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Review

Cytotoxic constituents of propolis inducing anticancer effects: a review

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Abstract

Objectives Propolis is a honeybee product used extensively in traditional medicine for its antioxidant, anti-inflammatory, immunomodulatory and anticancer effects. Propolis exhibits a broad spectrum of biological activities because it is a complex mixture of natural substances. In this review, the antitumour effects of propolis extracts and its constituents (e.g. flavonoids, terpenes and caffeic acid phenethyl ester) are discussed.

Key findings The effect of propolis on experimental carcinogenesis is discussed, as well as its possible mechanisms of action against tumours, involving apoptosis, cell cycle arrest and interference on metabolic pathways. Propolis seems to be efficient against different tumour cells both *in vitro* and *in vivo*, which suggests its potential in the development of new anticancer drugs.

Summary Propolis extracts may be important economically and would allow a relatively inexpensive cancer treatment. Preclinical investigations are needed to further elucidate the benefits of propolis and its antitumour properties.

Keywords caffeic acid phenethyl ester; cancer; diterpenes; flavonoids; propolis

Introduction

Natural products have afforded a rich source of compounds that have found many applications in cancer chemotherapy. Furthermore, the vast structural spectrum of natural compounds can provide lead compounds for therapeutic improvement by molecular modification. Over 70% of anticancer compounds are either natural products or substances derived from natural products. Also, conjugation of toxic natural products to monoclonal antibodies or polymeric carriers can lead to more efficacious targeted therapies. Since less than 15% of higher plants have been systematically investigated, the research into natural products as chemotherapeutic agents requires further attention and multidisiplinary scientific collaboration.[1]

Propolis is a honeybee product with many biological properties, including immunomodulatory, anti-inflammatory, antioxidant, antibacterial, antiviral, antifungal and antiparasite activities, among others.[2] The antitumour activity of propolis has been recently reviewed.[3] In fact, propolis is frequently mentioned in the literature as an antitumoural and immunomodulatory agent. In recent years, in-vitro and in-vivo assays have provided new information concerning its mechanisms of action, and data have been compiled from several laboratories focusing on its chemical composition, botanical sources and biological properties.[3,4]

In-vitro assays have shown the cytotoxic action of propolis and its isolated compounds on various tumour cells.[5,6] Propolis and its isolated constituents also exert antitumoural effects *in vivo*, caused by an immunomodulatory action,[7] mainly due to the augmentation of non-specific antitumour immunity via macrophage activation, which in turn could produce soluble factors and interfere directly with the tumour cells or in the function of other immune cells.[8]

The great number of publications on the antitumour action of propolis and its compounds reveals their potential in the development of new antitumour agents.[2] Since propolis administration to humans or rats does not lead to side-effects,[9–12] propolis could potentially

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be a relatively inexpensive cancer treatment. In this review, we describe the antitumour potential not only of propolis extracts but also of flavonoids, terpenes and caffeic acid phenethyl ester (CAPE).

Propolis Extracts from Different Geographic Regions

Propolis has received significant attention in recent years from researchers and the food industry.[13] The composition of propolis is very complex and it contains mainly waxes, resin and volatiles. The main chemical groups present in propolis resin comprise phenolic acids or their esters, flavonoids (flavones, flavanones, flavonols, dihydroflavonols and chalcones), terpenes, aromatic aldehydes and alcohols, fatty acids, stilbenes and β-steroids.[9,14]

Different solvents solubilize and extract different compounds, with ethanol, methanol and water being the most common extracts used in biological assays.[15] The ethanolic extract of propolis (EEP) is one of the richest sources of phenolic acids and flavonoids. EEP and its phenolic compounds have shown various biological activities, including immunomodulatory, chemopreventive and antitumour effects.[3]

Studies have been carried out using propolis from different geographic regions. Although interesting, this makes the standardization of biological assays difficult, since different propolis samples may have different chemical compositions and so do not allow comparison between results.[2] As reported elsewhere,[4] a universal standardization would be impossible and propolis biological action should be linked to its chemical composition and plant sources.

