**In vitro Antineoplastic Activity of Dye Compounds on Human Glioblastoma Cells**

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Dyes are an important class of natural and synthetic compounds, recently studied as potential anticancer drugs. Among various natural dye molecules, Curcumin was extensively studied in treatment of malignant gliomas, a highly incurable disease. Curcumin was reported to induce cell death in malignant gliomas by induction autophagy and apoptosis. We have previously reported that Helianthin, a synthetic dye compound, also induced apoptotic cell death in high grade glioma cells. In this study we evaluated the antiproliferative and the apoptotic effect of Curcumin and Helianthin on a human low passage glioblastoma cell line. We found that both compounds displayed antiproliferative properties on glioblastoma cells, however, at equimolar concentrations, Helianthin induced more cytotoxic effect than Curcumin. IC50 value is considered a good indicator of drug efficacy. We found that Helianthin required a lower concentration to achieve IC50 (16.9±735 ±14.8μM) than Curcumin (68.5±12.3μM). We also found that Curcumin and Helianthin treatment induced caspase 3, 8 and 9 activation in glioblastoma cells. This study may lead to a widespread search for dye agents that may represent an untapped source of drugs for cancer treatment.

Keywords: dye compounds, Curcumin, Helianthin, apoptosis, glioblastoma, therapy

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Dyes compounds, are either natural (e.g. Curcumin, Quercetin) or synthetic (e.g. Helianthin, Methyl Yellow, Methyl Red) products that represent a new promising class of anti-tumor drugs. In the last years, these substances gained attention as chemotherapeutic agents because of their cytotoxic effect on malignant cells as well as their favorable toxicity profile in vivo.

Dye compounds have been demonstrated to have antiproliferative effects on cancer cells, by interfering with receptor tyrosine kinases (RTKs) signaling, some of them inducing apoptosis in malignant cells [1-3].

Natural products are considered a very important source of antineoplastic agent, a vast numbers of drugs used in inducing apoptosis in malignant cells [1-3]. Receptor tyrosine kinases (RTKs) signaling, some of them exhibit antineoplastic effect on malignant cells as well as their favorable toxicity profile in vivo.

The efficacy of Curcumin in glioblastoma treatment was investigated by many research groups. It’s antiproliferative effects on glioblastoma cells first demonstrated in vitro by Ambegaokar et al. in 2003 [12]. Next years, other scientists demonstrated the efficacy of Curcumin used either alone or in combination to other chemotherapeutics or radiation therapy [13]. Turmeric is able to initiate apoptosis in glioblastoma cells through the activation of poly ADP ribose polymerase (PARP) and caspases activation [14]. Also, in glioblastoma cells, Curcumin acts as inhibitor of growth protein 4 (ING4) signaling pathway, activates a non-apoptotic autophagy signal, induces differentiation cascade, activates proteolytic pathways and induces apoptosis mediated through apo2 ligand and tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL/Apo2L) [15, 16].

Curcumin was tested in vivo and, in spite the limited number of studies, the results are encouraging. In 2012 Wolff et al. published the preliminary promising data of a clinical trial which used curcumin in association with other treatments for a personalized and targeted therapy in pediatric brain tumors [17].

The antimicrobial properties of synthetic dye compounds (i.e., methylene blue, trypan red, Acridine) were known from the middle 19th century. Now researchers began to ask whether synthetic dye moieties have anticancer properties. Acridine and its derivatives were shown to exhibit antineoplastic effect on cervical and lung cancer cells in vivo [18].

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Further use of this type of dye substances was investigated as a treatment for other tumor cells with a very promising early result [19].

In our previous work, we also found that Helianthin (methyl orange), a synthetic azo-dye compound, induced cell death in high grade glioma cell lines. We also found that the substance induced apoptosis by PARP degradation, without affecting the expression of B-cell lymphoma 2 (Bcl-2) [2]. In this paper, we evaluate and compare the antiproliferative and the apoptotic effect of Curcumin and Helianthin on a human low passage glioblastoma cell line. Unlike established cell lines, low-passage cell cultures seem to preserve in a better way the features of the original tumor.

