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Original Article

Hypericin Induces Apoptosis in MDA-MB-175-VII Cells in Lower Dose Compared to MDA-MB-231

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Abstract

Background: Breast cancer is the major cause of death from cancer among women around the world. Given the drug resistance in the treatment of this disease, it is very important to identify new therapies and anticancer drugs. Many studies demonstrated that hypericin could induce apoptosis in different cancer cell lines; however, the underlying mechanism is not well understood yet. Therefore, this study aimed to evaluate the anticancer effect of hypericin in two breast cancer cell lines, one with wild type P53 and the other with mutant P53.

Methods: In this study, the MDA-MB-231 and MDA-MB-175-VII cell lines were treated with different concentrations of hypericin for 24 and 48 hours. The measurement of cell death was performed by MTT assay. The cell apoptosis rate was measured using annexin V/propidium iodide assay through flow cytometry. The level of expression in P21 and P53 genes was evaluated by real time PCR. Immunocytochemistry (ICC) analysis was performed for P21 (direct target for P53 protein) to confirm the results.

Results: The results showed that hypericin could have dose-dependent cytotoxic effects on the MDA-MB-231 and MDA-MB-175-VII cell lines, and its cytotoxicity is much higher in the latter cells. According to flow cytometry results, 86% of MDA-MB-175-VII cells underwent apoptosis with IC50 dose of hypericin for MDA-MB-231 cells after 24 hours. Moreover, after 24 hours of exposure to hypericin with MDA- MB-231 IC50 concentration, the expression of P53 and P21 genes upregulated in MDA-MB-175-VII much more than MDA-MB-231 when both cell lines were treated with 24 hours IC50 dose of MDA-MB-231. The ICC analysis on P21 confirmed that by treating both cell lines with MDA-MB-231 IC50 dose of hypericin for 24 hours, this protein is overexpressed much more in MDA-MB-175-VII cells.

Conclusion: The results of this study demonstrated that hypericin's apoptotic and cytotoxic effects on cancer cells may be mediated via P53 overexpression, cell cycle arrest and the subsequent apoptosis. Therefore, it is of great importance to consider that hypericin would have better impact on cells or tumors with wild type P53.

Keywords: Anticancer drug, Hypericin, MDA-MB-175-VII, MDA-MB-231, P53 wild type

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Introduction

Breast cancer is the major cause of death from cancer in women worldwide.[1] The onset of this type of cancer may not have any symptoms, and it is usually diagnosed using mammography after it is developed.[1,2] To confirm breast cancer, samples are collected from suspected mass. Common treatments include the use of one or more simultaneous surgical procedures, radiotherapy and chemotherapy.[3] A major problem with breast cancer is disease relapse after treatment and its high metastasis, especially to the liver, lungs, brain and bones.[4,5] Therefore, if it is detected in advanced stages, it is practically untreatable. According to gene expression patterns, breast cancers are categorized into different types including Luminal A/B, Basal, claudin-low, and human epidermal growth factor receptor 2 positive (HER2 +).[6-8] The MDA-MB-231 and MDA-MB-175-VII cell lines are 2 examples of breast cancer cell lines with different mutations in P53.[9,10] Unfortunately, there is still no proper treatment for these types of breast cancers.[10,11] Therefore, it seems necessary to find a proper treatment with no significant side effects on normal cells in breast

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tissue including fibroblast cells.[12]

The use of medicinal plants in terms of their side effects has always been a concern. Several plants have been identified for the treatment of certain diseases so far.[13-16] Identification of anti-cancer and anti-metastatic effects of medicinal plants has always been considered.[17] Some studies have shown the cytotoxic effects of hypericin in *Hypericum perforatum* plant on cancer cells.[12] This plant is a member of Hypericaceae species, which is commonly found in the wheat and corn fields.[18] Various studies have been carried out on the effects of its extract in the treatment of some diseases such as depression.[19] However, the identification of other therapeutic effects of this extract can be highly valuable.[12] Considering the cytotoxicity of this plant this study was conducted to investigate the underlying mechanism of action of hypericin in inducing apoptosis in cancerous cells. P53 is a well-known gene that is mutated in over 50% of different cancers which reflects its true name, the "*gate keeper*".[20,21] In this study we investigated different effects of hypericin in two breast cancer cell lines: the MDA-MB-231 (with mutated P53) and MDA-MB-175-VII (with wild type P53).[22] Our results showed that hypericin could induce apoptosis in MDA-MB-175-VII cells in a lower dose than MDA-MB-231.