As an example of the action of propolis from different regions, Orsolic and co-workers have been investigating the immunomodulatory action of Croatian and Brazilian propolis in tumour models. Treatment of mice with the aqueous extract of propolis (AEP) modified macrophage tumouricidal activity, increasing the production of lymphocyte activating factors and inhibiting the human cervical carcinoma cell line (HeLa) and Chinese hamster lung fibroblast (V79). Propolis-treated mice also showed an elevated splenocyte response to polyclonal mitogens. They also noticed that propolis and some isolated polyphenolic compounds decreased the number of tumour nodules in the lung, and the antimetastatic effectiveness of propolis was higher than that achieved by its constituents.[7,16,17]

The treatment of rats with Brazilian EEP increased the cytotoxic activity of natural killer cells against murine lymphoma.[18] Experiments by our group were designed to evaluate the effects of EEP in melanoma-bearing mice submitted to stress. After the inoculation of B16F10 cells subcutaneously, it was verified that stress induced a higher tumour area, while propolis-treated mice, stressed or not, showed melanoma development similar to the control. Propolis induced higher levels of interleukin-1β and interleukin-6 in melanoma-bearing mice submitted or not submitted to chronic stress. Since propolis also stimulated Th1 cytokine production (interleukin-2 and interferon-γ) in these mice, one may speculate that a synergistic effect of interferon-γ and pro-inflammatory cytokines could inhibit tumour growth *in vivo* by inducing the production of antiangiogenic factors.[19,20]

Brazilian EEP inhibited the proliferation of human prostate cancer cells.[21] Inoue *et al*.[22] observed that Japanese AEP inhibited S-180 mouse sarcoma growth *in vitro*. Moreover, the administration of AEP significantly inhibited the growth of transplanted tumour in mice.

The anticarcinogenic properties of Turkish propolis has been reported,[23,24] and its antitumour effect could be due to the flavonoids inhibiting the incorporation of thymidine, uridine and leucine into the carcinoma cells, leading to an inhibition of DNA synthesis. Erhan Eroğlu *et al*.[25] reported that the decreasing mitotic index rates in tissue cultures of bladder cancer indicated that propolis may be both an anticarcinogenic and antimitotic agent, which would be of interest with regard to human health.[23]

The methanolic extract of Brazilian red propolis displayed 100% cytotoxicity against the human pancreatic cancer PANC-1 cell line, and a detailed phytochemical investigation led to the isolation of 43 compounds including three new ones. All the isolated compounds were tested against PANC-1 cells under nutrient-deprived conditions. One compound, (6aR,11aR)-3,8-dihydroxy-9-methoxypterocarpan, displayed the most potent (100%) preferential cytotoxicity in a time-dependent manner via a non-apoptotic pathway that does not lead to fragmentation of DNA but is accompanied by necrotic-type morphological changes.[26]

Chinese and Brazilian EEP exerted anticancer activities in four human colon carcinoma cell lines, namely CaCo2, HCT116, HT-29 and SW480, and both extracts caused marked dose-dependent growth inhibition.[27] In the HCT116 cell line, Chinese propolis extract induced apoptosis and caused a dose-dependent increase in cellular mRNA levels of proteins associated with tumour suppression (p21CIP1 and p53) in the HCT116 cell line. The antiproliferative and cytotoxic effects of propolis from Thailand were investigated on the colon carcinoma cell line SW620, verifying that the water extract of propolis showed greater antiproliferative activity than the methanolic extract.[28]

EEP from Tunisia exhibited an antiproliferative action against HT-29, A549, HEp-2, RAW 264.7 and Vero cancer cell lines.[29] The butanolic extract and the isolated constituents, mainly diterpenes and flavonoids, from Greek propolis have been assessed for their cytostatic activity against human malignant and normal cell strains. The butanolic extract of propolis was cytotoxic for HT-1080 human fibrosarcoma and HT-29 colon adenocarcinoma cells, but showed no cytotoxic action against normal human skin fibroblasts.[30]

Portuguese propolis has been reported for its biological activity and, using human erythrocytes and the A-498 cell line as a cellular model, it was demonstrated that propolis extracts at very low concentrations can inhibit or reduce lipid peroxidation and hemolysis induced by peroxyl radicals.[13] In addition, propolis displayed effective antiproliferative activity against human renal cancer cells. Furthermore, the comparison of the in-vitro response of normal and malignant cells to propolis extracts revealed growth inhibition only of cancer cells. Overall, these findings suggested that Portuguese propolis is a promising therapeutic agent in the prevention of