**Experimental part**

**Materials and methods**

**Cell lines and cell culture.** The human primary glioblastoma cell line GB10B, is a low passage primary brain tumor cell line derived from fresh glioblastoma tissue. The tumor was collected from a patient diagnosed with glioblastoma and surgically operated, following standard procedures at Bagdasar-Arseni Hospital in Bucharest. The informed consent of the patient and the ethical approval for the project (Commission for Ethics and Deontology UMF of Craiova) were obtained prior to surgery and any experiment. The tissue was mechanical and enzymatic digested and after the triburation and filtration, the sample was centrifuged for 10 min and the cells were resuspended and cultured using standard procedures. The cell line was grown in minimum essential medium eagle (MEM) containing 10% fetal bovine serum (FBS), 2mM glutamine and antibiotic (100 UI/mL penicilline and 100 UI/mL streptomycine). The cells were incubated at 37 and 5% CO₂ in a humidified incubator (CO₂ incubator Innova CO₂ incubated at 170) in 25 cm² culture cell bottles.

**Curcumin preparation**

Curcumin was purchased from Sigma Aldrich. The Curcumin powder was dissolved in Dimethyl Sulfoxide (DMSO) in order to obtain a 100mM stock solution. The solution was stored at -20°C, protected from light. We used different concentrations (0.1 µM, 1 µM, 2 µM, 5 µM, 10 µM, 20 µM, 50 µM, 100 µM, 150 µM) of Curcumin by diluting the stock solution with standard medium.

**Helianthin preparation**

Helianthin (Methyl Orange), was purchased from Santa-Cruz. The Helianthin powder was dissolved in H₂O and in order to obtain a 100µM stock solution. The solution was stored at -20°C, protected from light. We used different concentrations (0.1 µM, 1 µM, 2 µM, 5 µM, 10 µM, 20 µM, 50 µM, 100 µM, 150 µM) of Helianthin by diluting the stock solution with standard medium.

**MTT cellular proliferation assay**

The antiproliferative effect of Curcumin and of Helianthin, was examined using MTT assay (Sigma Aldrich). The assay is based upon the cleavage of the yellow tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan crystals by metabolically active cells. Tests were conducted with 4000 cells/well, plated in 200 µL media in 96-well plates, with six replicates. Cells proliferation was quantified 72 h after treatment, MTT reagent (10 µL) was added to each well and then incubated for 4h at 37°C. After that, cells were lysed by addition of 100 µL solubilization buffer. Optical density was measured using a spectrophotometer at 595 nm and relative cell viability was expressed as percentage of that in untreated control cultures.

**IC50 calculation.** To estimate the inhibitory concentration that kills 50% of cells (IC50), the formula used was:

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IC50 = \frac{(150-X)/(Y-X)) \times (W-Z) + Z.
\]

where X is the first point on the curve, expressed as percent inhibition, that is less than 50%, Y is the first point on the curve, expressed as percent inhibition, that is greater than or equal to 50%; Z is the concentration of inhibitor that gives X% inhibition; and W refers to the concentration of inhibitor that gives Y% inhibition. IC50 value was calculated from the dose-response curves for each compound [20, 21].

**Cell apoptosis assay**

Apoptosis was analyzed using ApoTarget Caspase-3 (CPP32) Colorimetric Protease Assay kit, ApoTarget Caspase-8 (FLICE) Colorimetric Protease Assay kit, ApoTarget Caspase-9 (Mch6/Apaf-3) Colorimetric Protease Assay Kit using the manufacturer’s recommendation (Invitrogen, Life Tefnologies, USA). Caspases are cysteine protease, which exist as inactive pro-forms. By inducing apoptosis, they are cleaved to active form. For experimental purposes we seeded into 10 cm Petri dishes. The cells treated with IC50 concentration of the Curcumin and Helianthin (taken from dose response) for 4, 8, 24, 48 h or untreated cells were trypsinized and the cytosolic fraction was isolated at a concentration of 3 x 10⁶ per sample. The samples were transferred in 96-well plates, after adding 50 µL of 2× Reaction Buffer/DTT (10 mM dithiothreitol) to each sample and 5 µL of 4mM EDT-Pna (Ile-Glu-Thr-Asp/p-nitroanilide) substrate per sample the plates were incubated at 37°C in a dark incubator for 2 h. The samples were read at an absorbance of 405 nm in a 96-well microplate reader at a spectrophotometer Star Fax-2100 (AWARENESS TECHNOLOGY INC).

