FOLIA HISTOCHEMICA
ET CYTOBIOLOGICA
Vol. 50, No. 1, 2012
pp. 25–37

REVIEW

VIA MEDICA

The anticancer activity of propolis

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Abstract: Propolis and its compounds have been the subject of many studies due to their antimicrobial and anti-inflammatory activity; however, it is now known that they also possess antitumor properties. This review aims to summarize the results of studies on the mechanism of activity of propolis and its active compounds such as CAPE and chrysin in the apoptotic process, and their influence on the proliferation of cancer cells. Our review shows that propolis and its presented compounds induce apoptosis pathways in cancer cells. The antiproliferative effects of propolis, CAPE or chrysin in cancer cells are the result of the suppression of complexes of cyclins, as well as cell cycle arrest. The results of *in vitro* and *in vivo* studies suggest that propolis, CAPE and chrysin may inhibit tumor cell progression and may be useful as potential chemotherapeutic or chemopreventive anticancer drugs. *(Folia Histochemica et Cytobiologica 2012, Vol. 50, No. 1, 25–37)*

Key words: cancer, propolis, CAPE, chrysin, apoptosis, proliferation

Introduction

Propolis, also called bee putty or bee glue, is a substance produced by bees from the resin collected from trees and shrubs, which combines with beeswax and secretions from the bee's salivary glands rich in enzymes. It can be yellow, brown or almost black, depending on the plants from which the resinous substance is collected. The smell of propolis is intense and aromatic [1].

The use of propolis by humans has a long history. The Egyptians used it for embalming the body because it was the perfect plastic material that further protected the mummy from bacteria, fungi and viruses [2]. Propolis has been the subject of many studies due to its antibacterial, antifungal [3–11], antiviral [12–14] and hepatoprotective activity. Water- or alcohol-soluble propolis and its many compounds have been used in the treatment of inflammation, for immunostimulation [11], and as an anticancer agent. The above-mentioned properties of propolis make it an unusual material of natural origin, characterized by a specific composition.

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Chemical composition of propolis

The diversity of propolis's chemical composition is presented in Table 1. In general, the main components of propolis are fatty, aliphatic and aromatic acids, flavonoids, alcohols, terpenes, sugars and esters. Several studies have confirmed the differences in percentages of individual components of propolis, depending on the origin of the plants from which the resin is collected [15, 16] and the species of bees [17].

In vitro studies

The influence of propolis and its compounds on the apoptotic process in cancer cells

The positive effect of anticancer therapy is seen in the ability to initiate apoptosis in cancer cells [26]. Apoptosis is a genetically regulated cell death. In general, there are two main pathways of apoptosis.

Table 1. Chemical composition of propolis based on: [18–25]

Compounds (percentage of content)		
Fatty and aliphatic acids (24–26%)	**Flavonoids (18–20%)**	**Microelements (0.5–2.0%)**
Butanedioic acid (Succinic acid)	Astaxanthin	Aluminum (Al)
Propanoic acid (Propionic acid)	Apigenin	Copper (Cu)
Decanoic acid (Capric acid)	Chrysin	Magnesium (Mg)
Undecanoic acid	Tectochrysin	Zinc (Zn)
Malic acid	Pinobanksin	Silicon (Si)
D-Arabinoic acid	Squalene	Iron (Fe)
Tartaric acid	Pinostrobin chalcone	Manganese (Mn)
Gluconic acid	Pinocembrin	Tin (Sn)
a-D-Glucopyranuronic acid	Genkwanin	Nickel (Ni)
Octadecanoic acid (Stearic acid)	Galangin	Chrome (Cr)
Hexadecanoic acid	Pilloin	
b-D-Glucopyranuronic acid	Acacetin	
9,12-Octadecadienoic acid	Kaemferide	
Tetradecanoic acid	Rhamnocitrin	
Pentanedioic acid	7,4'-dimethoxyflavone	
Glutamic acid	5-hydroxy-4'7-dimethoxyflavone	
2,3,4-trihydroxy butyric acid	5,7-dihydroxy-3,4'dihydroxyflavone	
Phosphoric acid	3,5-dihydroxy-7,4'-dimethoxyflavone	
Isoferulic acid	**Sugars (15–18%)**	**Others (21–27%)**
	Sorbopyranose	Cyclohexanone
	D-Erythrotetrofuranose	3-methyl,antitricyclo undec-3-en 10-one
	D-Altrose	Cyclohexane
	D-Glucose	Cyclopentene
	Arabinopyranose	5-n-propyl-1,3 dihydroxybenzene
	d-Arabinose	Butane
	a-D-Galactopyranose	2(3H)-Furanone
	Maltose	L-Proline
	a-D-Glucopyranoside	2-Furanacetaldehyde
	D-Fructose	2,5-is-3-phenyl-7-pyrazolopyrimidine
Aromatic acids (5–10%)	**Esters (2–6%)**	Cliogoinol methyl derivative
Benzoic acid	Caffeic acid phenethyl ester	Fluphenazine
Caffeic acid	4,3-Acetyloxycaffeate	4,8-Propanoborepinoxadiborole
Ferulic acid	Cinnamic acid,	1,3,8-Trihydroxy-6-methylanthraquinone
Cinnamic acid	3,4 dimethoxy-trimethylsilyl ester	1-5-oxo-4,4-diphenyl-2-imidazolin-2-yl guanidine
	3-Methoxy-4-cinnamate	3,1,2-Azaazoniaboratine/Piperonal
	Cinnamic acid 4 methoxy 3 TMS ester	3-Cyclohexene
	2-propenoic acid methyl ester	1H-Indole
Alcohol and terpens (2–3.3%)	**Vitamins (2–4%)**	1H-Indole-3-one
Glycerol	A, B_1, B_2, E, C, PP	2-Furanacetaldehyde
Erythritol		Guanidine
a-Cedrol		2(3H)Furanone
Xylitol		1,3,8-trihydroxy-6-meyhylanthraquinone

Table 1. Chemical composition of propolis based on: [18–25] cont.

Alcohol and terpens (2–3.3%)
Germanicol
Stigmast-22-en-3-ol
Farnesol
Pentitol
Ribitol
Vanilethanediol
Bicyclohept-3-en-2-ol

The first is induced by an external signal (extrinsic) stimulated by receptors of tumor necrosis factor (TNF): Fas (TNF receptor superfamily, member 6), TRAIL-R1 and R-2 (TNF-related apoptosis-inducing ligand-R1 and R2).