Materials and Methods

Materials and Reagents

MDA-MB-231 and MDA-MB-175-VII cell lines were purchased from Pasteur Institute of Iran (Tehran, Iran). Hypericin, cisplatin and MTT (3-[4,5-Dimethyl-2-thiazolyl]-2,5-diphenyl-2-tetrazolium bromide) assay kit were obtained from Sigma Aldrich (USA). The medium utilized for culturing was high glucose Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum, 100 mg/mL streptomycin and 100 U/mL penicillin (all from Gibco, USA). Rabbit polyclonal anti-P53 antibody, goat anti-Rabbit IgG Fc (FITC) and DAPI which used for immunocytochemistry (ICC) were purchased from Abcam (UK). All other reagents were obtained from Sigma Aldrich.

Cell Culture and MTT Assay

The breast cancer cell lines utilized in this study were cultured in cell culture incubator (37°C with 5% CO2) in a complete medium as described in the previous section. Cell culture medium above the cells was changed with fresh medium every 4 days. When confluency of cells reached into 90%, cells were detached by trypsin and were counted after centrifugation. 8000 and 6000 cells were seeded in each well of 96-well plates for 24 and 48 hours MTT assay, respectively. Post 24 or 48 hours medium of each well was replaced with fresh medium and an appropriate concentration of MTT solution based on manufacturer's manual. Formazan (the purple sediments) appeared after 3 hours. MTT containing media in the wells were depleted and 200 µL of Dimethyl sulfoxide (DMSO) was added to each well to dissolve formazan crystals. Afterward, absorption of each well was revealed by Biotek ELX800 microplate reader (BioTek Instruments, Inc.) at 570 nm.

Apoptosis Assay by Flow Cytometry

To further investigate whether cellular death which occurred after treatment of MDA-MB-231 and MDA-MB-175-VII cells with hypericin was kind of apoptosis or not, annexin V/propidium iodide (annexin V/ PI) apoptosis assay kit (Roche Company, Switzerland) was used. We followed exactly the procedure described in the kit instruction manual. To specify the number of cells which undergo apoptosis, we treated MDA-MB-175-VII cells with 24 hours half maximal inhibitory concentration (IC50) dose of hypericin for MDA-MB-231 cells which attained from prior MTT assay.

Real-Time Polymerase Chain Reaction

Total RNA extracted by TRIzol was purchased from Invitrogen. Quality and concentration of extracted RNAs were determined using spectrophotometry (treated cells received hypericin with a concentration equal to the 24 hours IC50 of MDA-MB-231). The same amounts of RNAs were then utilized for cDNA synthesis (Takara, Japan). These cDNAs were used for real time PCR reaction in Q5 plex rotor gene Qiagen device (USA) with the following program: 1- Holding Stage: 95°C/5 min. 2- Cycling Stage: denaturing step: 95°C/15 s, followed by annealing step 60°C/30 s, amplification step 72°C/20 s (number of cycles: 40). 3- Melt Curve Stage. Primers designed to specifically amplify Bax, Bcl2 and P53 (we used Gapdh as the internal control).

Immunocytochemistry

To perform ICC test, cells were seeded in 24-well plates. These cells were treated with specific dose of hypericin (treated cells received hypericin with a concentration equal to the 24 hours IC50 of MDA-MB-231) and then fixed by 4% paraformaldehyde at room temperature for 10 minutes. After that, these cells were incubated with anti-p21 antibody at 4°C. After 16 hours of incubation with primary anti-p21 antibody, cells were washed with PBS and then incubated for 1 hour with FITC (samples without primary antibody were used as negative controls). Cells nuclei were stained by DAPI.

Statistical Analysis

We used GraphPad Prism software version 6.01 to

perform statistical analysis. Error bars represent mean ± SD and *P* value less than 0.05 was considered as significant. For comparison between groups, unpaired *t* test was performed.