**Statistical analysis**

All data are represented as mean ± SEM. Data were analysed using ANOVA two-tailed t-test for analysis. P < 0.05 values were considered statistically significant.

**Results and discussions**

**The effect of Curcumin and of Helianthin on glioblastoma cell viability.**

In the present work we investigated the ability of Curcumin and of Helianthin to inhibit GB10B cells growth *in vitro*. The cells were exposed to increasing doses of Curcumin and Helianthin respectively. The concentrations of the two studied dye compounds varied from 0.1µM to 150 µM. The proliferation rates were evaluated 3 days after treatment by performing the MTT assay (Material and methods section).

Our results showed that Curcumin reduced cell survival by a maximum of 77% when compared with control cells. The concentration of Turmeric that induced this cytotoxic effect was of 100µM. This was statistically significant compared with normal controls (p<0.05) (fig. 1). Minimum inhibitory concentration of Curcumin was 0.1 µM and provoked only approximately 2% GB10B cell death. Higher Curcumin concentrations did not induce more cytotoxicity (fig. 1). Helianthin treatment also had antiproliferative effect on GB10B cells. While minimum inhibitory concentration (0.1 µM) induced only approximately 10% cells death, 100 µM Helianthin reduced cell survival by approximately 85%
when compared with untreated control glioblastoma cells. Similar results were obtained when used 150 \( \mu M \) Helianthin when the decrease of cells survival was of approximately 86.5%. This decrease in cell survival was statistically significant compared with untreated control (\( p<0.05 \)).

**IC50 calculation**

The IC50 value is very important in evaluation of the drug cytotoxic potency. The IC50 value is also important when comparing drug effect on different compounds.

We determined the half inhibitory concentration to induce 50% cell death (IC50) was determined in GB10B cell line for Curcumin and also for Helianthin. We found that IC50 value was 68.5 ±12.3 \( \mu M \) for Curcumin, and 16.9 ±7.35 \( \mu M \) for Helianthin. Our result showed that the most potent among two tested dye compounds was Helianthin.

**Activation of caspase3, 8 and 9 by Curcumin and Helianthin drugs-induced cell death.**

To evaluate whether the cytotoxicity of Curcumin and Helianthin induced apoptotic cell death, GB10B cells were exposed to IC50 concentration of each drug (taken from dose response) and apoptos induction was determined by caspase 3, 8 and 9 assay.

**Caspase 3 activation**

Caspase 3 is an executioner caspase involved in the coupling between the extrinsic and the intrinsic pathways of apoptosis. The activation of caspase 3 is subsequent to the activation of caspase 8 and 9. Several research studies demonstrated that Curcumin treatment activates caspase 3 in glioblastoma cells [22, 23].

In this part of the study, the cells treated with 68.5 \( \mu M \) Curcumin (IC50) or 16.9 \( \mu M \) Helianthin (IC50) for 4, 8, 24, 48 h or untreated cells were assayed. for activation of caspase 3 (fig. 2).

We found that 63.8 \( \mu M \) Curcumin induced caspase 3 activation at 12h after the treatment and remained active at 24h and 48h after drug administration (fig. 2A). The treatment with 16.9 \( \mu M \) Helianthin induced caspase 3 activation with a peak at 12 h returned to base-line at 24 and h after the treatment (fig. 2B).

**Caspase 8 activation.**

Caspase 8 is an initiator caspase. This proapoptotic protease activation precedes the activation of caspase 3.There are a few number of studies that demonstrated the activation of caspase 8 by Curcumin administration in glioblastoma cells [14, 16, 24].

