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Review Article

Chrysin and its potential antineoplastic effect

Patrycja Chylińska-Wrzos, Marta Lis-Sochocka, Barbara Jodłowska-Jędrych

Chair and Department of Histology and Embryology with Experimental Cytology Unit, Medical University of Lublin, Radziwiłłowska 11, 20-080 Lublin, Poland

* Corresponding author: Patrycja Chylińska-Wrzos; Phone: 0048 81 448 61 58; E-mail: patrycja.wrzos@umlub.pl

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ABSTRACT

In 2012, in Europe, there were noticed over 3 million new cases of cancer and 1.75 million of deaths from cancer. Numerous anticancer agents are cytotoxic, can damage normal cells, and they can cause serious side effects. Currently, natural and non-toxic agents are being sought that reduce the cost of therapy, are more effective and targeted, and do not damage healthy cells. Chrysin which belong to flavonoids family as natural substance, has multiple anticancer activities. It has been reported that chrysin can induce apoptosis in tumour cells by different mechanism. In our work we demonstrated the potential use of chrysin in gastrointestinal, breast, cervical, and lung cancer. In conclusion it is proven that chrysin or combination of chrysin with other related drugs can effectively improve the effectiveness of anticancer therapy. Furthermore, new agents, such as nanoparticles, may show greater efficacy, and better targeting, hence, less side effects on healthy cells. Based on these results, nanochrysin it offers as new and effective drug delivery system. Moreover, it has been reported that chrysin is a potential antitumor but also an adjuvant agent that can be used in combination with other antimetastatic substances to reduce tumor metastasis.

Keywords: Chrysin; Flavonoids; Propolis; Anticancer activity.

1. INTRODUCTION

Propolis, or 'bee glue', is natural sticky plant product created by bees which colour varies from yellowish-green to dark brown, depended from its origin and age [1-3]. Propolis can be used as a built material and as biological weapon because of its antibacterial, antifungal, antiviral, cytotoxic, anti-oxidant, anti-inflammatory and immunomodulatory effects [4-9].

The chemical composition of propolis is complex and very different, as well as being dependent upon the geographic region, botanical origin and collecting bee species [1, 7, 10]. These factors have influence on its biological activity. In most European countries, propolis is collected by bees from black poplar, birch, alder, pine and willow species buds [2, 7]. Each source generates a different propolis. In the most common types, such as poplar propolis, flavones, flavanones, phenolic acids and their esters, predominate. In the birch propolis, flavones and flavonols (but not the same as in the poplar type) dominate. In the green propolis, we find mainly prenylated p-coumaric acids and diterpenic acids, while in red propolis, we see polyprenylated benzophenones [1].

Researchers working between 2000 and 2012, identified about 300 compounds in the various propolis, including flavonoids, terpenes, phenolics and their esters, lipid-wax substances, beeswax, sugars, hydrocarbons and mineral elements, vita-

mins, proteins, and amino acids [2, 10]. In general, however, raw propolis is composed of waxes (30%), resins and vegetable balsam (50%), essential and aromatic oils (10%), pollens and other substances (5%) [6, 7, 9, 10].

The flavonoids are a major chemical component of propolis. These flavonoids are a group of polyphenols with different structures and properties [2]. Flavonoids can be found in fruits, vegetables, grains, nuts, seeds, tea and herbs. In the plants, flavonoids are concentrated mainly in the leaves and flowers [11-14]. Chemically, they have structure of 15 carbon atoms, described as C6-C3-C6, with a benzoic ring and a phenylopropane unit [2, 11, 14-16]. The double band between C2 and C3 in the C ring influences the antioxidative activity of the flavonoids. This can be affected by way of glycolysation at position C3 [2, 11, 14, 17]. In accordance to the chemical structure, flavonoids may be classified into: flavones, flavonols, flavanones, flavanonols, chalcones, dihydrochalcones, isoflavones, isodihydroflavones, flavans,isoflavans, and neoflavonoids [10, 14, 16].