Results

MTT Assay and Flow Cytometry

To determine IC50 dose for hypericin (concentration of hypericin in which 50% of cells die), MTT assay was performed. The IC50 dose for hypericin was determined to be 2 µg/mL and 1 µg/mL for 24 and 48 hours, respectively, for MDA-MB-175-VII (Figure 1). The IC50 dose for hypericin was defined to be 5 µg/mL and 3 µg/mL for 24 and 48 hours, respectively (Figure 2). Treatment of both cell lines with 24 hours IC50 of MDA-MB-231, induced about 50% apoptosis in MDA-MB-231, and about 86% apoptosis in MDA-MB-175-VII (Figure 3).

Real-Time Polymerase Chain Reaction

The expression level of P53 and Bax genes significantly increased after treatment of cells by IC50 dose of hypericin for 24 hours. The Bcl2 gene expression level decreased after treatment which confirms that these cells underwent apoptosis (Figure 4) (Gapdh was utilized as the internal control).

Immunocytochemistry

As shown in Figure 5, after treatment of cells with 24 hours IC50 of MDA-MB-231, p21 protein expression level increased much more in MDA-MB-175-VII cells compared with MDA-MB-231

Discussion

More than one million new cases of breast cancer are diagnosed every year in the world, while more than 600 000 cases lead to death.[1,23] Based on the progress of the disease, different methods are suggested to treat breast cancer.[1,7,8] The most common ways to treat breast cancer include surgery and breast discharge, radiotherapy and chemotherapy. Each of these treatments has several complications, and resistance is likely to occur with each method.[24,25] Therefore, choosing alternative therapies is very important.[26] The effects of the extracts of medicinal plants on the treatment of many diseases have been proven. Many plants have proven anti-cancer and

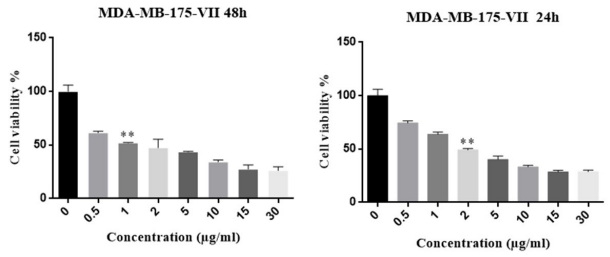

Figure 1. MTT assay results revealed that the IC50 dose for treatment of MDA-MB-175-VII cells with hypericin is 2 (µg/mL) (*P* value = 0.002) and 1 (µg/mL) (*P* value= 0.001) for 24 and 48 hours, respectively. Left diagram shows the results for MTT assay 48 hours after treatment of these cells with different doses of hypericin, and right diagram, shows that of 24 hours.

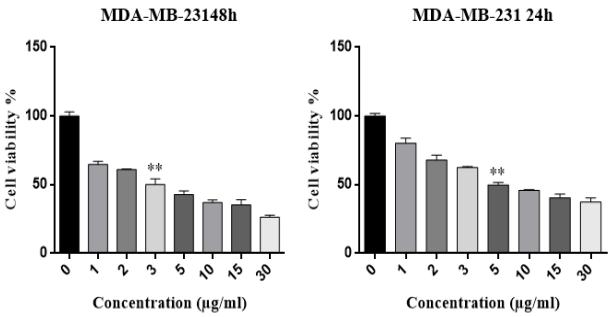

Figure 2. The IC50 dose for treatment of MDA-MB-231 with hypericin was calculated to be 5 (µg/mL) and 3 (µg/mL) for 24 and 48 hours, respectively. Left diagram; MTT assay results for 48 hours treatment of MDA-MB-231 cells with different doses of hypericin, right diagram; that of 24 hours.

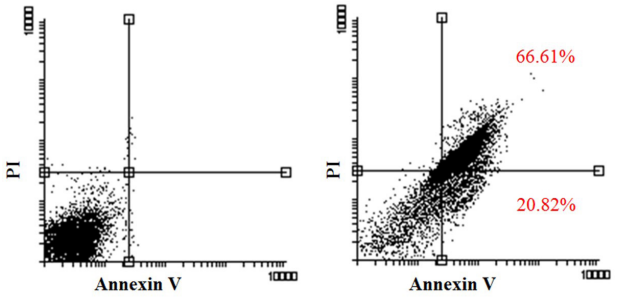

Figure 3. Annein/PI flow cytometry results demonstrated that treatment of MDA-MB-175-VII cells with 5 (µg/mL) hypericin for 24 hours induces apoptosis, and not necrosis, in these cells (right diagram, upright and downright quadrants), compared to untreated MDA-MB-175-VII cells (left diagram).