For caspase 8 evaluation, the cells were treated with IC50 concentration of the Curcumin or Helianthin for 4, 8, 24, 48 h and activation of caspase 8 was determined spectrophotometrically, as indicated in the section Material and Methods.
The treatment with 16.9 µM Helianthin induced early caspase 8 activation at 4 h, remained active at 8 h and progressively increased at 12, 24 and 48 h after treatment (fig. 3B). In contrast to Helianthin, 4 and 8 h incubation with Curcumin at IC50 concentration did not show a significant (p ≥ 0.05) increase of caspase 8 in glioblastoma cell (fig 3A). The treatment induced a significant increase (p ≤ 0.05) at 12 h and return to base-line at 24 and 48 h after the treatment (fig. 3A).

Caspase 9 activation

Caspase 9 activation occurs early in apoptosis preceding both caspase 8 and caspase 3. In fact, caspase 9 is an initiator caspase involved in the intrinsic pathway of apoptosis. Curcumin treatment proved to induce caspase 9 activation in glioblastoma cells [14, 16, 23].

In our experiments we evaluated caspase 9 activity by treating the cells with IC50 concentration of the Curcumin or Helianthin for 4, 8, 24 and 48 h respectively. The activation of caspase 8 was determined spectrophotometrically, as indicated in the section Methods.

Both dye compounds activated caspase 9 at 4 h after administration. Caspase 9 remained active up to 48 h after incubation with 65.8 µM Curcumin (fig. 4A) and 16.9 µM Helianthin (fig. 4B). The results were statistically significant (p≤0.05).

Glioblastomas, a type IV malignant glioma, are very aggressive brain tumors. In spite of current advances in multimodality therapy the prognosis of these patients remains extremely poor. Recently there are attempts to improve the present therapeutic approaches for these tumors. Among them is the discovery of novel anti-neoplastic drugs that are more efficient and less aggressive with the healthy tissues [25].

In the last years, literature enriched with reports showing that natural products extracted from plants or synthetic structures derived from natural compounds represent a hope for cancer patients. Nowadays, there are publications that underline the reasoning for using those new drugs as anti-tumor therapy alone or in combination with other treatments [5, 26, 27]. Among those new drugs are dyes, a major class of natural and synthetic compounds with a variety of therapeutic properties [27]. In recent years, some azo dye products have shown promise both as a potential antitumour agents alone or in combination with conventional treatment for several types of malignancies. These natural (e.g. curcumin, quercetin, saffron etc), or synthetic (e.g. helianthin etc) compounds proved to have antiproliferative effect on neoplastic cells and low toxicity in vivo. They have been demonstrated to have antiproliferative effects on cancer cells, by inhibiting RTKs signaling and inducing apoptosis [2, 12].
In our study we analyzed the antiproliferative and apoptotic effect of Curcumin and Helianthin treatment on GB10B low passage glioblastoma cell line in vitro. In fact, clinical protocols are preceded by preclinical studies of cytotoxicity using immortalized cell lines. However, established cell lines are often unable to reproduce the original tumor characteristics. Also, long-time culture cancer cells tend to accumulate a series of DNA mutations, cells morphology changes, growth rates alterations and expression of dysfunctional proteins [28, 29]. Here, we used a low-passage glioblastoma cell line obtained in our laboratory from fresh tumor tissue.

Several reports sustain the cytotoxic effect of Curcumin on various types of solid tumors, including glioblastomas. The first study that demonstrated the capacity of Curcumin to kill glioblastoma cells was published in 2003 [12]. Since then, various studies were made on different glioblastoma cell lines [22]. Although administration of Turmeric showed encouraging results in vitro, until now the drug was used only in a few clinical trials including one clinical trial of pediatric brain tumors. The preliminary results of this trial are promising but the substance is not yet used as a standard treatment for humans [17]. Our experiments demonstrated that the administration of Curcumin to GB10B culture induced cytotoxicity in a dose dependent manner (fig. 1). The maximum antiproliferative effect of the drug was obtained at 100 ¡M and determined a reduction in cell viability up to 77%. This Curcumin concentration of 100µM seems to be safe as Turmeric is used in Indian diet for thousands of years and proved no toxicity when administered at doses up to 10 g/day.

