Review

Revista Actualidades Biológicas

Actual. Biol. 47(123): e4709, 2025 | Jul-Dec | Medellin DOI:10.17533/udea.acbi/v47n123a09

Flavonoids use in glioblastoma multiforme experimental models

Uso de los flavonoides en modelos experimentales de glioblastoma multiforme

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Abstract

Glioblastoma Multiforme (GBM) represents tumors that develop in the Central Nervous System (CNS) with the highest malignancy, incidence and prevalence. Flavonoid therapy has been gaining strength as an alternative adjuvant that counteracts the appearance and development of various cancers, including GBM. The objective of this review is to analyze the use of flavonoids in GBM therapy in a ten-year window (2013-2023). Fifty-one articles were reviewed, including original articles and bibliographic reviews. Flavonoids have been tested in different GBM *in vitro* models, decreasing proliferation and angiogenesis, activating signaling pathways and redirecting cell activity to alternative pathways that promote apoptosis and cell repair.

Keywords: cell culture, cellular therapy, flavonoids, glioblastoma multiforme, *in vitro* models, phytotherapy

Resumen

El Glioblastoma Multiforme (GBM) representa al tumor con mayor malignidad e incidencia y prevalencia de aquellos que se desarrollan a nivel de Sistema Nervioso Central (CNS). El uso de flavonoides ha venido tomando fuerza de forma alternativa y coadyuvante para contrarrestar la aparición y el desarrollo de diversos tipos de cáncer, dentro de ellos el GBM. El objetivo de esta revisión fue analizar el uso de flavonoides de diferentes fuentes vegetales en modelos experimentales de GBM en una ventana de búsqueda de diez años (2013-2023). Se revisaron cincuenta y un artículos dentro de los que se encuentran artículos originales y revisiones bibliográficas. En conclusión, los flavonoides han sido probados en diferentes modelos *in vitro* de GBM, disminuyendo la proliferación y la angiogénesis, activando vías de señalización y redireccionando la actividad celular a vías alternas que evoquen apoptosis y reparación celular.

Palabras clave: cultivo celular, fitoterapia, flavonoides, glioblastoma multiforme, modelos *in vitro*, terapia celular

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Received: August 8, 2024; accepted: March 23, 2025; published: June 10, 2025.

INTRODUCTION

Glioblastoma multiforme (GBM) is the most common and aggressive primary brain cancer. GBM can be classified between those with glial origin and those without. Depending on the origin, GBM is further divided into three subclasses: astrocytomas, ependymomas and oligodendrogliomas (Batash et al., 2017; Hanif et al., 2017; Mahmoud et al., 2022). Common genetic alterations include the loss of heterozygosity of chromosome 10q, with an occurrence of 60 to 90% of GBM cases (Verdugo et al., 2022; Waugh 2016), deletions in the p53 gene, alterations in epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGFR) with occurrence rates in primary GBM of approximately 86%, 50%, and 60%, respectively. Additionally, alterations in the Receptor Tyrosine Kinase/Rat Sarcoma/Protein Rat Sarcoma/Phosphatidyl Inositol 3 Kinase (RTK/Ras/PI3K) signaling pathways, recently reported by the Cancer Genome Atlas Research Network, also contributes to disease development in 79% of evaluated cases (Hanif et al., 2017; Waugh, 2016). Finally, mutations in the murine double minute protein homolog 2 (MDM2) and phosphatase and tensin homolog (PTEN) genes (Waugh, 2016) have been reported as therapeutic targets.

To counteract the effects of GBM development, the disease is approached from three points: tumor dissection, radiotherapy and chemotherapy, but these do not increase the life expectancy or survival time of GBM patients (Ozdemir & Regad, 2017). As for chemotherapy, temolozomide, also known as Temodal® or Temodar®, is the alkylating agent used in conjunction with radiation and even in combination with surgical resection, because it has been shown to increase survival time from 12 to 16.4 months and because it is one of the few chemotherapeutic agents that cross the blood-brain barrier (Ozdemir-Kainak et al., 2018). However, the cellular mechanisms that led to it being one of the most widely used chemicals in treatment for first-degree brain tumors such as GBM are not fully understood. Furthermore, prolonged use of temolozomide is related to healthy cell dysfunction and therapeutic resistance of the disease (Ströbele et al., 2015).