Table 1 Propolis or its constituents and tumour cells used in different studies

Reference	Propolis extract or compounds	Cell lines[a]
Li *et al*.[21]	Ethanolic extract (Brazil)	DU-145, PC-3, RC-58 t/h/SA#4
Erhan Eroğlu *et al*.[25]	Aqueous extract (Turkey)	Tissue from patients with bladder carcinomas
Inoue *et al*.[22]	Aqueous extract	S-180
Ishihara *et al*.[27]	Ethanolic extract (China and Brazil)	CaCo2, HCT116, HT-29, SW480
Szliszka *et al*.[32]	Ethanolic extract	HeLa
Kouidhi *et al*.[29]	Ethanolic extract (Tunisia)	HT-29, A549, HEp-2, RAW 264.7, Vero
Seda *et al*.[33]	Ethanolic extract (Turkey)	MCF-7
Valente *et al*.[13]	Methanolic extract (Portugal)	A498
Huang *et al*.[34]	Propolin G (Taiwan)	C6, DBTRG-05MG
Diaz-Carballo *et al*.[35]	Mucronulatol (Caribbean)	HCT8, MDR1-/ MDR3+
Izuta *et al*.[36]	Chrysin, galangin, pinocembrin, caffeic acid, caffeic acid phenethyl ester (China)	SH-SY5Y
Josipović & Orsolić[37]	Quercetin, caffeic acid, chrysin, naringenin, naringin	MOLT, JURKAT, HL 60, RAJI, U937
Sha *et al*.[38]	Flavanol racemates and flavanol racemic mixture (China)	HeLa
Ha *et al*.[39]	Chrysin	BV-2
Li *et al*.[40]	Flavonoids, phenolic acid derivatives, glycerides (Mexico)	26-L5, B16-BL6, LLC, A549, HeLa, HT-1080
Pichichero *et al*.[41]	Chrysin	B16-F1, A375
Pratsinis *et al*.[30]	Extract and diterpenes (Greece)	HT-29
Hernandez *et al*.[42]	Caffeic acid phenethyl ester	A549, LS 180, HeLa, NCTC clone L 929, M12.C3.F6
Chen *et al*.[43]	Caffeic acid phenethyl ester	BxPC-3, PANC-1
Lee *et al*.[44]	Caffeic acid phenethyl ester	SK-Hep1
Lee *et al*.[45]	Caffeic acid phenethyl ester	HepG2
Lee *et al*.[46]	Caffeic acid phenethyl ester	Daoy
Jin *et al*.[47]	Caffeic acid phenethyl ester	U937
Jung *et al*.[48]	Caffeic acid phenethyl ester	MCF7, MDA 231
Lin *et al*.[49]	Caffeic acid phenethyl ester	C6

[a]Cell lines: 26-L5: colon carcinoma; A375: human melanoma; A498: human renal carcinoma; A549: human lung adenocarcinoma; B16-BL6: murine melanoma; B16-F1: mouse melanoma clone F1; BL6: melanoma; BV-2: mouse microglial cell; BxPC-3: human pancreatic cancer cell; C6: rat glioma; CaCo2: human colon carcinoma; Daoy: human medulloblastoma; DBTRG-05MG: human glioblastoma; DU-145: human prostate cancer; HCT8: human colon cancer; HCT116: human colon adenocarcinoma; HeLa: human cervical adenocarcinoma; HepG2: human hepatocellular liver carcinoma; HEp-2: human laryngeal epidermoid carcinoma; HL60: promyelocytic leukaemia cell; HT-29: human colon adenocarcinoma; HT-1080: human fibrosarcoma; JURKAT: leukaemic human T cell line; LLC: Lewis lung carcinoma; LNCaP: human prostate adenocarcinoma; LS 180: human colonic adenocarcinoma; M12.C3.F6: murine B-cell lymphoma; MCF-7: human breast adenocarcinoma; MDA 231: human breast adenocarcinoma; MDR: multidrug resistant cell; MOLT: human malignant T-lymphoblastic cell; NCTC clone L 929 (normal subcutaneous connective tissue; PANC-1: human pancreatic cancer; PC-3: human prostate cancer cell; RAJI: human Burkitt's lymphoma cell line; RAW 264.7: mouse leukaemic monocyte macrophage; RC-58T/h/SA#4: telomerase-immortalized primary human prostate cancer-derived cell line; S-180: mouse sarcoma; SH-SY5Y: neuroblastoma; SK-Hep1: hepatocellular carcinoma; SW480: human colon adenocarcinoma; U937: human leukaemic monocyte lymphoma; Vero: kidney epithelial cells of the African green monkey.

diseases mediated by free radicals and particularly in the chemoprevention of renal cancer cells.