Compared with Curcumin, synthetic azo-dye compound Helianthin was until now studied only on high grade glioma cell lines in vitro. In fact, we have previously shown that the substance induced apoptosis in several high grade glioma cell cultures. Now we found that 100 ¡M Helianthin drastically induced decrease in cell viability, killing 85% of GB10B cells. This concentration of Helianthin seems to be safe as it was proved most azo-dyes have a median lethal dose(LD50) values between 250 and 2000 mg/kg body weight in the mammal tests [30]. Thus, our data showed that synthetic dye Helianthin had a greater cytotoxic effect on GB10B cells when compared with natural dye compound Curcumin. Consistently with our previous work, we observed that 1M Helianthin killed approximately 40% of our GB10B cells [2].

It is already known that programmed cell death (apoptosis) is a vital process in all living cells. However, in glioblastoma cells apoptosis is widely deregulated [31]. In glioblastoma, the intrinsic apoptosis pathway is regulated by the pro-apoptotic protein Bcl2, reported to determine the increase of membrane permeability, which subsequently determines the release of cytochrome 3. It also induces caspase 9 activation and subsequent activation of caspase 3 [31]. The extrinsic pathway in glioblastoma involves the induction of the death receptors trimerisation, results in the recruitment and activation of caspase 8 and 10. Activation of these caspases leads in the cleavage of target substrates by caspase 3. In that way the 2 pathways are linked [32, 33].

In our work we investigated the capability of Curcumin and of Helianthin to induce apoptosis in GB10B cells. It is already known that Curcumin is capable to induce apoptosis in glioblastoma by activation of pro-apoptotic proteins and inhibition of anti-apoptotic signals. The drug is also involved in the activation of non-apoptotic autophagy, induces differentiation cascade signaling, inhibits matrix metalloproteinases (MMPs) and glucose-6-phosphate transporter (G6PT) gene expression and also activates proteolytic pathways [34, 35]. Here, we observed that after treating GB10B cells with Curcumin, caspase 3 activated at 12h after the treatment and remained active up to 48h after the administration of the drug. Caspase 8 was active only at 4h and 12h after Turmeric administration while caspase 9 activated at 4h after the administration of the drug. Caspase 9 remained activated up to 48h after Curcumin administration.

In our previous studies we reported that Helianthin treatment was associated with PARP degradation without affecting expression of Bcl-2 in high grade glioma cell lines [2]. In the present study, we observed that Helianthin activated caspase3 in GB10B cells only at 12h after the administration of the drug (fig. 2B). Caspase 8 was first activated at 4h after administering the drug and remained active up to 48h after. In contrast to caspase 3 and 8, after Helianthin treatment, early caspase 9 activation was detected (4 hours after the treatment), that successively increased until the end of treatment. These results suggest that Curcumin and Helianthin treatment may induce death in GB10B cells by activation of both extrinsic and intrinsic pathways. However further studies are necessary to better understand the apoptotic mechanisms induced by dye compounds in glioblastoma.

Conclusions
In conclusion, this study demonstrates that Curcumin and Helianthin treatment induces cell death in glioblastoma cells in vitro and Helianthin displayed better antiproliferative capacity than Curcumin. We also found that Curcumin and Helianthin treatment determined caspase 3, 8 and 9 activation in GB10B cells. Further on more studies are necessary to conclude whether Curcumin and Helianthin might be effective chemotherapeutics agents in vivo. More importantly, this study may lead to a widespread search for dye agents that may represent an untapped source of drugs for cancer treatment.