On the other hand, the use of antiangiogenic drugs has also been proposed, including Bevacizumab, an immunodrug composed of a recombinant humanized monoclonal antibody that neutralizes Vascular Endothelial Growth Factor A (VEGFAa) and inhibits interaction with the signal-receptor molecule. In the cell, this factor promotes survival, proliferation and migration through its paracrine release from Stem-like glioblastoma cells (Yang et al., 2017).

In addition to the above, radiation is also one of the therapies used. However, clinical reports have pointed out that its use in other tumors in both children and adults is associated with a threefold increase in the risk of developing a GBM, even after the original tumor has been removed (Mahmoud et al., 2022; Messaoudi et al., 2015; Ramezani et al., 2019). Currently, even as adjuvant therapy, the use of biomolecules such as flavonoids, as well as their application using nanotechnology (e.g., flavonoid nanoencapsulations), are being investigated as novel therapeutic strategies. These alternatives have shown negative effects upon overexpression of the previously mentioned protein receptors, in addition to positively influencing the percentage and time of survival. In this context, the present article aims to compile and analyze scientific evidence published in the last decade (2013-2023) that explore the therapeutic potential of flavonoid-based phytotherapy as a complementary strategy to inhibit tumor progression.

MATERIALS AND METHODS

Definition of the research topic

The topic of interest is the "Use of flavonoids in experimental models of glioblastoma multiforme". Within this, subtopics such as the study of natural compounds (flavonoids) and their application in a specific type of brain cancer (glioblastoma multiforme) by means of experimental models was considered.

Search engine selection

Articles searches were performed in biomedical search engines such as PubMed and Google Scholar, using the keyword combinations: "Flavonoids, Glioblastoma multiforme, Experimental models, Flavonoids and Glioblastoma multiforme, Flavonoid therapy in Cancer. To optimize the results, combinations of keywords with Boolean operators (AND, OR) were used as follows: -

"Flavonoids AND Glioblastoma multiforme", "Flavonoids AND experimental models AND Glioblastoma", "Flavonoids AND treatment AND brain cancer", "Flavonoids OR natural flavonoids AND Glioblastoma multiforme".

Inclusion and exclusion criteria

Inclusion criteria

Articles were chosen that had the following characteristics: original articles published in journals indexed and approved by PUBLINDEX of MinCiencias, studies involving experimental models (*in vitro* or *in vivo*), publications in English and Spanish, and studies addressing the use of flavonoids in the treatment of glioblastoma multiforme with an observation window of ten years (2013-2023).

Exclusion criteria

Articles with the following characteristics were eliminated or not taken into account: systematic reviews and meta-analyses, opinion articles, editorials or commentaries, studies that did not involve experimental models, and research that did not directly address the use of flavonoids in glioblastoma.

Search procedure

Execution of the search

Searches were performed in the selected engines using the established keyword combinations. The search was limited to articles published with an observation gap from 2013 to 2023.

Review of results

The results obtained were initially screened by titles and abstracts to identify potentially relevant studies. Selected articles were reviewed in full text to confirm their inclusion based on the defined criteria.

Summary of information

Relevant data from the included articles were synthesized and analyzed to evaluate the use of flavonoids in experimental models of glioblastoma multiforme, highlighting key findings, methodologies employed and conclusions of the studies.

RESULTS

Results by search engine

A total of 24,357 articles were obtained from Google Scholar and 540 from PubMed. Taking into account the inclusion and exclusion criteria detailed previously described, fifty-three articles were considered for this article, the analysis of which is shown in Table 1.

Flavonoids

Flavonoids are secondary metabolites consisting mainly of a benzopyrone ring with one or more phenolic groups in different positions (Zheng et al., 2017). Flavonoids are most commonly found in fruits, stems, herbs, cereals, nuts, vegetables, flowers, and seeds (Shan et al., 2017). Flavonoid use ranges from natural dyes to skin care products (Villela et al., 2019). Recently, flavonoid use has been directed towards the medical field in antitumor, anticancer, antimicrobial, antiviral, antioxidant and neuroprotective therapy (Ullah et al., 2020).