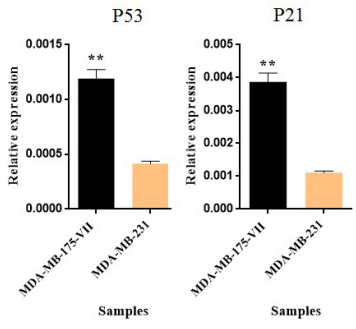

Figure 4. Real-time PCR results demonstrated that treatment of MDA-MB-175-VII and MDA-MB-231 cells with IC50 dose of hypericin induces upregulation of P53 (left plot) (*P* value = 0.004) and P21 (right plot) (*P* value = 0.002) mRNA expression level much more in MDA-MB-175-VII cells than MDA-MB-231.

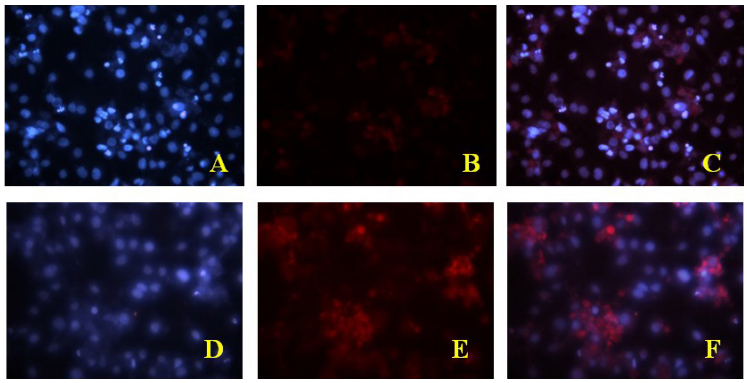

Figure 5. The ICC Results. A-C treated MDA-MB-231 cells and D-F MDA-MB-175-VII cells which treated with IC50 dose of MDA-MB-231 for 24 hours. A and D are cells nuclei stained with DAPI. B and E are cells which are stained with antibody against P21 protein-secondary antibody-FITC. Red dots which show P21 proteins expression, are much more in MDA-MB-175-VII samples compared with MDA-MB-231 (200X).

cytotoxic properties.[13] *H. perforatum* is one of the medicinal plants with numerous proven therapeutic effects.[12,27,28] Hypericin is the active ingredient of this plant, and some studies have reported its efficacy in the treatment of some cancers.[12,27-29] Although the mechanism of action of hypericin in the treatment of cancers is not clear, it is believed that this substance affects cell survival and death in several cell lines.[12]

The results of this study showed that hypericin has significant cytotoxic effects on MDA-MB-231 cancer cell line. Accordingly, IC50 for this extract was reported 2 µg/mL 24 hours after exposure, while it was reported 2 µg/mL 48 hours after exposure. The results indicated a high cytotoxic effect of hypericin on MDA-MB-231 cancer cells, and also a significant decrease in IC50 of hypericin compared with cisplatin. A very important point in previous studies is the lack of effect of hypericin on fibroblastic cells even at high doses which indicates that this substance has no effect on healthy cells.[12,14,30]

Moreover, to determine the apoptosis rate and measure cytotoxicity in this study, MDA-MB-175-VII and MDA-MB-231 cancer cells were exposed to hypericin. Flow cytometry and ICC were used, and the expression of P21 and P53 genes as determinant genes in cell survival and death, were measured through real time PCR.

The findings of this study suggest that over 86% of apoptosis is induced by hypericin exposure of MDA-MB-175-VII cancer cells to 24-hour IC50 dose of MDA-MB-231 cells. The results also indicate an increase in genetically induced cell death (P53 and P21). The results were confirmed by ICC assay on P21 protein.