List of abbreviations

- RTK-receptor tyrosine-kinase
- PARP-poly ADP ribose polymerase
- ING4-inhibitor of growth protein 4
- TNF-tumor necrosis factor
- Bcl-2-B-cell membrane protein
- MEM-minimum essential medium eagle
- FBS-fetal bovine serum
- DMSO-Dimethyl Sulfoxide
- MMP-matrix metalloproteinases
- G6PT-glucose-6-phosphate transporter
- PARP-poly ADP ribose polymerase
- ING4-inhibitor of growth protein 4
- TNF-tumor necrosis factor
- Bcl-2-B-cell membrane protein
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- FBS-fetal bovine serum
- DMSO-Dimethyl Sulfoxide
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References
1. MEHTA, K., PANTAZIS, P., MCQUEEN, T., AGGARWAL, B.B., Antiproliferative effect of curcumin (diferuloyl methane) against human breast tumor cell lines, Anticancer Drugs, 8, 1997, p. 470-481.
2. ALEXANDRU, O., DRAGUTESCU, L., TATARANU, L., ET AL., Helianthin induces antiproliferative effect on human glioblastoma cells in vitro, J. Neurooncol., 102, 2011, p. 9-18.
3. MUKHOPADHYAY, A., BUÉSU-RAMOS, C., CHATTERJEE, D., ET AL., Helianthin downregulates cell survival mechanisms in human prostate cancer cells in vitro, Oncogene, 20, 2001, p. 7597-7609.
4. NOBILI, S., LIPPI, D., WITORT, E., ET AL., Natural compounds for cancer treatment and prevention, Pharmacol. Res., 59, 2009, p. 365-378.
5. AMIN, A., GALI-MUHTASIB, H., OCKER, M., SCHNEIDER- STOCK, R., Overview of major classes of plant-derived anticancer drugs, Int. J. Biomed. Sci., 5, 2009, p. 1-11.

6. AMMON, H.F., WAHL, M.A., Pharmacology of Curcuma longa, Planta Med., 57, 1991, p. 1-7.

7. GUPTA, S.C., PATCHVA, S., AGGARWAL, B.B., Therapeutic roles of curcumin: lessons learned from clinical trials, AAPS J., 15, 2013, p. 195-218.

8. ALINEJAD, B., GHORBANI, A., SADEGHHNA, H.R., Effects of combinations of curcumin, linalool, rutin, safranal, and thymoquinone on glucose/serum deprivation-induced cell death, Avicenna J. Phytomed., 3, 2013, p. 321-328.

9. NOORAFSHAN, A., ASHKANI-ESFAHANI, S., A review of therapeutic effects of curcumin, Curr. Pharm. Des., 19, 2013, p. 2032-2046.

10. BAYET-ROBERT, M., KWIATKOWSKI, F., LEHEURTEUR, M., ET AL., Phase I dose escalation trial of docetaxel plus curcumin in patients with advanced and metastatic breast cancer, Cancer Biol. Ther., 9, 2010, p. 8-14.

11. IDE, H., TOKIWA, S., SAKAMAKI, K., ET AL., Combined inhibitory effects of soy isoflavones and curcumin on the production of prostate-specific antigen, Prostate, 70, 2010, p. 1127-1133.

12. AMBEGAOKAR, S.S., WU, L., ALAMSHAHI, K., ET AL., Curcumin inhibits dose-dependently and time-dependently neuroglial cell proliferation and growth, Neuro. Endocrinol. Lett., 24, 2003, p. 469-473.

13. DHANDAPANI, K.M., MAHESH, V.B., BRANN, D.W., Curcumin suppresses growth and chromoresistance of human glioblastoma cells via AP-1 and NFκB transcription factors, J. Neurochem., 102, 2007, p. 522-538.

14. KARAMARKAR, S., BANIK, N.L., RAY, S.K., Curcumin suppressed anti-apoptotic signals and activated cysteine proteases for apoptosis in human malignant glioblastoma U87MG cells, Neurochem. Res., 32, 2007, p. 2103-2113.

15. LIU, E., WU, J., CAO, W., ET AL., Curcumin induces G2/M cell cycle arrest in a p53-dependent manner and upregulates ING4 expression in human glioma. J. Neurooncol., 85, 2007, p. 263-270.

16. KARAMARKAR, S., BANIK, N.L., PATEL, S.J., RAY, S.K., Curcumin activated both receptor-mediated and mitochondria-mediated proteolytic pathways for apoptosis in human glioblastoma T98G cells, Neurosci. Lett., 407, 2006, p. 53-58.

17. WOLFF, J.E., BROWN, R.E., BURYANEK, J., ET AL., Preliminary experience with personalized and targeted therapy for pediatric brain tumors, Pediatr. Blood Cancer, 59, 2012, p. 27-33.