Flavonoids can be divided into flavones (apigenin and luteolin), flavonols (quercetin, kaempferol, myricetin and fisetin), flavonones (flavonone, hesperetin and narigenin), isoflavonoids (genistein, orobol and diadzein), anthocyanidins (apigenidin, luteolinin and cyanidin) and aurones (sulfuretin and leptosidin) (Shan et al., 2017; Villela et al., 2019).

Among the most important differences in the chemical structure of each group of flavonoids are the following: substituents, functional groups, degree of oxidation, dimeric and polymorphic forms (Zheng et al., 2017), addition or reduction of hydroxyl groups, methylation of these same groups and of the flavonoid nucleus to produce flavonoids o-glycosides or C-glycosides, bringing low reactivity and higher solubility, as well as dimerizations and bisulfate formation (Kumar & Pandey 2013).

These metabolites are found in plants as flavonoids or glycosides, in which one or more hydroxyl groups

Table 1. Number of articles obtained by search engine and keyword

Key words	Google Scholar	Pubmed
Flavonoids AND Glioblastoma multiforme	2900	300
Flavonoids AND experimental models AND Glioblastoma.	0	0
Flavonoids AND treatment AND brain cancer	3180	0
Flavonoids OR natural flavonoids AND Glioblastoma multiforme	177	120
Glioblastoma multifome AND Experimental models.	18,100	0
Total	24,357	540

of the flavonoid nucleus are linked to sugars (Table 2). The most common sugars present are glucose, galactose, rhamnose and xylose, and rutinose (Chong-Tuesta, 2019; Neri-Numa et al., 2020).

Recently, the use of flavonoids in health science research has increased interest in their consumption. Nowadays, people tend to include foods rich not only in flavonoids, but also in prebiotic carbohydrates and carotenoids, due to their metabolic benefits (Table 2) (Suhail et al., 2023; Ullah et al., 2020).

Therapeutic use of flavonoids

At present, the therapy proposed to treat GBM is based on surgical resection and chemotherapy. However, due to the variability in the results obtained and the recurrence of the disease, interest in alternative therapies such as the one described in this article has been growing. The following are different studies where the antitumor action of flavonoids is evident in experimental models *in vivo* and *in vitro*.

1. Flavones

Flavones are characterized by a double bond between carbon 2 (C2) and C3 in the C ring and by the attachment of the benzene B ring to C2. Luteolin, chrysin and apigenin differ in the number of hydroxyl groups attached to the flavone backbone (Ponte et al., 2021). The diversity of flavones, like other flavonoids, is achieved by chemical modifications and conjugations including hydroxylations, O-methylation or binding to sugars such as O- or C-glycosides, O- or C-glucuronides, among others (Kariagina & Dossef, 2021).

1.1 Luteolin

Luteolin or luteolin (3,4,5,7-tetrahydroxyflavone) is a natural flavonoid, widely found in fruits and vegetables such as celery, chrysanthemum flowers, cucumbers, carrots, onions, broccoli and garlic, and has been reported to have antihypertensive, anti-inflammatory and anticancer actions (Imran et al., 2019; Neri-Numa et al., 2020).

Wang et al. (2017) reported that luteolin exposure to GBM-derived U251MG and U87MG cell lines induced apoptosis mediated by the endoplasmic reticulum stress response and mitochondrial dysfunction due to an increase in reactive oxygen species (ROS), which increased with increasing luteolin concentration (doses used: 0 µM-8 µM). Other associated cellular events were linked to the phosphorylation of protein kinases such as protein kinase-like endoplasmic reticulum kinase (PERK), eukaryotic initiation factor 2 (eIF2α), activating transcription factor 4 (ATF4), DNA damage inducible transcript (CHOP or DDIT3) and cleavage of caspase 12, suggesting a decrease in cell proliferation at the previously mentioned doses (Wang et al., 2017).