The second pathway (intrinsic) is mediated by mitochondria and pro-apoptotic proteins including cytochrome c [27]. Apoptosis induction is one of the mechanisms proposed for the therapeutic effects of propolis [28, 29]. The mechanism of apoptosis induced by propolis seems dependent on the kind of compounds and the concentration of the propolis extract. Recent studies suggest that the astaxanthin and flavonoids in propolis can protect SH-SY5Y cells from beta-amyloid (A beta) (25-35) induced apoptotic death [23].

The effect of propolis on the apoptotic process in cancer cells

In vitro studies show different sensitivities of tumor cells to extracts of propolis in the context of apoptosis.

The antitumor effect of water-soluble derivatives of propolis (WSDP) from Croatia and Brazil on mammary carcinoma cells (MCA), human epithelial carcinoma cell line (HeLa), and Chinese hamster lung fibroblast cells (V79) have been studied by Orsolic and Basic [30]. Their study showed that the percentage of apoptotic MCA cells increased from 20% (in controls) to 24% and 26% after exposure to 50 μg/ml of Brazilian and Croatian propolis, respectively. The percentage of apoptotic HeLa cells (2% in controls) was 10% for Croatian propolis and 9.5% for Brazilian propolis. However, the percentage of apoptotic V79 cells treated with both Brazilian and Croatian propolis was smaller than in non-treated cells. These results indicate different degrees of sensitivity to propolis among cancer cells and normal fibroblasts.

Similarly, a study by Orsolic et al. [31] showed a slight increase in the percentage of apoptotic MCa cells from 0.56% after 3 h to 6.02% after 15 h of incubation with tested WSDP (50 μg/ml) and, within the same two periods of time, a significant increase in the level of cells necrosis was observed. The authors suggested that WSDP induced low apoptotic effect on MCa cells may be the result of complexity, i.e. the large number of components might have an antagonistic effect on one another.

A more significant (2.1–40.1%) proapoptotic effect of propolis (in the concentration-dependent manner: 0.015–0.5 μl/ml) has been observed in human histiocytic lymphoma cells (U937) [29]. In the same concentration range, propolis also inhibited cell growth in the studied cells.

Antitumor activity of ethanol extract of propolis (EEP), which is one of the richest sources of phenolic acids and flavonoids from Turkey, on the percentage of TUNEL positive human breast cancer cell line MCF-7, shows that apoptosis induction is strongly dependent on the concentration and dilutions of EEP. In the antitumor activity, dilutions of EEP 0.125 and 0.063 mg/ml were more effective (17.5–100%) than dilutions of EEP 0.25 and 0.5 mg/ml (5.11–18.97%) [32].

These observations are of great interest, and many researchers have tried to specify the mechanism of the proapoptotic effect of propolis. Aso et al. [29] were the first to show that propolis induces apoptosis through activating caspase-dependent pathway. They investigated the effect of caspase inhibitor Z-Asp-CH_2-DCB on the DNA fragmentation stimulated by propolis (0.1 μl/ml). Complete prevention of DNA fragmentation of U937 cells and J447.1, P388, HL-60 and Jurkat leukemia cell lines suggests that such an effect is not cell-specific [29].

The study by Motomura et al. [33] indicates that methanol extracts of propolis (300 μg/ml and 500 μg/ml) increase apoptosis in U937 cells due to the activation of caspase-3 and down-regulation of Bcl-2 protein. The overactivity of caspase-6 induced by EEP is stronger than the induced activity of caspase-8 and -9 in MCF-7 cells, which confirms the involvement of intrinsic caspase pathway of apoptosis and the antitumor activity of propolis [32]. Additionally, the above observation is in line with results of a study in which propolis induced apoptosis through the release of cytochrome c from mitochondria to the cytosol and through activating caspase-3 in human leukemia HL-60 cells in a concentration-dependent manner (3–50 μg/ml for cytochrome c and 10–50 μg/ml for caspase-3) [34]. The cell death induced by propolis was largely prevented by treating HL-60 cells with 30 μM z-DEVD-fmk, a caspase-3 inhibitor [23].

The extrinsic pathway of apoptosis was also induced by propolis. Results obtained by Szliszka et al. [35] indicate that EEP (50 μg/mL) markedly augmented tumor necrosis factor related apoptosis inducing ligand (TRAIL) in HeLa cell line. TRAIL, as a member of TNF superfamily, induces programmed death in cancer cells through its interaction with the death-domain containing receptor TRAIL-R1 (death receptor 4 — DR4) and/or TRAIL-R2 (death receptor 5 — DR5) [36, 37].

The mechanism of propolis-induced apoptosis appears to be independent of the kind of cancer cells studied, but dependent on the concentration of propolis extract. Studies available in the literature indicate that propolis induces apoptosis through the release of cytochrome c from mitochondria to the cytosol, through the caspase cascade and TRIAL signal. All these effects of propolis are summarized in Figure 1.

Despite the results on the proapoptotic activity of propolis, the study by Nadia et al. [38] demonstrates the antiapoptotic effect of propolis. Induced permeability transition pore (PTP) opening in the rat liver mitochondria after exposure to ferulenol, a sesquiterpene prenylated coumarin derivative isolated from the plant Ferula vesceritensis [38], was restored by propolis and in this way the apoptotic process was prevented. Since opposite effects of propolis on apoptosis have been published, further studies are necessary to understand the exact influence of propolis on the apoptotic pathways in cancer cells.

The effect of propolis compounds on apoptosis pathways in cancer cells

The search for active compounds of propolis led to the extraction of CAPE and chrysin, which are believed to be mainly responsible for the antitumor therapeutic activities of propolis. The cancer inhibitory effects of CAPE and chrysin have been confirmed in a variety of culture cell lines.

The major activity of propolis is the result of the presence of CAPE in propolis. A particularly high concentration of this compound was found in the New Zealand propolis called BIO 30. Previous studies have shown the usefulness of CAPE through its anti-inflammatory [39–41], immunostimulatory [31, 42], and antitumor activity.

CAPE exhibits strong antitumor effects in oral cancer cells: fibroblasts from oral submucous fibrosis (OSF), neck metastasis of Gingiva carcinoma (GNM) and tongue squamous cell carcinoma (TSCCa) [43]. Orsolic et al. [31] observed significant enhancement of apoptosis to 31.24% in MCa cells with CAPE (5 or 10 μg/ml) after 15 h of incubation.