Chrysin (5,7-dihydroxyflavone or 5,7-dihydroxy-2-phenyl-4Hchromen-4-one) is one of the flavones which can be found in passion flower (*Passifloracaerulea),* in honey, in propolis and in bee pollen (Fig 1.) [12, 17, 18]. The chemical structure of chrysin, with the presence of a double band between C2-C3 in ring C, and the lacking of oxygenation at C3 (Fig 1.), is associated with numerous pharmacological properties. These include anti-microbial, anti-inflammatory, anti-spasmodic, anxiolytic, anthelmintic, anti-cancer, hypoglycemic, antiatherogenic, and anti-HIV [12, 14, 18-21].

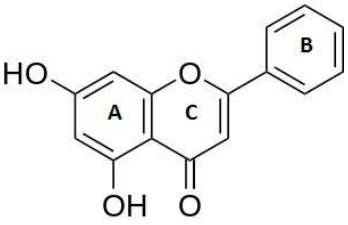

Figure 1. Structure of chrysin.

The effectiveness of all drugs and natural substances is dependent of their bioavailability and the solubility of these agents, but is also associated with their cytotoxicity [12]. Chrysin has a relatively low solubility and is poorly absorbed in the intestine which may limit its bioavailability and made it less useful during any therapy [22, 23]. The cytotoxicity of chrysin is dependent of used dosage [24].

In 2012, in Europe, there were noticed over 3 million new cases of cancer and 1.75 million of deaths from cancer. The most common types of cancer and most common causes of death were: female breast, colorectal, prostate and lung cancers [25]. Cancer as a multifactorial disease, may have a genetic background and be caused by harmful environmental factors [26, 27]. Numerous anti-cancer agents are cytotoxic, can damage normal cells, and they can cause serious side effects [26]. Currently, natural and non-toxic agents are being sought that reduce the cost of therapy, are more effective and targeted, and do not damage healthy cells. Flavonoids as natural substances, are regarded as safe and easy to obtain, so they are good candidates to anticancer therapy in clinical treatment [13].

The multiple anticancer activities of chrysin has drawn our attention. It has been reported that chrysin can induce apoptosis in different tumour cells [21, 26, 28-30], inhibit cell proliferation and block the cell cycle [31]. Moreover, it can activate notch 1 signalling [32], and it is a histone deacetylase inhibitor which can significantly inhibit tumour growth [33]. However, Song et al. [21] report that the addition of amino acids reduce the anti-cancer activity of chrysin.

In this review we looked for potential antineoplastic action of chrysin or chrysin in combination with other active substances in selected cancer diseases. In addition, evaluating and investigating the pathway and mechanism of action of this substance on cancer cells may be of great importance when planning antineoplastic therapy.

2. USE OF CHRYSIN IN SELECTED CANCER DISEASES

2.1. Chrysin in gastric cancer

According to published statistics, gastrointestinal cancers are one of the leading causes of cancer deaths in the world. One of the major clinical

problems is the late recognition and lack of effective antineoplastic therapy [25, 34].

Chrysin and triphenylgermanium Bromide (Chry-Ge) induced apoptosis in Colo205 cells by way of the intrinsic pathway. Moreover, it led to the reorganization of cytoskeleton and was evidenced of damaging the nucleus in Colo205 cells [35]. Other authors have investigated the effects of chrysin on MMP-9 (matrix metalloproteinase-9) expression and activity in AGS gastric cancer cells. In such studies, they found that chrysin can decrease cancer invasiveness in cells by controlling MMP-9 expression through the suppression of the JNK/c-Jun and ERK/c-Fos signaling pathways [36]. Moreover, chrysin can suppress RON (Recepteur d'origine Nantais) in the AGS cells, which, as a consequence, decreases cell invasion [37]. The phenolic compounds in New Zealand propolis, as well as chrysin alone, showed anti-proliferative and anti-inflammatory assays against three gastro-intestinal cancer cell lines; HCT-116 colon carcinoma, KYSE-30 oesophageal squamous cancer, and NCI-N87 gastric carcinoma [38]. In the Leòn et al. [39] study, the authors investigated the mechanisms of action of two flavonoids:silibinin (VOsil), and chrysin (VOchrys) in a human colon adenocarcinoma cell line (HT-29). Their results indicated that the complexation of the flavonoids inhibited the viability of HT-29 cells in a dose dependent manner. Moreover, the anticancer effects of VOchrys were mediated by a decrease of the GSH (glutathione) levels and by cell cycle arrest.