Speaking of anticancer activity, luteolin has been reported to have negative effect on decreasing cell viability in breast, prostate, osteosarcoma and leukemia cancer cell culture lines, among others. In a study by Anson et al. (2018), luteolin exposure to GBM U-87 MG and GBM U251 MG cell lines (0 µM-80 µM) inhibited cell proliferation by decreasing overexpression of EGFR and some of its target proteins such as AKT and MAPK and apoptosis via poly ADP-Ribose polymerase (PARP) and caspases.

Table 2. List of flavonoids, their group, source and cellular effect

Flavonoid	Group	Natural source	Reference
Luteolin	Flavona	Celery, chrysanthemum, cucumber, carrot, onion (raw), broccoli (steamed), garlic (cooked).	Neri-Numa et al., 2020.
Apigenin	Flavona	Fresh coriander, celery seeds, celery, Chinese celery, dried oregano.	Imran et al., 2019
Quercetin	Flavonol	Apples, cherries.	Babaei et al., 2013;
		Onions, asparagus and red lettuce leaves.	Wang et al., 2019
Kaempferol	Flavonol	Black and green tea leaves, mate leaves, beans, cabbage, grapes, broccoli, strawberries, kale, citrus fruits, brussels sprouts, apples, dried raspberries, chrysanthemum tomatoes, *Ginkgo biloba* L, *Tilia* spp., *Astragalus mongholicus*, *Equisetum* spp., *Moringa oleifera* and Japanese pagoda tree.	Silva dos Santos et al., 2021.
Miricetin	Flavonol	Wide distribution in blueberries, grapes, red onion, berries, nuts and green tea.	Jiang & Wang, 2019
Genistein	Isoflavones	Soy and its derivatives.	Liu et al., 2021

On the other hand, in a dose-dependent study (0 µM-80 µM), Wang et al. (2017) reported that luteolin decreased the phosphorylation of the PI3K/AKT/mTOR (p-IGF-1R/PI3K/AKT/mTOR)-mediated insulin-like growth factor-1 receptor (IGF-1R) signaling pathway, which is required for the activation of epithelial-mesenchymal transition and tumor cell migration. Additionally, they reported a reset in the balance between tissue inhibitor of extracellular matrix metalloproteinases/metalloproteinases (TIMP/MMPs) necessary to decrease the degradation of the extracellular matrix and consequently, the appearance of metastatic processes in this type of tumor was observed. Consequently, the levels of MMPs that were overexpressed diminished, while TIMP concentrations, which had been diminished, showed an increase.

A breakthrough in the administration of alternative flavonoid-based therapies was reported by Zheng et al. (2017). In *in vitro* (U87MG) and *in vivo* (zebrafish F, BALB/C mice and transgenic nude mice) experimental models, treatment with luteolin micelles decreased cell proliferation via Ki67 marker, inhibited RAS-RAF-MEK-MEK-MAPK mitogenic pathway activation and increased apoptosis through the suppression of Bcl family proteins, Bax and caspase 9 cleavage (Zheng et al., 2017).

The doses used in this study were between 0 µg/ml and 100 µg/ml and the results showed that cell viability was inversely proportional to luteolin concentration. Another flavonoid belonging to this group is apigenin.

1.2 Apigenin

Apigenin is found in many fruits and vegetables, such as parsley, celery and chamomile, which are the most common sources. Apigenin inhibited the proliferation and survival of high-grade adult-type diffuse glioma cells by suppressing Erk kinase expression in U87MG, T98G and U1242MG (LD5030 µM) glioblastoma cell lines with no effect on human astrocyte cells. The induction of apoptosis, with increased phosphorylation of p38, mitogen-activated protein kinase (MAPK) and activation of Jun-kinase one (JNK1) pathway were reported. Additionally, the combination of apigenin (LD50 35 µM) with temozolomide (TMZ) treatment significantly increased cell cycle arrest in the G2 phase through inhibition of p-AKT, cyclin D1, Bcl-2, MMP-2 and MMP-9 expression (Chen et al., 2021; Wang et al., 2021; Wong et al., 2023).