18. KUMAR, P., KUMAR, R., PRASAD, D.N., Synthesis and anticancer study of 9-aminoacridine derivatives, Arabian J. Chem., 6, 2013, p. 79-85.

19. KAWAKAMI, M., KOYA, K., UKAI, T., ET AL., Structure-activity of novel rhodacyanine dyes as antitumor agents, J. Med. Chem., 41, 1998, p. 130-142.

20. MAHATA, S., MARU, S., SHUKLA, S., ET AL., Anticancer property of Bryophyllum pinnata (Lam.) Oken. leaf on human cervical cancer cells. BMC Complement Altern. Med., 12, 2012, p. 15.

21. YAN, Z., CALDWELL, G.W., Evaluation of cytochrome P450 inhibition in human liver microsomes. In Optimization in Drug Discovery, Springer, 2004, p. 231-244.

22. ZANOTTO-FILOLO, A., BRAGANHOL, E., EDELWEISS, M.I., ET AL., The curry spice curcumin selectively inhibits cancer cells growth in vitro and in preclinical model of glioblastoma, J. Nutr. Biochem., 23, 2012, p. 591-601.

23. HUANG, T.Y., TSAI, T.H., HSU, C.W., HSU, Y.C., Curcuminoids suppress the growth and induce apoptosis through caspase-3-dependent pathways in glioblastoma multiforme (GBM) U8401 cells, J. Agric. Food Chem., 58, 2010, p. 10639-10645.

24. HOSSAIN, M., BANIK, N.L., RAY, S.K., Synergistic anti-cancer mechanisms of curcumin and paclitaxel for growth inhibition of human brain tumor stem cells and LN18 and U138MG cells, Neurochem. Int., 61, 2012, p. 1102-1113.

25. MAO, H., LEBRUN, D.G., YANG, J., ET AL., Deregulated signaling pathways in glioblastoma multiforme: molecular mechanisms and therapeutic targets, Cancer Invest., 30, 2012, p. 48-56.

26. RAJ, E., SANKARI, L.S., MALATHI, L., KRUPAA, J.R., Naturally occurring products in cancer therapy, J. Pharm. Bioallied. Sci., 7, 2015, p. S181-S183.

27. AGGARWAL, B.B., SUNDARAM, C., MALANI, N., ICHIKAWA, H., Curcumin: the Indian solid gold, Adv. Exp. Med. Biol., 595, 2007, p. 1-75.

28. SAMBUY, Y., DE ANGELIS, I., RANALDI, G., ET AL., The Caco-2 cell line as a model of the intestinal barrier: influence of cell and culture-related factors on Caco-2 cell functional characteristics, Cell Biol. Toxicol., 21, 2005, 1-26.

29. CHANG-LIU, C.M., WOLOSCHAK, G.E., Effect of passage number on cellular response to DNA-damaging agents: cell survival and gene expression, Cancer Lett., 113, 1997, p. 77-86.

30. ZEE, F., Anaerobic azo dye reduction, Ph. D. thesis, Wageningen University, Wageningen-Netherlands, 2002.

31. BRENNER, D., MAK, T.W., Mitochondrial cell death effectors, Curr. Opin. Cell Biol., 21, 2009, p. 871-877.

32. AGGARWAL, B.B., Signalling pathways of the TNF superfamily: a double-edged sword, Nat. Rev. Immunol., 3, 2003, p. 745-756.

33. GUICCIARDI, M.E., GORES, G.J., Life and death by death receptors, FASEB J., 23, 2009, p. 1625-1637.

34. NAGAI, S., KURIMOTO, M., WASHIYAMA, K., ET AL., Inhibition of cellular proliferation and induction of apoptosis by curcumin in human malignant astrocytoma cell lines, J. Neurooncol., 74, 2005, p. 105-111.

35. ROMERO-HERNANDEZ, M.A., EGUA-Agüilar, P., PÉREZ-PENASCIA-DIAZCONTI, M., ET AL., Toxic effects induced by curcumin in human astrocytoma cell lines, Toxicol. Mech. Methods, 23, 2013, p. 650-659.

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