Chrysin treatment induced Tnfα and Tnfβ gene expression and activated multiple TNF-mediated signaling pathways in Colon (HCT116, DLD1) and rectal (SW837) cancer cell lines leading to apoptosis. In addition, it has been suggested that this comes about by way of a novel pathway in which the transcriptional factor AHR (Aryl Hydrocarbon Receptor) is required. The cell viability in all cell lines was decreased at 50 μM and 100 μM chrysin [40]. In HCT116 cells, chrysin induced cell death by DNA damage dependent of used dosage, as well as by mitochondrial membrane perturbation accompanied by cytochrome c release, down-regulation of Bcl-2, the activation of BID and Bax, and caspase-3 activation [41]. In related work, Mohammadian et al. [42] revealed that chrysin inhibits the growth of the AGS human gastric cell line. In this study, the authors used a PLGA-PEG-chrysin complex, as well as free chrysin. Herein, the value of inhibitory concentration 50 (IC50) was calculated for each case. Their results showed that the IC50 value was significantly decreased in nanocapsulatedchrysin, in comparison with free chrysin. This finding directly indicated that capsulated chrysin is more effective than free.

In the Li et al. study [43], the authors investigated the influence of the combination of chrysin and cisplatin on Hep G2 cancer cells, and saw increased apoptosis in Hep G2 cancer cells. Furthermore, the combination of chrysin and cisplatin treatment increased the expression of proapoptotic proteins (p53, Bax, and DR5), while it decreased the expression of the antiapoptotic protein Bcl-2. In addition, the combination of chrysin and cisplatin promoted both extrinsic and intrinsic apoptosis pathways by activating caspase-8 and caspase-9 in the Hep G2 cells. Similar results were obtained by Zhang et al. [30] and Huang et al. [44]. Huang et al. [44] used chrysin combined with apigenin and observed that this combination can reduce HepG2 and MDA-MB-231 proliferation and cell motility, as well as induce apoptosis.

2.2. Chrysin in female reproductive system

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among women [25, 45]. Among the cancers of the female reproductive system, however, cervical, endometrial and ovarian cancers are predominant. Indeed, cervical cancer is the third most common type among women, worldwide [46].

Lirdprapamongkol et al. [47] analyzed the effect of chrysin and tectochrysin on hypoxic survival of 4T1 mouse breast cancer cells in vitro. After application of 40-100 μM of chrysin, they saw a decrease in hypoxic cell survival, while the application of tectochrysin in a 60 μM concentration did not show any changes. Because tumor hypoxia is correlated with metastasis, the authors also examined the influence of chrysin on the 4T1 cell line, via the spontaneous lung metastasis model. They found out that administration of 100 and 250 mg/kg of chrysin by day, did not show any significant effect on primary tumor growth, but, the number of metastatic colonies in the lung was

decreased, as was the total number of metastases, while the size of metastases were significant suppressed in a dose-dependent manner.

It was showed that 5,7-dihydroxy-8-nitro-chrysin (NOC), a novel synthetic chrysin analog, induce apoptosis in MDA-MB-453 human breast cancer cell line intrinsically, via activation of caspase-9. In the tested cells, apoptosis were induced by activation of the Akt/FOXO3a axis (Forkhead box O3a transcription factor), with increased Bim (B cell lymphoma 2 (Bcl-2) - aninteracting mediator of cell death) expression [48]. Similar results were seen in a 2014 follow-up study. Herein, the authors reported that LW-214 (a new flavonoid sourced from chrysin), activated the intrinsic mitochondrial apoptotic pathway in human breast cancer MCF-7 cells. However, after 24 h, decreased expression of Bcl-2 and increased expression of Bax was observed, in a dose-dependent manner [49]. Zhao et al. [48] also used a nude mice model bearing an inoculated MCF-7 tumor to determine the influence of LW-214 in vivo. In this experiment, they saw that LW-214 inhibited tumor growth. In H&E staining, noted no morphological changes observed in the organs, and no significant difference was seen in the average body weight of mice treated by LW-214, compared with a control group. In conclusion, it suggest that LW-214 has anticancer effects in the MCF-7 cell line in vivo and in vitro [48, 49].