Similarly, the anticancer effect of apigenin on the expression of the cell cycle inhibitor p21/WAF1, the functional reactivation of p53 and proapoptotic

caspases, and on DNA fragmentation of tumor cells, has been reported (Sung et al., 2016). Recently, flavones such as apigenin and acacetin were reported to induce cell cycle arrest in the G2/M phase through regulation of p21 interaction (activation and upregulation) with cyclins A1, B1 and CdK1 (downregulation), as well as activation of ROS-induced apoptosis mediated by increased expression of Bax, t-Bid, caspase 8, caspase 9, caspase 3, and PARP in U87MG cells (Shendge et al., 2021). The possible action of apigenin in suppressing NF-kB and consequent the survival of U87MG cells has also been mentioned (Baradaran-Rahimi et al., 2019).

On the other hand, Wang et al. (2021) mentioned that apigenin (LD50 50 µM) used together with temozolomide (TMZ), the chemical substance of greatest use in chemotherapy in GBM, reinforced the antiproliferative capacity of the tumor through the inhibition of the PI3K/AKT signaling pathway, activated apoptosis by increasing procaspase expression, decreased extracellular matrix remodeling through inhibition of MMPs 2 and 9, and arrested the cell cycle in G2 phase due to the suppression of cyclin D1.

2. Flavonols

Flavonols are mainly found in onions (1.2g/kg), leeks, broccoli, red wine, apricots, apples, and tomatoes, among others. Flavonol concentrations vary between zones of the same plant and during different stages of plant maturation. Flavonols actively participate in the metabolism of indole acetic acid, are involved in the transport of growth hormones such as auxin and in the protection of the plant from ultraviolet (UV) type A and B rays, which deteriorate genetic material, increase the production of waste substances and damage photosystem II (Zhang et al., 2013). Quercetin, kaempferol and myricetin are among the most representative flavonols.

2.1 Quercetin

Quercetin is found mainly in onions and tomatoes, which are commonly used vegetables in the daily lives of most households. According to Taylor et al. (2019), rat C6 and human T98G glioblastoma cell lines treated with specific concentrations of quercetin (24 µM), an increase in apoptosis was observed through increased expression of Bax, PARP and Caspase 3 proteins in the C6 cell line and Bax, Bcl-2, Caspase 3 and PARP in the T98G cell line.

According to Bispo da Silva et al. (2019), quercetin (30 uM) had an immunomodulatory effect on the C6 cell line through the positive regulation of tumor necrosis factor (TNF) and negative regulation of IL-10, which decreased the proliferation and migration of these cells. On the other hand, in human glioblastoma multiforme U251 and TG1 lines, quercetin modulated the expression of interleukins and TNF in the same way as in rat C6 cells (30 µM) (Bispo Da Silva et al., 2019).

Additionally, quercetin decreased tumorogenesis in Wistar rats, an *in vitro* model of GBM, when U251 cells were transplanted at the striatal level (30 µM) (Bispo Da Silva et al., 2020). Likewise, Jakubowicz et al. (2013), found that the combination of traditional drugs used to treat GBM, such as temolozomide, and quercetin, increased apoptosis through the intrinsic pathway in a dose-response study (0, 1, 5, 25, 50 µM of quercetin). Wanyu et al. (2023), found that quercetin (40 µM) induced apoptosis in the glioblastoma multiforme T98G cell line, increasing the ROS rate, superoxide dismutase (SOD) formation and consequently decreased mitochondrial membrane viability. In turn, the mitochondrial alterations were promoted by an imbalance between the Bax/Bcl-2 protein ratio followed by a reduction in the expression of caspases 9 and 3.

2.2 Kaempferol

Kaempferol can be isolated from green and black tea leaves and from mate leaves, as well as from grains, green vegetables, red and citrus fruits, chrysanthemum (*Chrysanthemum* spp.), ginkgo biloba (*Ginkgo biloba* L.), linden (*Tilia* spp.), Chinese milkvetch (*Astragalus mongholicus*), field horsetail (*Equisetum* spp.), moringa (*Moringa oleifera*) and Japanese pagoda tree (Silva dos Santos et al., 2020). The effects of kaempferol have been reported in various human pathologies including Parkinson's disease, Alzheimer's disease and glioblastoma.