To understand the pathways involved in the proapoptotic effect of CAPE, many studies have been conducted. In most of them, increased activity of caspase-3 or caspase-7 was presented in various types of cancer cells: HL-60, CI41, U937, human ovarian carcinoma SK-OV-3, human lung carcinoma

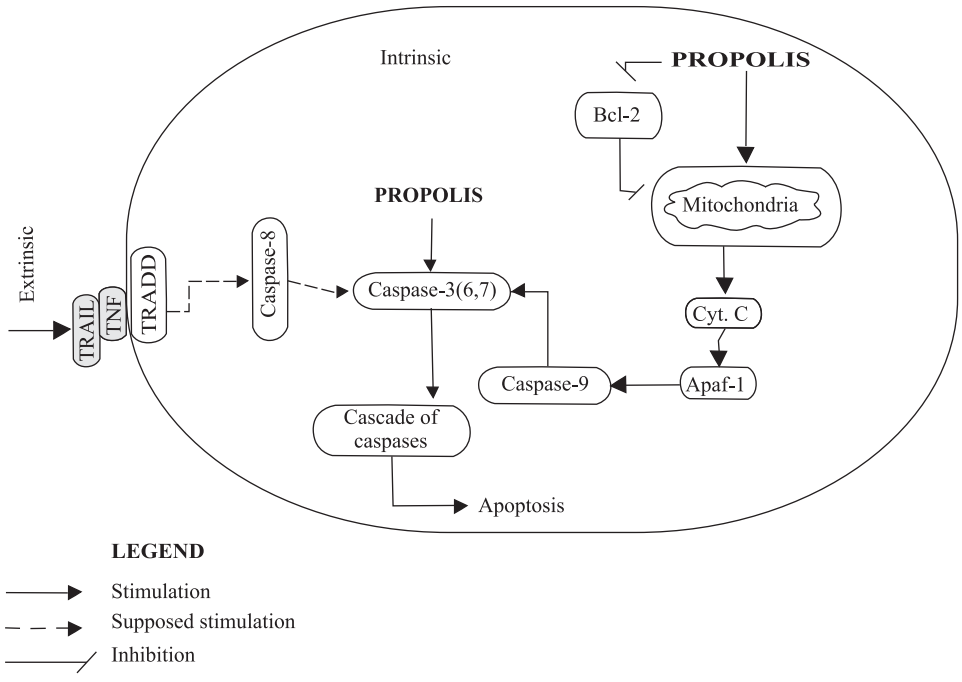

Figure 1. Effect of propolis on apoptosis pathways in cancer cells based on [24–29]. Bcl-2 — B cell lymphoma 2 protein; Cyt. C — cytochrome C; Apaf-1 — apoptotic protease activating factor 1; TNF — tumor necrosis factor; TRAIL — TNF related apoptosis-inducing ligand; TRADD — TNFR associated death domain protein

NCI-H358, human hepatocellular carcinoma HepG2, C6 glioma, human cervical cancer ME180, human pancreatic cancer PANC-1, and BxPC-3 cells [44–50]. It appears that the beneficial effect of CAPE is not dose- or concentration-dependent, since the increased activity of caspase-3 was observed when CAPE was used at a dose of 5 μg/mL, 6 μg/mL or 10 μg/ml [44] or at different concentrations: 5 μM [45], 50 μM [46] or 10–100 μM [48]. Additionally, CAPE induced activity of other proteins involved in the pro-apoptotic process, such as Bax or Bak, and reduced the expression of Bcl-2 which is an inhibitor of apoptosis [44, 46, 48, 49]. Also, CAPE, similarly to propolis, releases cytochrome c, which suggests that intrinsic pathways of apoptosis are affected by CAPE [46, 50]. Moreover, the number of apoptotic HeLa cells increased to 49.6% after treatment with 50 μg/ml CAPE and 100 ng/ml TRAIL [35]. This result indicates the extrinsic pathway of apoptosis involved in the action of CAPE.

Other studies have demonstrated that many proteins involved in the apoptotic process are affected by CAPE. The mechanisms of inhibition of tumor growth by CAPE are caused by the induction of the activity of p53 [28, 45, 46, 49], p21protein [47], p38 MAPK and JNK kinase [46, 47, 49] and a result of NF-κB inhibition [49, 50] associated with the downregulation of IAPs such as cIAP-1 and cIAP-2 expression [51].

CAPE induces apoptosis (Figure 2) by the activation of Bax, p53, p21 proteins, p38 MAPK, JNK, ERK kinases, the release of cytochrome c into the cytosol, and induction of caspase cascade activity. A study by Szliszka et al. [35] also suggests that CAPE inhibits NF-κB and enhances extrinsic pathway of apoptosis in cancer cells induced by TRAIL and Fas receptor stimulation. All these effects seem to be independent of the type of tumor cells and the concentration or dose of CAPE.

Chrysin (5,7-dihydroxyflavone) is another component of propolis that shows significant biological and pharmacological properties. It is a natural flavonoid found in plant extracts (Passiflora caerulea, Populus tremula) [52], honey and propolis. Chrysin has antioxidant and anti-inflammatory effects [53, 54]. The anti-cancer property of chrysin has previously been demonstrated, although the molecular mechanisms are still not clear. This flavonoid influences the apoptotic process in many types of cell lines.

Chrysin (5, 7.5 and 10 μM) induces apoptosis in U937 cells by the inactivation of PI3K/Akt signal pathway as well as downregulation of NF-κB and IAP activation, and in this way it stimulates caspase-3 which plays a crucial role in cell death [55]. An increase in the activity of PI3K and Akt is typically observed in cancer cells [56]. Additionally, U937 cells treated for 12 h with chrysin (7.5 and 10 μM) released cytochrome c from the mitochondria into the cytoplasm [55]. Woo et al. [55] concluded that chrysin, as a natural, nontoxic substance, is a potentially important agent to be used in prevention or therapy of patients with leukemia.

Other studies have shown that chrysin participates in the intrinsic pathway of apoptosis in human colorectal cancer cells HCT116, human liver cancer cell line HepG2, and human nasopharyngeal carcinoma cells CNE-1 [57]. The percentage of apoptotic HCT116, HepG2 and CNE-1 cells increased markedly after treatment with 1 ng/ml TNFα together with 10, 20 and 40 μM of chrysin. Chrysin significantly sensitizes TNF-α-induced apoptosis via a caspase cascade — activation of caspase-8 and caspase-3. Pretreatment of HCT116 cells with 40 μM chrysin and 1 ng/ml TNFα compared to the TNFα only group ($p < 0.01$) inhibits IκB kinase activity, NF-κB transcriptional activity and suppresses anti-apoptotic gene c-FLIP. The above cited study indicates that chrysin associated with TNFα in its inhibitory effect on NF-κB activation reduces c-FLIP expression in HCT116 cells.