The clinical application of propolis in cancer and other diseases has been limited due to its low aqueous solubility and, consequently, minimal systemic bioavailability. Nanoparticle-based delivery approaches have the potential to render hydrophobic agents such as propolis dispersible in aqueous media, thus avoiding possible low solubility. Propolis nanofood inhibits pancreatic cell growth in murine xenograft models; moreover, these effects were accompanied by a potent anti-angiogenic response and should facilitate the eventual clinical application of this well-known but underused therapeutic agent. No host toxicity was seen when maximal volumes were administered to mice. A similar therapeutic efficacy of free propolis against a panel of human pancreatic cancer cell lines was demonstrated *in vitro*,[31] as assessed by cell viability and clonogenicity assays.

All these data show that researchers from different countries have been interested in the antitumour properties of propolis and, in more recent years, the effects of isolated compounds have been investigated as well.

Table 1 shows the propolis extracts or constituents and tumour cells used by different researchers.

Flavonoids

Propolis is produced by bees from a mixture of resins, pollen and plant waxes, and flavonoids are its main group of compounds in some geographical regions.

Chrysin (5,7-dihydroxyflavone) is a natural and biologically active flavonoid compound extracted from honey, plants and propolis.[50,51] It possesses anti-inflammatory, anticancer, anti-allergic, anti-anxiolytic and antioxidant properties, and disturbs cell cycle progression.[52–56] However, the mechanism by which chrysin inhibits cancer cell growth and the intracellular pathways remain poorly understood. A significant decrease in human telomerase reverse transcriptase

expression levels was verified in cells from leukaemia patients treated with Manisa propolis, due to chrysin.[57]

The cytotoxic effect of polyphenolic/flavonoid compounds on different leukaemia cell lines was evaluated using five different flavonoids (quercetin, caffeic acid, chrysin, naringenin and naringin) and five leukaemia cell lines (MOLT, JURKAT, HL-60, RAJI and U937).[37] Quercetin exhibited the strongest cytotoxic effect on all cell lines, although caffeic acid and chrysin also showed a high cytotoxic level.

Several Brazilian red propolis constituents were investigated and 7-hydroxy-6-methoxyflavanone exhibited the most potent activity against B16-BL6, LLC and HT-1080 cancer cell lines, while mucronulatol was efficient against Lewis lung carcinoma and A549 cancer cell lines.[58] These data suggest that both components are promising for future anticancer drug development.

New flavanol racemates and a new flavanol racemic mixture were isolated from crude Chinese propolis, and the isolated compounds showed cytotoxic activity against the HeLa human cervical carcinoma cancer cell line.[38]

The natural flavones from Mexican propolis containing a 1-phenylallyl moiety ((7″R)-8-[1-(4′-hydroxy-3′-methoxyphenyl) prop-2-en-1-yl] galangin) displayed potent cytotoxic activity in the nutrient-deprived medium and triggered apoptosis-like morphological changes in PANC-1 cells.[59] Similarly, the phenylpropanoid-substituted flavanol (2R,3S)-8-[4-phenylprop-2-en-1-one]-4′,7-dihydroxy-3′,5-dimethoxyflavan-3-ol, showed potent cytotoxic action against A549 cells and HT-1080 cells, which was stronger than that of 5-fluorouracil, a clinically used anticancer drug.[40]

Terpenes

Some samples of propolis possess terpenes as major components. Thirteen cycloartane-type triterpenes and four prenylated flavanones isolated from Myanmar propolis were evaluated.[60] A cycloartane-type triterpene, 3α,27-dihydroxycycloart-24E-en-26-oic acid, showed potent cytotoxicity against B16-BL6 murine melanoma cells, and (2S)-5,7-dihydroxy-4′-methoxy-8,3′-diprenylflavanone exhibited strong cytotoxic action against human cancer cell lines (lung A549 adenocarcinoma, cervix HeLa adenocarcinoma and HT-1080 fibrosarcoma).[60]

A methanolic extract of propolis from Myanmar was found to inhibit PANC-1 human pancreatic cancer cells preferentially under nutrient-deprived conditions. Bioactivity-guided fractionation of the extract led to the isolation of cycloartane-type triterpenes (22Z,24E)-3-oxocycloart-22,24-dien-26-oic acid, which exhibited the most potent preferential cytotoxicity in a concentration- and time-dependent manner.[61]

The extract and the diterpenes from Greek propolis were found to be the most active against HT-29 human colon adenocarcinoma cells, without affecting normal human cells. Manool, a diterpene, was its most active compound, arresting the cancer cells at the G(2)/M phase of the cell cycle.[30]