In a study by Colombo et al. (2018), kaempferol nanoemulsions (LD50200 ng/mL) placed in the nasal mucosa of Wistar rats decreased the

progression and size of GBM tumor foci in this experimental model. On the other hand, in human umbilical cord endothelial cells (HUVECs), kaempferol (50 µg) initiated apoptosis through increased oxidative stress and consequent activation of protein kinase ATM and p53 phosphorylation, which in turn promoted apoptosis mediated by caspase 3, 8 and 9 activation (Lee et al., 2016).

In the liver cancer cell line HepG2, kaempferol (LD50 44 µM) was the only flavonoid capable of reducing the cell viability by blocking overexpression of P-glycoprotein (P-gp), responsible for chemoresistance in liver cancer, one of the deadliest cancers (Nair et al., 2020). Silva dos Santos et al. (2021) mention that in the GBM GL-15 cell line, a kaempferol concentration of 50 µM and 48 h exposure inhibited the activity of MMP 2 and 9, proteins associated with aggressiveness and progression of this type of tumor. Additionally, they decreased laminin and fibronectin expression. In the same article, decreased cell migration was shown to be mediated by inhibition of the PKCa-ERK-NFkB pathway in the GBM cell line GBM8401 in a dose-response study (10, 20 and 40 µM).

2.3 Myricetin

Myricetin is found in most fruits, vegetables, nuts and berries and in beverages such as tea and red wine. In the literature, its antioxidant and antiproliferative cellular effects are mentioned (Jiang et al., 2019).

In the GBM cell line DBTRG-05MG, the antiproliferative effect of myricetin is mediated by regulation of the PI3K-PTEN-Akt mitogenic pathway through the activation of sirtuin deacetylases of the nicotinic and adenine+/ sirtuin dinucleotide proteins (SIRTs), specifically SIRT3, responsible for regulating glucose and lipid metabolism in cells, but which in GBM cells promoted oxidative stress and consequently apoptosis, decreasing proliferation through inhibition of the previously mentioned signaling pathways.

In a study published by Wang et al. (2018), it was shown that myricetin (50 µg) arrested the cell cycle at the G2/M transition, cells were trapped between the G0 and G1 phase and apoptosis was consequently activated via caspases 3, 8 and 9 and p53.

On the other hand, Wang et al. (2016), used micelles loaded with myricetin at doses ranging from 25 to 200 µM in cells of the GBM cell line DB-TRG-05MG. Exposure was evaluated at three time points: 12, 24 and 48 h. The results show that myricetin encapsulations increased apoptosis of these cells from 50 µM through regulation of Bcl-2 expression and the functional increase of the apoptotic proteins BAD and BAX.

3. Isoflavones

Isoflavones are mainly found in legumes of the *Papilonidae* family. Among its representatives are biochanin A, genistein, daidzein, glycitein and formononetin (Ahmed et al., 2020). For the purposes of this review, we will focus on the role of genistein in GBM therapy.

3.1 Genistein

Genistein is the isoflavone with the highest content in soybean and soy-based food products. A wide range of antioxidant, anti-inflammatory, antiangiogenic, proapoptotic and antiproliferative properties of genistein are reported in the literature (Ahmed et al., 2020; Liu et al., 2021). By loading beads with 0.2 mg of this isoflavone, Liu et al. (2021) determined that genistein inhibited migration in the human GBM cell lines M059K, M059J and U87MG by decreasing the functionality of DNA-dependent protein kinases (DNA-PKcs), which promote cell migration through the activation of AKT 1, 2 and 3; genistein inhibited DNA-PKcs interaction with the AKTs.

In an *in vitro* study with U87MG, rat glioma F98 and rat EGFRVIII-specific overexpressing F98 cells (F98 EGFRvIII) (also from GBM), Liu et al. (2015) found that genistein blocked C/EBPb protein expression. Further, in an in vivo model with female BALB/C nude mice (100 µg/100 µL of genistein/day), genistein blocked IL-6 expression. Additionally, De Azambuja Borges et al. (2019) mention that use of liposomes with different concentrations of genistein (0-3.9 mg/mL), an antioxidant effect was reflected by 70.36% compared to 58% of genistein-free liposomes. Similarly, they evaluated the antitumor effect on

C6 cells, finding that after 48 h of exposure to 10 µM of genistein, cell viability was decreased by 95% (De Azambuja-Borges et al., 2019).