This study advances our understanding of the molecular mechanism involved in the anti-cancer activity of chrysin.

Chrysin induces apoptosis in cancer cells by activation of caspases, suppression of anti-apoptotic proteins such as IAP, c-FLIP, PI3K/Akt signal pathway, inhibition of IKK and NF-κB activity (Figure 3). Despite the results of studies presenting the proapoptotic activity of chrysin, it has been shown that Chinese propolis inhibited apoptosis in neuroblastoma cell line SH-SY5Y, caspase-3 activity and cytochrome c releasing into the cytosol [58]. Further studies are necessary to understand the exact mechanism of chrysin-induced apoptosis in cancer cells.

In summary, the induction of apoptosis in various cancer cells by propolis extracts (EEP or WSDP) and its extremely active compounds, such as CAPE and chrysin, depends on the concentration of the natural products used. Propolis and the presented compounds induce the intrinsic pathway of apoptosis through the release of cytochrome c from mitochondria to the cytosol, through caspase cascade and activation of pro-apoptotic proteins: Bax, Bad, p53, and p21.

Moreover, propolis, as well as CAPE and chrysin, enhances extrinsic pathway of apoptosis in cancer cells stimulated by TRIAL (TNF) or Fas receptors. CAPE, through activating p38 MAPK, JNK, ERK kinases confirms the involvement of the intrinsic pathway of apoptosis in the mechanism of the anticancer activity. The suppression of anti-apoptotic proteins IAP,

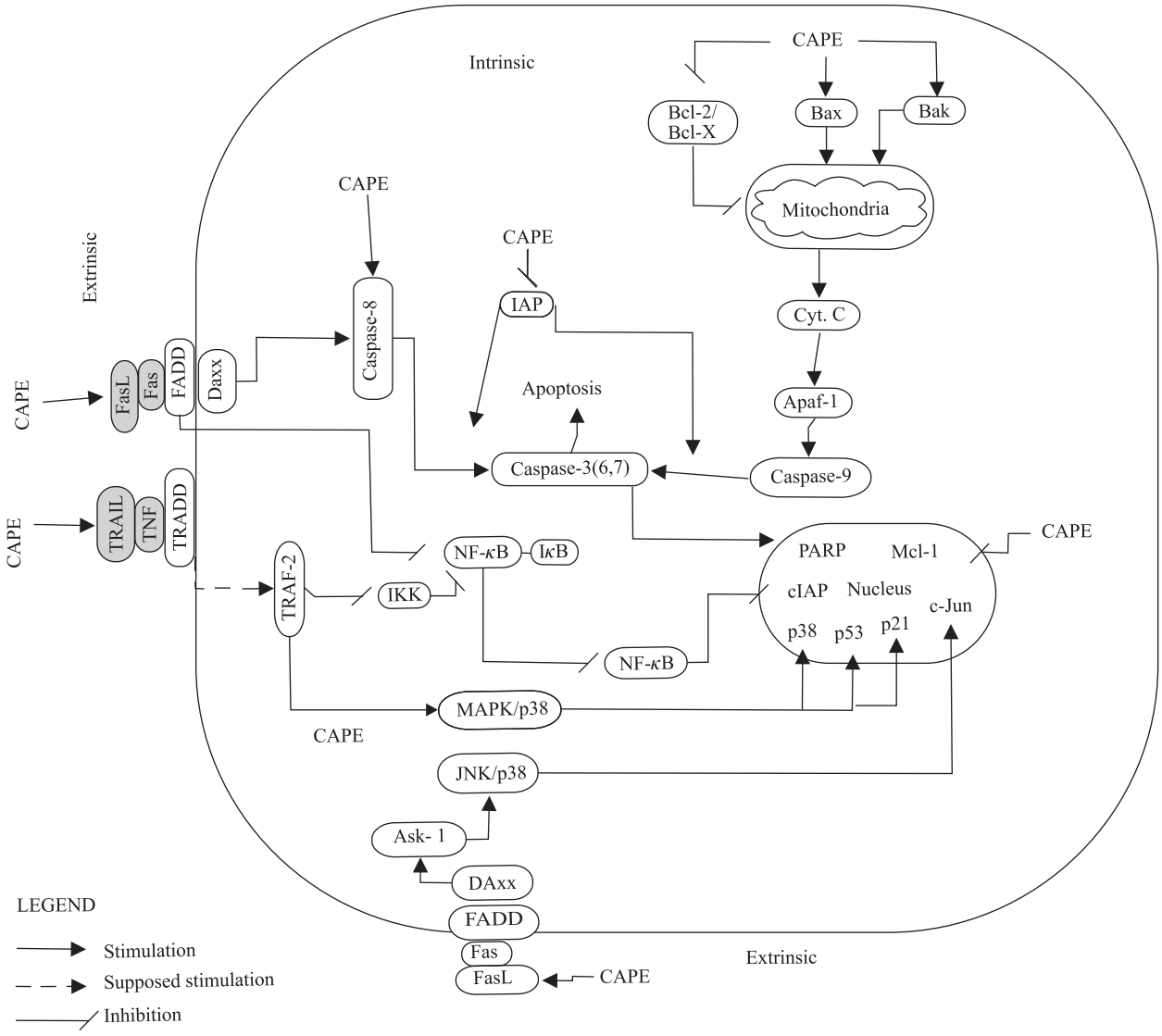

Figure 2. Targeting the apoptosis pathways in cancer cells stimulated by CAPE based on: [22, 23, 30, 39–46]. Bcl-2/Bcl-X — B cell lymphoma 2 protein/B cell lymphoma X protein; Bax — Bcl-2 associated X protein; Bak — Bcl-2 homologous antagonist/killer protein; Cyt. C — Cytochrome C; Apaf-1 — apoptotic protease activating factor; IAP — inhibitor of apoptosis proteins, Fas — Fas 'fatty acid synthase' associated protein, FasL — Fas associated protein ligand; FADD — Fas protein associated with death domain; Daxx — death domain associated protein; TNF — tumor necrosis factor; TRAIL — TNF related apoptosis-inducing ligand; TRADD — TNFR associated death domain protein; TRAF — TNF receptor-associated factor-2; NF-κB — nuclear factor-kappaB; IKK — kappa B kinase; IκB — inhibitor of nuclear factor kappaB; MAPK/p38 — mitogen-associated protein kinase and p38 pathways; JNK/p38 — c-Jun N-terminal kinase and p38 pathways; Ask-1 — apoptosis signal-regulating kinase 1; PARP — poly(ADP-ribose) polymerase; Mcl-1 — myeloid leukemia cell differentiation protein; p21, p38, p53 — 21, 38, 53 protein

c-FLIP, Bcl-2, PI3K/Akt kinase and inhibition of NF-κB activity by chrysin have also been presented. All these pathways are presented in Figure 3. Although many studies have shown the proapoptotic effects of propolis, CAPE and chrysin, there are also results that suggest their antiapoptotic action. These contradictory results suggest that further studies are needed to precisely define the mechanism of action of propolis, CAPE and chrysin in the apoptotic pathways.