Some studies have investigated the mechanism of action of hypericin in induction of cancer cell death and its cytotoxic effects. However, the exact mechanism of action of hypericin is not clear yet. For example, in a study by Jendzelovsky et al it was stated that hypericin can increase expression of 2 genes: "multidrug resistance-associated protein 1 (MRP1)" and "breast cancer resistance protein (BCRP)". These 2 genes can be related to drug resistance receptors, ABC transporter proteins and Proadifen (SKF-525A).[31] In terms of molecular structure, there is the possibility of interactions between Ca^{2+} pump SERCA and hypericin. In another study, some appropriate sites in cell membrane lipid are identified for binding to hypericin, while an important part of these sites are in the endoplasmic membrane.[32] This association increases the intracellular oxidative stress and decreases the mitochondrial membrane potential which activates caspases 3 and 9, and ultimately leads to cell death.[31] Moreover, the study of Barliya et al showed that hypericin could play a crucial role in induction and expression of hypoxia-inducible factor 1α which can remove von-Hippel Lindau protein from cancer cells, and prevent the growth of these cells.[33] According to our knowledge, no study has investigated different effects of hypericin on various breast cancer cells with wild type or mutated P53 gene.

Hamilton et al showed that low concentrations of hypericin could inhibit growth of AtT-20 and GH4C1 cell lines.[30] Mirmalek et al also reported that hypericin could have dose-dependent cytotoxic and apoptotic effects on a breast cancer cell line (MCF-7). Moreover, hypericin's caused a death rate of 52% on MCF-7 cell line. In addition, the results of this study showed that the expression of P53 and bax genes (apoptosis-inducing genes) increased after 24 hours of exposure to hypericin, and the expression of the Bcl2 gene (apoptosis-inhibiting gene) decreased. These results confirm that the induction of apoptosis and cytotoxic effects of hypericin on the studied cell line may be mediated with P53 overexpression. These results show that the cytotoxic effects of hypericin on cancer cells are better than Cisplatin. So it can be considered as an appropriate candidate in treatment of this type of cancer.[12] These findings are consistent with the results of the study by Acar et al in which hypericin increased P53 gene expression which induces cell death

and thereby increases the apoptosis rate.[29]

These findings along with similar studies, suggest that hypericin induces apoptosis by increasing the expression of cell death-inducing genes and preventing cell division by expression of cell survival genes.[12,34] However, according to the study of Roscetti et al hypericin has weak inhibitory effects on cell growth and has no cytotoxic effects on cancer cells.[28]

In conclusion, the results of this study indicate that hypericin is a cytotoxic agent that induces cell death through upregulation of P53 in MDA-MB-175-VII breast cancer cells. In addition, the cytotoxic effects of this substance on these cells are higher than that of MDA-MB-231 cells. It seems that this substance, with appropriate anticancer effects, can be suggested as a suitable treatment for cancers with wild type P53.

Authors' Contribution

MAJ contributed to the designing of the study, statistical analysis and drafting of the manuscript. NAG, MR, SGh, FSh, HZM, HA, and EG contributed to data collection and manuscript drafting. MAJ supervised the study.

Conflict of Interest Disclosures

The authors have no conflicts of interest.

Ethical Statement

Not applicable.