Another type of a new chrysin analog, 8-bromo-7-methoxychrysin (BrMC), induced intrinsic apoptosis in a time-dependent manner, via the Akt/FOXO3a axis in cisplatin (DDP)-sensitive (A2780) and -resistant (A2780/DDP) ovarian cancer cell lines. The effect of BrMCis greater that natural chrysin [50].

Mohammadinejad et al. [51], on a T47D breast cancer cell line, examined the effect of encapsulated chrysin in PLGA-PEG (poly (D, L-lactic-co-glycolic acid) and poly (ethylene glycol) as compared to pure chrysin. They saw that loaded chrysin in PLGA-PEG increases its solubility and tolerance, and decreases the side effects of the drug. In addition, the authors observed that pure chrysin and chrysin nanoparticles inhibited cell proliferation in a dose dependent manner. As a result of such, they suggested that placing the chrysin into nanoparticles improves its effectiveness on cell growth inhibition, and it strongly decreases the cyclin D1 expression. This study was continued by Eatemadi et al. [52] and Anari et al. [53]. Anari et al. [53] evaluated the cytotoxicity of chrysin nanoparticles and pure chrysin on two human breast cancer cell lines: T47D and MCF7. They confirmed the results of Mohammadinejad et al. [51] in that nanochrysin has a positive effect on the breast cancer cell lines (T47D and MCF7) in a dose-dependent and time-dependent manner. Still, they noted that the MCF7 cell line is less sensitive to chrysin than is the line T47D. Eatemadi et al. [52] have also shown that nanochrysin has a time-dependent cytotoxic effect, plus they found that it increased the expression of the BRCA1 gene and reduced the expression of the hTERT and FTO genes in the T47D cell line. By the way, chrysin inhibited the migration and invasion of MDA-MB-231 and BT-549 cell lines by way of the down-regulation of MMP-10 (matrix metalloproteinase-10). In both lines, MDA-MB-231 and BT-549, chrysin treatment increased the expression of E-cadherin, while it decreased the expression of vimentin, snail and slug. This suggests that chrysin has a reversal effect on the epithelial-mesenchymal transition [54].

Co-treatment therapy with chrysin and 1,2,3,4,6-penta-O-galloyl-b-D-glucose (5GG) induced apoptosis, cell cycle arrest, inhibited cell proliferation and colony formation in AU565 and MDA-MB-231 human breast cancer cells. The combination of chrysin and 5GG decreased the growth of tumor by down-regulation of the phospho-LRP6 (pLRP6) and Skp2 proteins [55]. Another combination of chrysin, chrysin and apigenin reduced cell viability and cell motility, as well as induced apoptosis in a dose- and time-dependent manner in MDA-MB-231 breast cancer cell line. Herein, co-treatment for 36 h synergistically decreased cell line motility but not viability, but significant cytotoxicity was observed for 72 h of co-treatment [44].

In another work, the authors examined the anticancer activity of *S. discolor* (*Scutellaria discolor* Colebr., SDE) on different cancer cell lines, and they isolated the substance which is responsible for this action. They discovered that SDE induced cell death in a concentration dependent-manner by up-regulation of APAF1, BAX, BCL2L11, caspase

9, DFFA, GADD45A and TP53, and increased the expression of caspase-3mRNA. In their work, they saw that the stronger effects were observed in the cervical cancer cell line, HeLa. This result was confirmed by utilizing the lines ME180 and Bu25TK. In addition, spectroscopic methods indicated that chrysin was the major compound of SDE that had showed the antiproliferative activity [56].

2.3. Chrysin in respiratory system

According to data, lung cancer is one of the leading causes of cancer death in developed countries [25, 57].