Effect of propolis and its compounds on cancer cells proliferation

Cancer cells are characterized by uncontrolled growth and development as a result of abnormal function of genes responsible for cell cycle regulation controlled by complexes of cyclins and cyclin-dependent protein kinases which stimulate the cell to move at the next phase of the cell cycle. The cell cycle is a process regulated also by p53 protein, which as a result of DNA

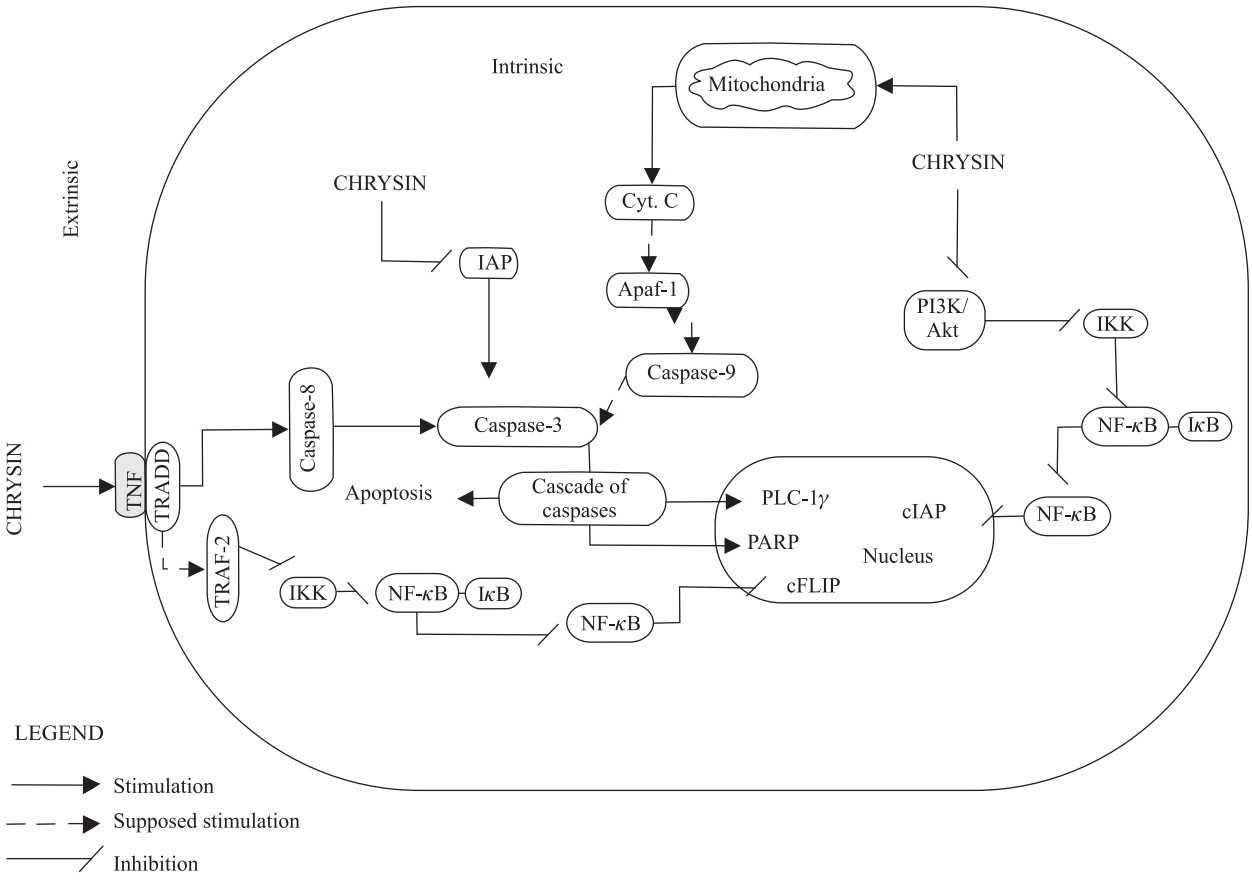

Figure 3. Targeting the apoptosis pathways in cancer cells stimulated by chrysin based on: [50–52]. Cyt. C — Cytochrome C; Apaf-1 — apoptotic protease activating factor; IAP — inhibitor of apoptosis proteins; TNF — tumor necrosis factor; TRADD — TNFR associated death domain protein; TRAF-2 — TNF receptor-associated factor-2; NF-κB — nuclear factor-kappa B; IKK — kappa B kinase; IκB — inhibitor of nuclear factor kappa B; PARP — poly(ADP-ribose) polymerase; PI3K — phosphoinositide 3-kinase; Akt — altered PI3 kinase; PLC-1g — phospholipase c-1g; cIAP — cellular inhibitor apoptosis protein; cFLIP — cellular Flice inhibitory protein

damage increases the levels of cyclin-dependent kinase (Cdk) inhibitors such as p16, p21 and p27 proteins [59–61]. There are suggestions that the genes responsible for the synthesis of cyclins are potential oncogenes, as their deregulation or excessive expression leads to continuous Cdk activity which phosphorylates Rb protein [62]. The process of cancer cell proliferation was studied after the administration of propolis and its compounds.

Effect of propolis on cancer cells proliferation

The antiproliferative activity of propolis on U937 cells was observed by Motomura et al. [33]. Methanol extract of propolis used at a concentration of 100–1,000 μg/ml significantly inhibited the growth of U937 cells in a dose-dependent manner. Flow cytometric analysis indicated an increase to 32.8% and 37.7% in the number of U937 cells at the G2/M phase, for 300 μg/ml and 500 μg/ml of propolis, respectively, as a result of down-regulation of cyclin A, cyclin B, CDK2 expression and increasing the level of p21 and p27 proteins. Blocking cell cycle progression at the G2 phase is important, because the cell does not go to the next M phase in which cancer cell division occurs.