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References

1. Zare Marzouni H, Lavasani Z, Shalilian M, Najibpour R, Saadat Fakhr M, Nazarzadeh R, et al. Women's Awareness and Attitude Toward Breast Self-Examination in Dezful City, Iran, 2013. Iran Red Crescent Med J. 2015;17(1):e17829. doi: 10.5812/ircmj.17829.
2. Raghavendra A, Sinha AK, Le-Petross HT, Garg N, Hsu L, Patangan M, Jr., et al. Mammographic breast density is associated with the development of contralateral breast cancer. Cancer. 2017;123(11):1935-40. doi: 10.1002/cncr.30573.
3. Shahzamani K, Zare Marzouni H, Tarkhan F, Lashgarian H. A Study of Mechanism and Rate of PC12 Cancer Cell Destruction Induced by Lysine-Coated Gold Nanoparticle. Journal of Babol University of Medical Sciences. 2016;18(8):41-7. doi: 10.22088/jbums.18.8.41.
4. Schiavon G, Hrebien S, Garcia-Murillas I, Cutts RJ, Pearson A, Tarazona N, et al. Analysis of ESR1 mutation in circulating tumor DNA demonstrates evolution during therapy for metastatic breast cancer. Sci Transl Med. 2015;7(313):313ra182. doi: 10.1126/scitranslmed.aac7551.
5. Hughes R, Qian BZ, Rowan C, Muthana M, Keklikoglou I, Olson OC, et al. Perivascular M2 Macrophages Stimulate Tumor Relapse after Chemotherapy. Cancer Res. 2015;75(17):3479-91. doi: 10.1158/0008-5472.can-14-3587.
6. Liu JC, Egan SE, Zacksenhaus E. A Tumor initiating cell-enriched prognostic signature for HER2+:ERalpha- breast cancer; rationale, new features, controversies and future directions. Oncotarget. 2013;4(8):1317-28. doi: 10.18632/oncotarget.1170.
7. Miller SM, Goulet DR, Johnson GL. Targeting the Breast Cancer Kinome. J Cell Physiol. 2017;232(1):53-60. doi: 10.1002/jcp.25427.
8. Prat A, Fan C, Fernandez A, Hoadley KA, Martinello R, Vidal M, et al. Response and survival of breast cancer intrinsic subtypes following multi-agent neoadjuvant chemotherapy. BMC Med. 2015;13:303. doi: 10.1186/s12916-015-0540-z.
9. Skandalis SS, Afratis N, Smirlaki G, Nikitovic D, Theocharis AD, Tzanakakis GN, et al. Cross-talk between estradiol receptor and EGFR/IGF-IR signaling pathways in estrogen-responsive breast cancers: focus on the role and impact of proteoglycans. Matrix Biol. 2014;35:182-93. doi: 10.1016/j.matbio.2013.09.002.
10. Wei W, Tweardy DJ, Zhang M, Zhang X, Landua J, Petrovic I, et al. STAT3 signaling is activated preferentially in tumor-initiating cells in claudin-low models of human breast cancer. Stem Cells. 2014;32(10):2571-82. doi: 10.1002/stem.1752.
11. Yamamoto M, Hosoda M, Nakano K, Jia S, Hatanaka KC, Takakuwa E, et al. p53 accumulation is a strong predictor of recurrence in estrogen receptor-positive breast cancer patients treated with aromatase inhibitors. Cancer Sci. 2014;105(1):81-8. doi: 10.1111/cas.12302.
12. Mirmalek SA, Azizi MA, Jangholi E, Yadollah-Damavandi S, Javidi MA, Parsa Y, et al. Cytotoxic and apoptogenic effect of hypericin, the bioactive component of Hypericum perforatum on the MCF-7 human breast cancer cell line. Cancer Cell Int. 2015;16:3. doi: 10.1186/s12935-016-0279-4.
13. Kooti W, Servatyari K, Behzadifar M, Asadi-Samani M, Sadeghi F, Nouri B, et al. Effective Medicinal Plant in Cancer Treatment, Part 2: Review Study. J Evid Based Complementary Altern Med. 2017;22(4):982-95. doi: 10.1177/2156587217696927.
14. Javidi MA, Zolghadr F, Babashah S, Sadeghizadeh M. Introducing Dendrosomal Nanocurcumin as a Compound Capable of in vitro Eliminating Undifferentiated Stem Cells in Cell Therapy Practices. Exp Clin Endocrinol Diabetes. 2015;123(10):632-6. doi: 10.1055/s-0035-1555775.
15. Behbahani GD, Ghahhari NM, Javidi MA, Molan AF, Feizi N, Babashah S. MicroRNA-Mediated Post-Transcriptional Regulation of Epithelial to Mesenchymal Transition in Cancer. Pathol Oncol Res. 2017;23(1):1-12. doi: 10.1007/s12253-016-0101-6.
16. Javidi MA, Ahmadi AH, Bakhshinejad B, Nouraee N, Babashah S, Sadeghizadeh M. Cell-free microRNAs as cancer biomarkers: the odyssey of miRNAs through body fluids. Med Oncol. 2014;31(12):295. doi: 10.1007/s12032-014-0295-y.
17. Safarzadeh E, Sandoghchian Shotorbani S, Baradaran B. Herbal medicine as inducers of apoptosis in cancer treatment. Adv Pharm Bull. 2014;4(Suppl 1):421-7. doi: 10.5681/apb.2014.062.
18. Bartos M, Tropek R, Spitzer L, Padysakova E, Jansta P, Straka J, et al. Specialization of pollination systems of two co-flowering phenotypically generalized Hypericum species (Hypericaceae) in Cameroon. Arthropod Plant Interact. 2015;9(3):241-52. doi: 10.1007/s11829-015-9378-8.
19. Cirak C, Radusiene J, Jakstas V, Ivanauskas L, Seyis F, Yayla F. Secondary metabolites of seven Hypericum species growing in Turkey. Pharm Biol. 2016;54(10):2244-53. doi: 10.3109/13880209.2016.1152277.
20. Yang HW, Chung M, Kudo T, Meyer T. Competing memories of mitogen and p53 signalling control cell-cycle entry. Nature. 2017;549(7672):404-8. doi: 10.1038/nature23880.
21. Merkle FT, Ghosh S, Kamitaki N, Mitchell J, Avior Y, Mello C, et al. Human pluripotent stem cells recurrently acquire and expand dominant negative P53 mutations. Nature. 2017;545(7653):229-33. doi: 10.1038/nature22312.
22. Lacroix M, Toillon RA, Leclercq G. p53 and breast cancer, an update. Endocr Relat Cancer. 2006;13(2):293-325. doi: 10.1677/erc.1.01172.
23. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin. 2016;66(1):7-30. doi: 10.3322/caac.21332.