Shao et al. [58] reported that chrysin induces growth inhibition and apoptosis in the A549 cultured lung cancer cells. They also put forward that the actuation of AMP-activated protein kinase (AMPK) may have contributed to this process, as their Western-blot analysis results demonstrated a significant AMPK activation after chrysin treatment in A549 cells. Moreover, inhibition of AMPK by shRNA-mediated gene silencing, or by its inhibitor, diminished chrysin-induced A549 cell growth inhibition and apoptosis. Furthermore, forced activation of AMPK by introducing a constitutively active form of AMPKa (CA-AMPKa), or by its activators, mimicked the chrysin effect. In addition, as they found that chrysin inhibited the Akt/mammalian target of rapamycin (mTOR) activation, knocking down of AMPK by shRNA almost reversed this effect. Finally, they observed that a relative low dose of chrysin enhanced doxorubicin-induced AMPK activation, hence promoting A549 cell apoptosis.

Kasala et al. [59] investigated the chemo-preventive role of chrysin against benzo(a)pyrene [B(a)P] induced lung carcinogenesis in Swiss albino mice. In their work, they administered B(a)P orally (50 mg/kg body weight) twice a week for four weeks to induce lung cancer in the test mice. They reported that administration of B(a)P resulted in increased lipid peroxides and carcinoembryonic antigens, with concomitant decrease in the levels of both enzymatic and non-enzymatic antioxidants. Chrysin supplementation down-regulated the expression of PCNA, COX-2 and NF-kB and maintained cellular homeostasis. This confirmed the chemopreventive potential of chrysin against B(a)P induced lung cancer in Swiss albino mice [59].

In A549 cells, in vitro, Lim et al. [60] investigated the combination of chrysin and docetaxel (DTX). As a result of this study, they saw increased cytotoxicity, suppressed cellular proliferation and induced apoptosis in the post-treatment of chrysin following prior DTX treatment. Moreover, in vivo, chrysin enhanced the tumor growth delay activity of DTX and increased DTX-induced apoptosis by way of the A549-derived xenograft model. Furthermore, chrysin prevented DTX-induced edema in ICR mouse-subjects. These results indicate that chrysin administration strengthened the therapeutic efficacy of DTX and diminished the adverse effect of DTX. This outcome suggests that chrysin could be exploited as an adjuvant therapy for NSCLC.

Brechbuhlf et al. [61] reported that treatment with chrysin resulted in significant and sustained intracellular flavonoid-induced glutathione (GSH) depletion. What is more, the GSH enzyme network in the four cancer cell types was predictive of the severity of chrysin-induced intracellular GSH depletion. Their gene expression data also indicated a positive correlation between basal MRP1, MRP3 and MRP5 expression, and total GSH efflux before and after chrysin exposure. In addition, Brechbuhlf et al. [61] saw that in all the four investigated cell lines, co-treating the cells for 72 hours with chrysin (5-30 μM) and doxorubicin (DOX) (0.025-3.0 μM) significantly enhanced the sensitivity of the cells to DOX, as compared to 72-hour DOX alone treatment. In this experiment, the maximum decrease in the IC50 values of cells treated with DOX alone compared to co-treatment with chrysin and DOX was 43% in A549 cells, 47% in H157 and H1975 cells and 78% in H460 cells. Hence, chrysin worked synergistically with DOX to induce cancer cell death. This approach could allow for use of lower concentrations of applied chemo-therapy agents, by sensitizing cancer cells that are typically resistant to therapy to such agents.

Moreover, propolis extract and chrysin sensitizes A549 human lung adenocarcinoma and HeLa human cancer cell lines to TRAIL-induced apoptosis. Moreover, the TRAIL sensitization effect of chrysin is not mediated by inhibition of TRAIL-induced NF-κB activation or by glutathione

depletion. In actuality, immunoblot analysis using a panel of anti-apoptotic proteins, revealed that chrysin selectively decreases the levels of Mcl-1 protein, by down-regulating Mcl-1 gene expression as determined by qRT-PCR. The contribution of Mcl-1 in TRAIL resistance was confirmed by si-Mcl-1 knockdown. Indeed, among the signaling pathways that regulate Mcl-1 gene expression, only that constitutive of STAT3 phosphorylation was suppressed by chrysin. The proposed action of chrysin in TRAIL sensitization by inhibiting STAT3 and down-regulating Mcl-1 was supported by using a STAT3-specific inhibitor, cucurbitacin-I, which decreased Mcl-1 levels and enhanced TRAIL-induced cell death, in a manner similar to that observed with chrysin treatment [62].