It is believed that cancer cells are immortal because their telomeres are not curtailed due to excessive telomerase activity. This allows tumor cells to avoid aging, which protects normal cells against malignant transformation and is considered to be a mechanism second in importance only to apoptosis [63]. EEP (0.03 g/mL) decreased the telomerase expression to 60–93% by suppression of the human telomerase reverse transcriptase (hTERT) activity in T-cell acute lymphoblastic leukemia CCFR-CEM cell line [64].

The studies discussed above show the antiproliferative effects of propolis on cancer cells as a result of DNA damage. Propolis inhibits the cell cycle by suppression of cyclin A, cyclin B, Cdk2 expression, increasing the level of p21, p27 proteins and inhibition of hTERT effect in tumor cells. Propolis can suppress cancer cell proliferation via two mechanisms: stopping proliferation at the G2 phase, and decreas-

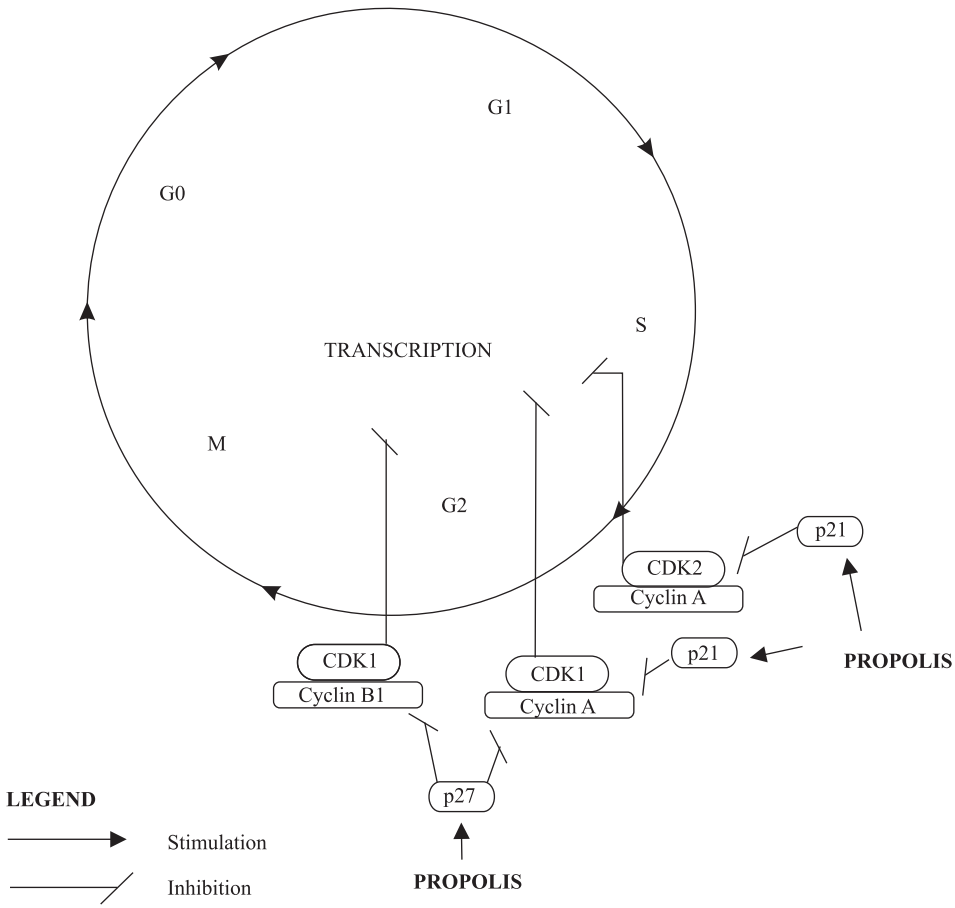

Figure 4. Effect of propolis on cancer cells cycle based on: [28]. G1 phase (Gap1) — cell increase; S phase (synthesis) — DNA replication; G2 phase (Gap2) — cell growth; M phase (mitosis) — cell division; G0 phase (Gap0) — end of cell division; CDK — cyclin-dependent kinase; p21, p27 — 21, 27 protein

ing telomerase activity. Both pathways are presented in Figure 4.

Effect of propolis compounds on cancer cells proliferation

The wingless-type glycoprotein (WNT) signaling pathway is involved in 90% of all colorectal cancer cases [65]. The adenomatous polyposis coli gene or axin which are WNT pathway suppressors, and the β-catenin oncogene are strongly connected with carcinogenesis [66]. Mutations in the β-catenin gene were discovered in colorectal, ovarian, pancreatic and prostate cancer [67]. Therefore, the phosphorylation of β-catenin by glycogen synthase kinase 3β or casein kinase 1α is required for proteasomal degradation of β-catenin and leads to cancer cell growth arrest. All the WNT signals have been studied after administration of CAPE in HCT116 cell line [68].

Flow cytometry and Western blotting assay indicated an increase in the number of HCT116 cells at G0/G1 phase and a decrease in the number of S phase cells by CAPE (2.5–10 mg/L) in a dose- and time-dependent manner. Wang et al. [68] suggested that the obtained results are due to the induction of suppression of β-catenin level in cytosol and nucleus. Similar results were achieved in a study by He et al. [69]. CAPE (2.5–80 mg/L) inhibited the proliferation of SW480 colorectal cancer cells by the reduction of β-catenin expression and suppression of cyclin D1 and c-myc protein expression. Also, it was found that CAPE inhibited the SW480 and HCT116 cell growth and induced cell cycle arrest at the G1 phase in a dose- and time-dependent manner. The obtained results were caused by the reduction of β-catenin expression in nucleus and cytoplasm, and downregulation of cyclin D1 and c-myc protein expression [70]. The above data strongly confirms that the antiproliferative effect of CAPE on colorectal cancer cells is associated with a decrease in β-catenin expression in cells. The described activities of CAPE in β-catenin signaling pathway are presented in Figure 5.