DISCUSSION

Recently, flavonoids, as secondary metabolites of plants, have been attracting increasing interest in the scientific community due to their various health-promoting properties. These compounds are found in a wide variety of fruits, vegetables, herbs, and seeds. Not only do they play a vital role in protecting plants against UV and other environmental stressors, but they also offer multiple therapeutic benefits for humans (Shan et al., 2017; Zheng et al., 2017). This dual role of flavonoids evidences their importance as both a defense mechanism in plants, as well as in their application in phytotherapy to promote human health. At present, although their action is supported by oral tradition, the scientific evidence relies on experimental models, both *in vitro* and *in vivo*, of diseases that have a greater incidence in humans.

The potential of flavonoids in modern medicine is increasingly recognized, especially for their use in antitumor, antioxidant and neuroprotective therapies (Ullah et al., 2020). Studies are constantly being reported showing the ability of these metabolites to combat cellular damage and neurodegenerative diseases, offering natural avenue to address complex health problems. This reinforces the importance of further exploring the medical applications of flavonoids.

A large part of the properties that plants have is due to the diversity of structures present in them, mainly flavonoids, which reflect their action simultaneously or individually because they can be classified into flavones, flavonols, flavanones, isoflavonoids, anthocyanidins and aurones. These diverse structures are responsible for their wide range of biological effects. Chemical modifications, such as hydroxylation and methylation, significantly influence their solubility and reactivity properties. This allows the creation of compounds such as flavonoids O-glycosides and C-glycosides, which exhibit increased solubility and reduced reactivity (Kumar & Pandey, 2013; Zheng et al., 2017). The complexity of these structures makes one reflect on the richness of natural chemistry and how each modification opens up new therapeutic possibilities.

Within therapeutic applications, flavonoids have shown remarkable anticancer potential. For example, luteolin, a component of flavones, has been shown to induce apoptosis in glioblastoma cells through mitochondrial dysfunction and endoplasmic reticulum stress. Furthermore, it inhibits signaling pathways crucial for cell survival, such as p-IGF-1R/PI3K/AKT/mTOR (Anson et al., 2018; Wang et al., 2017). This finding highlights the ability of flavonoids to counteract cancer progression from various access points, suggesting that this is an avenue for the development of new treatments. It is interesting to discover how seemingly simple natural compounds have the potential to dismantle complex cancer cellular networks.

Similarly, apigenin stands out for its ability to inhibit cell proliferation in high-grade gliomas by increasing p38 MAPK phosphorylation and activating the JNK1 pathway (Chen et al., 2021; Wang et al., 2021). This mechanism reveals a novel approach to combat one of the most aggressive cancers, highlighting the potential of flavonoids in oncology treatments. Understanding these mechanisms highlights the importance of further research into natural products, which often offer less toxic and more effective solutions than conventional therapies.

On the other hand, flavonols, such as quercetin and kaempferol, have also been shown to be effective in glioblastoma models. Quercetin, present in onions and tomatoes, enhances cell apoptosis by modulating proteins such as Bax and caspase 3. On the other hand, kaempferol, found in green and black tea, decreases cell viability and MMPs activity (Silva dos Santos et al., 2021; Taylor et al., 2019). The ability of these compounds to interfere with key cellular processes is impressive and shows the myriad of benefits that these types of compounds have.

Finally, isoflavonoids, such as genistein which are predominantly found in soybeans, have been the subject of extensive studies due to their antiproliferative and antioxidant properties. In *in vitro* and *in vivo* studies, genistein has been shown to inhibit cell migration and chemoresistance,

highlighting its potential as an adjunct in conventional cancer treatments (De Azambuja Borges et al., 2019; Liu et al., 2021). These findings underscore the importance of isoflavonoids in new therapeutic strategies, emphasizing the need to integrate these compounds into medical protocols. It should be noted that, although most of the flavonoids described here are evaluated individually, there are reports that highlight the importance of using flavonoids in synergy or pure extracts. A study by Hajimejdipoor et al. (2014) concluded that combinations of quercetin, rutin, caffeic acid, chlorogenic acid, gallic acid, and rosmarinic acid influenced antioxidant capacity. Therefore, to obtain the best antioxidant effects in products containing these materials, interactions among them should be considered. Likewise, Chunhua et al. (2015) emphasized that natural products work better when they are in their pure or raw state, without detracting from the individual function of flavonoids or the studies in which they have been evaluated.