24. Mallini P, Lennard T, Kirby J, Meeson A. Epithelial-to-mesenchymal transition: what is the impact on breast cancer stem cells and drug resistance. Cancer Treat Rev. 2014;40(3):341-8. doi: 10.1016/j.ctrv.2013.09.008.
25. Holohan C, Van Schaeybroeck S, Longley DB, Johnston PG. Cancer drug resistance: an evolving paradigm. Nat Rev Cancer. 2013;13(10):714-26. doi: 10.1038/nrc3599.
26. Song S, Xiong C, Zhou M, Lu W, Huang Q, Ku G, et al. Small-animal PET of tumor damage induced by photothermal ablation with 64Cu-bis-DOTA-hypericin. J Nucl Med. 2011;52(5):792-9. doi: 10.2967/jnumed.110.086116.
27. Momekov G, Ferdinandov D, Zheleva-Dimitrova D, Nedialkov P, Girreser U, Kitanov G. Cytotoxic effects of hyperatomarin, a prenylated phloroglucinol from Hypericum annulatum Moris subsp. annulatum, in a panel of malignant cell lines. Phytomedicine. 2008;15(11):1010-5. doi: 10.1016/j.phymed.2008.04.008.
28. Roscetti G, Franzese O, Comandini A, Bonmassar E. Cytotoxic activity of Hypericum perforatum L. on K562 erythroleukemic cells: differential effects between methanolic extract and hypericin. Phytother Res. 2004;18(1):66-72. doi: 10.1002/ptr.1369.
29. Acar M, Ocak Z, Erdogan K, Cetin EN, Hatipoglu OF, Uyeturk U, et al. The effects of hypericin on ADAMTS and p53 gene expression in MCF-7 breast cancer cells. J buon. 2014;19(3):627-32.
30. Hamilton HB, Hinton DR, Law RE, Gopalakrishna R, Su YZ, Chen ZH, et al. Inhibition of cellular growth and induction of apoptosis in pituitary adenoma cell lines by the protein kinase C inhibitor hypericin: potential therapeutic application. J Neurosurg. 1996;85(2):329-34. doi: 10.3171/jns.1996.85.2.0329.
31. Jendzelovsky R, Mikes J, Koval J, Soucek K, Prochazkova J, Kello M, et al. Drug efflux transporters, MRP1 and BCRP, affect the outcome of hypericin-mediated photodynamic therapy in HT-29 adenocarcinoma cells. Photochem Photobiol Sci. 2009;8(12):1716-23. doi: 10.1039/b9pp00086k.
32. Eriksson ES, Eriksson LA. Identifying the sarco(endo)plasmic reticulum Ca2+ ATPase (SERCA) as a potential target for hypericin--a theoretical study. Phys Chem Chem Phys. 2012;14(36):12637-46. doi: 10.1039/c2cp42237a.
33. Barliya T, Mandel M, Livnat T, Weinberger D, Lavie G. Degradation of HIF-1alpha under hypoxia combined with induction of Hsp90 polyubiquitination in cancer cells by hypericin: a unique cancer therapy. PLoS One. 2011;6(9):e22849. doi: 10.1371/journal.pone.0022849.
34. Peebles KA, Baker RK, Kurz EU, Schneider BJ, Kroll DJ. Catalytic inhibition of human DNA topoisomerase IIalpha by hypericin, a naphthodianthrone from St. John's wort (Hypericum perforatum). Biochem Pharmacol. 2001;62(8):1059-70.