In order to better define the mechanism of CAPE activity in cell arrest during proliferation, a study was conducted on C6 glioma cells. CAPE (10–100 μM)

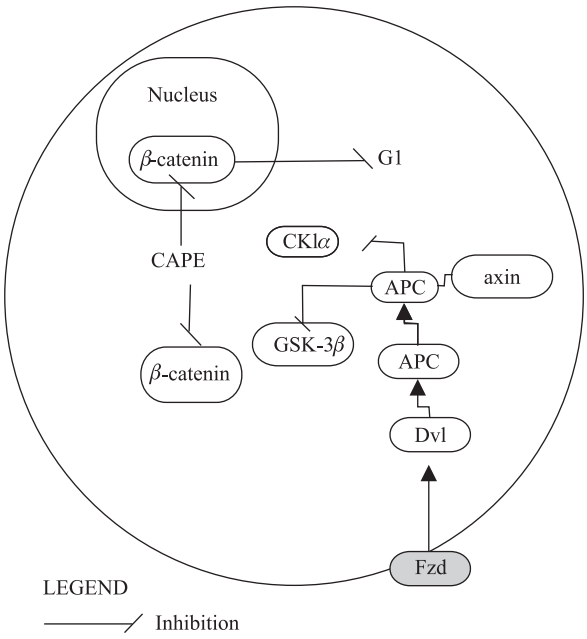

Figure 5. Effect of CAPE on β-catenin signaling pathway based on: [61–65]. Fzd — frizzled receptor; Dvl — dishevelled family proteins; APC — Adenomatous Polyposis Coli (APC) gene; GSK-3β — Glycogen Synthase Kinase 3β; CKIα — Casein Kinase 1α; G1 phase (Gap1) — cell increase

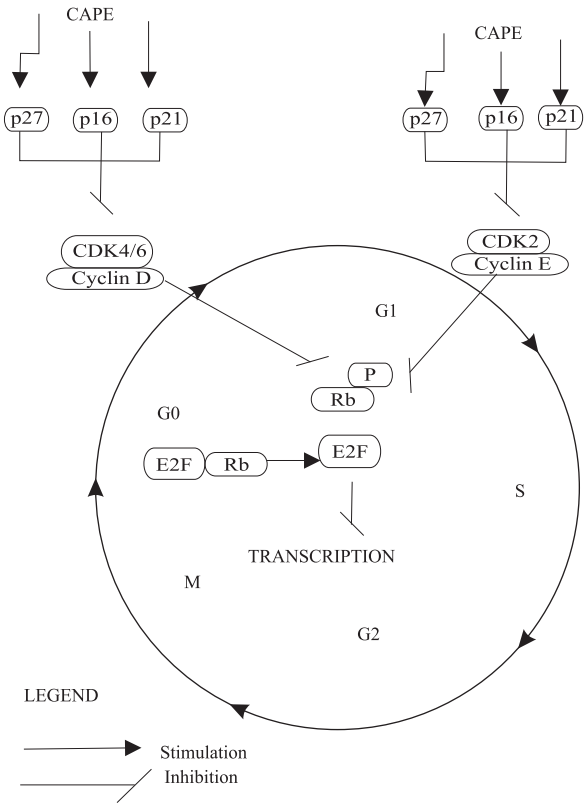

Figure 6. Effect of CAPE on process of transcription in cancer cell based on: [66]. G1 phase (Gap1) — cell increase; S phase (synthesis) — DNA replication; G2 phase (Gap2) — cell growth; M phase (mitosis) — cell division; G0 phase (Gap0) — end of cell division; CDK — cyclin-dependent kinase; E2F — transcription factor 2; Rb — retinoblastoma protein; p16, p21, p27 — 16, 21, 27 protein

caused a significant reduction in the number of C6 glioma cells compared to a control group ($p < 0.05$) [71]. Flow cytometric assay showed an increase in the percentage of the cells at G1 phase to 85%, and a decrease to 7–8% at S phase. Moreover, CAPE (50 μM) decreased CDK2/cyclin E and CDK4/cyclin D activity and the protein level of hyperphosphorylated pRb correlating with an increase of p21, p27, p16 expression in C6 glioma cells. This study [71] suggests that CAPE increases cell cycle arrest at the G0/G1 phase as a result of an inhibition of pRb phosphorylation (Figure 6).

In summary, the studies presented above identified that CAPE inhibits colorectal cancer cells proliferation and induces cell cycle arrest by downregulation of β-catenin protein expression and activation of the cyclin-dependent kinase inhibitors which prevent pRb phosphorylation.

The effect of chrysin on proliferation was studied in rat C6 glioma cells [72]. The proliferation and growth of C6 cells exposed to 10–50 μM chrysin for 72 h decreased by about 30–90% ($p < 0.001$). Additionally, chrysin at 30–50 μM concentration increased the number of C6 glioma cells at the G1 phase from 69% to 83% ($p < 0.001$), while the number of cells at the S phase decreased from 11.4 % to 2.8% ($p < 0.001$). Chrysin at 50 μM concentration reduced the activity of CDK2/cyclin E to 8.9% ($p < 0.001$) and CDK4/cyclin D to 5.7% as a result of significant overactivity of cyclin-dependent kinase inhibitor p21 in the C6 glioma cells, but the specific inhibitor (SB203580) of p38 MAPK downregulated the p21 protein expression and increased the C6 glioma cells proliferation. This study suggests that chrysin increases the p21 protein level by induction of the p38 MAPK kinase activity resulting in C6 glioma cell proliferation arrest (Figure 7).

The studies presented above indicate that a mechanism of tumor cell cycle arrest is observed after the administration of propolis, CAPE or chrysin. Propolis and the presented compounds induce the inhibition of cell proliferation through suppression of complexes of cyclins and cyclin-dependent protein kinases, and increase the level of protein inhibitors such as p21 and p27 in tumor cells.