Narayan and Kumar [63] explored the antineoplastic and immunomodulatory effects of chrysin (derived from an extract of *Achyranthes aspera*) (PCA) on urethane-induced lung cancer in vivo. In the study, PCA was fed orally to urethane (ethyl carbamate) primed lung cancerous mice at a dosage of 100 mg/kg body weight for 30 consecutive days. Herein, the enhanced activity and expression of the antioxidant enzymes GST, GR, CAT, SOD, as well as down-regulation of expression and activation of LDH enzymes in PCA were observed. What is more, PCA fed urethane-primed lung tissues showed down-regulated expression of the pro-inflammatory cytokines IL-1b, IL-6 and TNF, along with that of TFs, NF-jB and Stat3, while the expression of the proapoptotic proteins Bax and p53 was enhanced. In related experimental work, FTIR and CD spectroscopy data revealed that PCA resisted the urethane mediated conformational changes of DNA. This was made evident by the shift in guanine and thymine bands in FTIR, from 1,708 to 1,711 cm^{-1} and 1,675 to 1,671 cm^{-1}, respectively. The present study suggests that PCA components have a synergistic anti-cancerous and cytokine based immunomodulatory roll. Moreover, they have DNA conformation restoring effects.

Table 1. The type of active substances and the cell line on which they act.

Substances	**Cell lines of the digestive system**
Chrysin	Colo205, AGS, HCT-116, KYSE-30, NCI-N87, HT-29, DLD1, SW837
Chrysin and triphenylgermanium Bromide (Chry-Ge)	Colo205
Silibinin (VOsil)	HT-29
PLGA-PEG-chrysin complex	AGS
Chrysin and cisplatin combination	HepG2
Chrysin and apigenin combination	HepG2
Substances	**Cell lines of the female reproductive system**
Chrysin	4T1, T47D, MCF7, MDA-MB-231, BT-549
Chrysin and apigenin combination	MDA-MB-231
5,7-dihydroxy-8-nitrochrysin (NOC) (synthetic chrysin analog)	MDA-MB-453
LW-214 (flavonoid sourced from chrysin)	MCF-7
8-bromo-7-methoxychrysin (BrMC) (chrysin analog)	A2780, A2780/DDP
PLGA-PEG-chrysin complex	T47D, MCF7
Chrysin and 1,2,3,4,6-penta-O-galloyl-b-D-glucose (5GG)	MDA-MB-231, AU565
Scutellaria discolor Colebr., SDE*	HeLa, ME180, Bu25TK
Substances	**Cell lines of the respiratory system**
Chrysin	A549, lung cancer in Swiss albino mice induced by benzo(a)pyrene [B(a)P], urethane-induced lung cancer in vivo
Chrysin and docetaxel combination	A549
Chrysin and doxorubicin combination	A549, H157, H1975, H460

*Chrysin is a major compound of SDE with antiproliferative activity

3. CONCLUSION

The mechanism of action of chrysin is based on the induction of apoptosis in tumor cells, whereas in the initiation of this process various proteins and enzymes may be involved i.a.Bax, Bcl-2, caspases: 3, 8 and 9, p53 protein, and cytochrome C. In addition, chrysin exhibits an anti-inflammatory and anti-proliferative effects, it inhibited cancer cells growth and also reduces viability and motility of various tumor cells. Numerous studies have shown that the use of chrysin, chrysin analogues or chrysin combinations and other related drugs can effectively improve the effectiveness of anticancer therapy (Table 1.). Furthermore, new agents, such as nanoparticles, may show greater efficacy, and better targeting, hence, less side effects on healthy cells (Table 1.). Based on these results, nanochrysin offers new and effective drug delivery system. Moreover, it has been reported that chrysin is a potential antitumor but also an adjuvant agent that can be used in combination with other antimetastatic substances to reduce tumor metastasis.

AUTHORS' CONTRIBUTION

PC-W: concept of the work, collection and analysis of literature, text translation, wrote the manuscript. ML-S: collection and analysis of literature, preparation of literature, wrote the manuscript. BJ-J: critical evaluation of work, edited the manuscript. The final manuscript has been read and approved by all authors.

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The authors declare that they have no competing interests.

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