In conclusion, flavonoids represent a promising class of natural compounds with significant therapeutic applications in the treatment of cancer and other diseases. However, continued research is essential to better understand their mechanisms of action and to optimize their clinical use. As we move forward in this exploration, it is critical to maintain a balanced approach that considers both the potential and limitations of flavonoids in modern medicine. Clearly, although there are some doubts about their application in the clinical phase, the implementation of this type of therapy in humans will provide important insights into the role of natural compounds in human health.

CONCLUSIONS

The richness and distribution of flavonoids in plant species are evidence of the deep connection between humans and the plant kingdom. Key therapeutic responses to treat various human diseases are found in plants, with secondary metabolites standing out as valuable tools in mitigating the progression of pathologies. These molecules have shown potential antihypertensive, antiglycemic and, in particular, antitumor effects. The scientific literature supports this phenomenon, evidencing processes such as the induction of apoptosis in tumor cells, the activation of previously silenced signaling pathways and the regulation of essential target proteins by alternative pathways. Without exposure to adequate doses of these metabolites, many of these processes would be difficult to achieve.

In the specific case of glioblastoma multiforme, the flavonoids identified in this review stand out for their significant therapeutic activity. Luteolin showed proapoptotic and antiproliferative effects at doses of 0-80 µM, modulating critical pathways such as EGFR, AKT and MAPK. Apigenin, at doses of 30-35 µM, induced apoptosis through MAPK/JNK1 activation and cell cycle arrest in G2, with synergistic results when combined with temozolomide. Quercetin, in the range of 24-40 µM, upregulated Bax, Bcl-2 and caspases, and also inhibited cell migration. Likewise, kaempferol (10-50 µM) and myricetin (50 µM) promoted apoptosis through oxidative stress and caspase activation and reduced cell migration by inhibiting MMPs 2 and 9. Finally, genistein (0.2 mg and 10 µM) stood out for its potent antioxidant activity and for significantly decreasing cell viability by blocking DNA-PKcs and AKT. These results underline the modulatory role of flavonoids in key tumor processes, although their efficacy needs to be validated in further preclinical and clinical studies.

Furthermore, studies in *in vitro* and *in vivo* models of glioblastoma multiforme confirm that flavonoids have a considerable antitumor impact, decreasing proliferation and angiogenesis, activating signaling pathways and redirecting cellular activity towards apoptosis and repair processes. In this context, flavonoids not only position themselves as promising therapeutic agents, but also highlight the importance of promoting healthy eating habits, including fruits, vegetables and nuts in the daily diet. A return to a closer interaction with nature could significantly contribute to overall health recovery.

Finally, flavonoids emerge as promising therapeutic agents against glioblastoma multiforme, with specific mechanisms of action ranging from the induction of apoptosis to the inhibition of cell proliferation and migration. Their ability to modulate key signaling pathways and their antioxidant activities position them as natural compounds of high value in oncology research.

However, their transition from the laboratory to the clinic requires additional studies to validate their efficacy, safety and applicability in humans. This knowledge reinforces the need to value the richness of bioactive compounds in nature and to promote dietary habits that, in addition to preventing diseases, contribute to the overall improvement of health.

ACKNOWLEDGMENTS

Special thanks to the Faculty of Sciences of the Universidad del Tolima. The authors of this manuscript declare that no funding of any kind was obtained for this study, and there are no organizations or companies with interests linked to it.

CONFLICT OF INTEREST

There is no conflict of interest.

AUTHORS' CONTRIBUTION

JPAA: study conception, contribution in writing and proofreading. APR and LFT: literature search and proofreading in writing.

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