Additionally, propolis shows an antitumor effect as a result of inhibition of the hTERT effect in U937 cells. Moreover, it has been suggested that the antiproliferative effect of CAPE is associated with the downregulation of the β-catenin expression in SW480

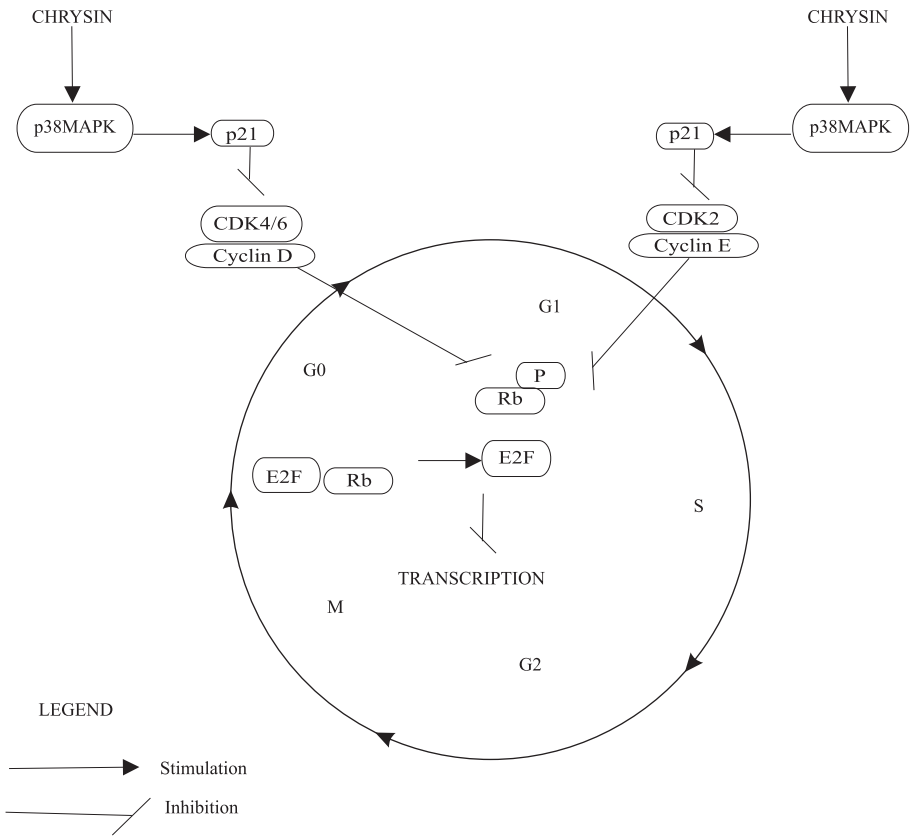

Figure 7. Influence of chrysin on cell cycle based on: [67]. G1 phase (Gap1) — cell increase; S phase (synthesis) — DNA replication; G2 phase (Gap2) — cell growth; M phase (mitosis) — cell division; G0 phase (Gap0) — end of cell division; p38MAPK — mitogen-activated protein kinase and p38 pathway; CDK — cyclin-dependent kinase; E2F — transcription factor 2; Rb — retinoblastoma protein; p21 — 21 protein

and HCT116 colorectal cancer cells. Based on the presented results, propolis inhibits cell proliferation due to the influence at the G2 phase. CAPE and chrysin are involved at the G0/G1 phase of cell proliferation. It is possible that the effect of propolis on cell proliferation is not only done by CAPE or chrysin.

In vivo studies

The results of the *in vitro* studies presented above suggest that propolis, CAPE and chrysin have cytotoxic properties against cancer cells through the induction of apoptosis or cell division and cell growth arrest. Nevertheless, *in vitro* studies do not always reflect the behavior of the tested product in the body. Therefore, studies in a living organism are an important element of the research of chemotherapeutic agents.

The results of an *in vivo* study presented by Borrelli et al. [73] showed the efficacy of CAPE in limiting tumor growth in a rat model of colon cancer. Azoxymethane (AOM) administered intraperitoneally induced formation of aberrant crypt foci (ACF) and tumors in the rat colon. CAPE administered intraperitoneally at a dose of 50 mg/kg significantly ($p < 0.01$) reduced the effects of AOM, thus avoiding the development of ACF and tumors in the colon. It is important to note that propolis without CAPE gives no positive effect. These results may suggest that the potential antitumor effect of propolis is dependent on the presence of CAPE.

Another experiment demonstrated that propolis administered at a dose of 160 mg/kg to mice with Ehrlich ascitis carcinoma (EAC) cell line increased the survival rate of the animals to 30% [74]. Propolis significantly reduced the tumor viability and volume. Flow cytometric analysis showed that propolis used 22 h before inoculation of EAC led to reduction of the number of tumor cells at the S phase cell cycle, thus arresting the tumor cells proliferation.

CAPE has an inhibitory effect on the growth of C6 glioma cells in BALB/c-*nu* mice [71]. Its administration at doses of 1 mg/kg, 5 mg/kg and 10 mg/kg to mice after tumor inoculation caused a marked reduction of tumor volume to 39.7%, 63.4% and 78%, respectively, compared to control animals. Histological analysis presented a decrease in the number of mitosis positive C6 glioma cells after application of

CAPE, while cells in the control group showed a complete proliferation.

In vivo studies confirm the *in vitro* observations. Both studies show significantly beneficial effects of propolis, CAPE and chrysin in stopping cancer progression.

Conclusions

With an increasing incidence rate of cancer worldwide, new anticancer agents are still required. One of the benefits of anticancer therapy is the ability to initiate apoptosis and cell cycle arrest in cancer cells. The studies presented in this review suggest that propolis and its compounds, CAPE and chrysin, may inhibit cell cycle proliferation or induce apoptosis in tumor cells. They induce the apoptotic process by activation of Bax, p53, p21 proteins, p38 MAPK, JNK, ERK kinases, release of cytochrome c into the cytosol, and activation of caspase cascade. These effects are not dependent on the type of tumor cell. The results of the studies cited in this review also suggest the inhibition of NF-κB activation, the suppression of antiapoptotic proteins, such as IAP, c-FLIP, Akt kinase, and the initiation of extrinsic pathway of apoptosis by induction of TRAIL and Fas receptor stimulation in cancer cells.

Propolis and its compounds described above induce the inhibition of cell proliferation by the suppression of complexes of cyclins and cyclin-dependent protein kinases, as well as by increasing the level of protein inhibitors such as p21, p16 and p27 in tumor cells, and inducing cell cycle arrest by decreasing β-catenin protein expression. Although many studies have shown the inhibitory effects of propolis and its compounds on growth and cancer cell proliferation, further research is necessary to understand the efficiency and mechanism of their beneficial effects.

Propolis, as a component of many active substances, possesses anticancer properties. The beneficial antiproliferative and antiapoptotic activities of CAPE and chrysin are not always based on similar mechanisms as propolis. Propolis and its compounds could be potentially useful as chemotherapeutic or chemopreventive anticancer drugs.

Acknowledgements

This work was supported by Grant N N405 625438 from the National Research Committee, Warsaw, Poland. The authors declare no conflict of interest.

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Submitted: 3 February, 2011
Accepted after reviews: 14 November, 2011