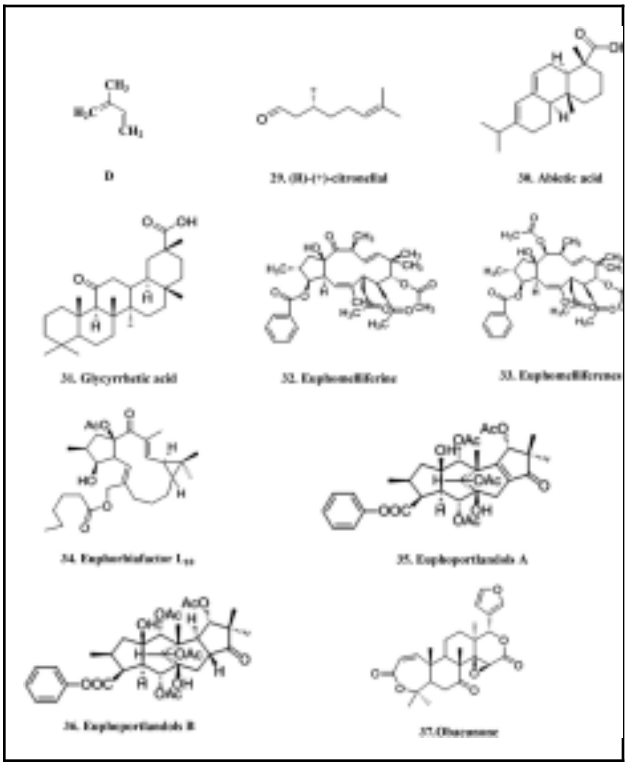

Figure 8: Basic Terpoid Structures that can Inhibit P-gp.

In a study by Tatiana N. Pashirova and her research team, terpene-modified lipid nanosystems for TMZ were examined as a potential combination therapy that could increase toxicity against cancer cells. Abietic acid, the terpene used in the study, was shown to inhibit cancer cell growth as well as modulate cell membrane permeability. However, there is still more research to be done in identifying the mechanisms through which abietic acid-lipid systems containing TMZ lead to anticancer activities such as inducing the overexpression apoptosis genes and downregulating oncogenic genes.[31]

[31] Pashirova, Tatiana N., Andrey V. Nemtarev, Daina N. Buzyurova, Zukhra M. Shaihutdinova, Mudaris N. Dimukhametov, Vasily M. Babaev, Alexandra D. Voloshina, and Vladimir F. Mironov. 2024. "Terpenes-Modified Lipid Nanosystems for Temozolomide, Improving Cytotoxicity against Glioblastoma Human Cancer Cells In Vitro" *Nanomaterials* 14, no. 1: 55. https://doi.org/10.3390/nano14010055

Discussion

Curcumin and flavonoids shed light on the potential of natural medicines as alternative treatment options for glioblastoma (GBM), an aggressive and lethal brain tumor. Curcumin, a phytochemical and polyphenol, demonstrated significant promise in inhibiting the growth and proliferation of GBM cells. By targeting key pathways like Retinoblastoma (RB) and Sonic hedgehog (Shh) pathways, curcumin can regulate cell cycle progression, induce apoptosis, and inhibit tumor cell migration. Curcumin could potentially overcome chemoresistance by downregulating P-glycoprotein expression on the blood-brain barrier, enhancing the efficacy of anticancer drugs. In addition, curcumin has been shown to induce the expression of p21, which effectively arrests the cell cycle and thus inhibits cell growth.

Flavonoids are another group of natural compounds with therapeutic potential against GBM. Quercetin demonstrates selective cytotoxicity towards GBM cells while sparing normal astrocytes. The ability to penetrate the blood-brain barrier and target specific signaling pathways during GBM pathogenesis is shown to be a promising therapeutic agent. Though these natural compounds are generally regarded as safe, flavonoids have been associated with potential toxic interactions with other drugs and adverse effects such as liver failure and dermatitis.

Though there is compelling evidence that supports the efficacy of natural medicines in GBM treatment, there is still a lack of extensive pre-clinical and clinical studies done on curcumin and flavonoids in order to validate the therapeutic benefits in human patients. The bioavailability of these natural compounds poses a significant challenge as curcumin, specifically, suffers from poor solubility, absorption, and rapid metabolism, limiting its effectiveness in vivo. However, drug delivery systems like micelles, nanoparticles, and liposomes offer promising strategies to improve the bioavailability of these natural compounds.

Combination therapy offers some promise as more research is being done on combining natural products, nanocarriers, and TMZ. For instance, terpenoids are naturally occurring, plant-derived compounds that work as inhibitors of P-glycoprotein in the BBB, which may help increase the cerebral influx of drugs and thus improve their affectivity. By combining well-studied lipid transport nanosystems with TMZ and the advantages of terpenes, there is potential for the creation of treatments that may overcome the issue of BBB impermeability in the CSCs of GBM.

Conclusion

Natural alternatives to traditional chemotherapy treatments are gradually gaining traction in the medical field. Amongst them, phytochemicals such as curcumin, flavonoids, quercetin, and terpenoids are well-known for their ability to bypass the blood-brain barrier and specifically target glioblastoma multiforme cells. Curcumin was able to deter the advancement of glioma growth by moderating the Sonic hedgehog and Retinoblastoma pathways. Flavonoids encompass a broader umbrella of plant metabolites that possess antioxidative properties and are commonly found in everyday fruits and vegetables. One specific example would be quercetin, which possesses the ability to selectively target GBM cells and induce trigger apoptosis. Terpenoids also aid in the suppression of glioblastoma by reducing the expression of the P-glycoprotein, thus increasing the body's ability to raise cell membrane permeability and allow medications to pass through. Although there are some setbacks, such as low bioavailability and limited amounts of clinical research, phytotherapy shows promise and should be further studied as a form of GBM treatment.

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