Caffeic acid phenethyl ester

Caffeic acid phenethyl ester (CAPE), a phenolic component of propolis, has been widely studied.[62] This compound has several biological properties including antioxidant, anti-inflammatory, antiviral, immunomodulatory, anti-angiogenic, anti-invasive, antimetastatic and carcinostatic activity. The cytotoxic action of CAPE on tumour cells but not on normal cells has been reported. Grunberger *et al.*,[63] through a bioguided assay using a propolis sample from Israel, identified CAPE as a cytostatic agent and reported its differential cytotoxicity in normal rat/human versus transformed rat/human melanoma and breast carcinoma cell lines. Tumour cell lines displayed a significantly greater sensitivity to the action of CAPE than analogous normal ones. Propolis from Iran also showed a cytotoxic action on tumour cells but not on normal cells due to CAPE.[64]

CAPE, galangin, xanthomicrol and chrysin showed a significant antiproliferative activity on several cancer cells.[42] DNA harvested from cancer cell cultures treated with propolis exhibited a ladder of internucleosomal DNA cleavage characteristic of apoptosis.

Oxidative stress is the major cause of cellular injuries in a variety of chronic health problems, such as carcinogenesis and neurodegenerative disorders. It has been suggested that CAPE is a potent exogenous cytoprotective and antigenotoxic agent against cell oxidative damage that could be used as a template for designing novel drugs to combat diseases induced by oxidative stress components, such as various types of cancer.[65]

The effect of CAPE was investigated on cytochrome P450 (CYP), which is involved in diethylnitrosamine metabolism during the initiation stage of chemical hepatocarcinogenesis.[66] CAPE modified the enzymatic activity of CYP isoforms involved in the activation of diethylnitrosamine, such as CYP1A1/2 and CYP2B1/2, suggesting an alternative mechanism for the protective effect of CAPE against chemical hepatocarcinogenesis.

The mechanism of CAPE-induced apoptosis in human myeloid leukaemia U937 cells was investigated.[47] A DNA fragmentation assay revealed the typical ladder profile of oligonucleosomal fragments in CAPE-treated U937 cells. It was also observed that the nuclear condensation, a typical phenotype of apoptosis, was found in CAPE-treated U937 cells, followed by the release of cytochrome C, reduction of Bcl-2 expression, increase of Bax expression, activation/cleavage of caspase-3 and activation/cleavage of PARP in U937 cells, but not by Fas protein, an initial mediator in the death signalling, or by phospho-eIF2α and CHOP, crucial mediators in estrogen receptor (ER) mediated apoptosis. Authors concluded that CAPE induces the mitochondria-mediated apoptosis but not death receptor- or ER-mediated apoptosis in U937 cells.

CAPE exerts an antimetastatic action by inhibiting matrix metalloproteinase-2 and -9 expression, possibly by targeting nuclear factor-κB in hepatocellular carcinoma. The protective effects of CAPE were investigated on *tert*-butyl hydroperoxide (*t*-BHP)-induced hepatotoxicity in a cultured HepG2 cell line and in rat liver.[45] CAPE was found to significantly reduce *t*-BHP-induced oxidative injury in HepG2 cells, as determined by cell cytotoxicity, lipid peroxidation and reactive oxygen species levels in a dose-dependent manner. In-vivo studies showed that pretreatment with CAPE prior to the administration of *t*-BHP significantly and dose-dependently prevented increases in the serum levels of hepatic enzyme

markers (alanine aminotransferase and aspartate aminotransferase) and reduced lipid peroxidation in rat liver. The protective effects of CAPE against *t*-BHP induced hepatotoxicity may be, at least in part, due to its ability to scavenge reactive oxygen species and to protect DNA from oxidative stress-induced damage.

CAPE induced apoptosis of human pancreatic cancer cells involving caspase and mitochondrial dysfunction.[43] CAPE resulted in marked inhibition of viability of BxPC-3 and PANC-1. CAPE induced a time-dependent increase in percentage of hypodiploid cells and a significant decrease in mitochondrial transmembrane potential in BxPC-3 cells. It induced morphological changes typical of apoptosis, without DNA fragmentation as seen by DNA electrophoresis.

In an attempt to identify the estrogenic properties of propolis, Jung *et al*.[48] verified that CAPE showed a selective binding affinity to human estrogen receptor β (hERβ) rather than hERα. CAPE also reduced ERα expression in MCF-7 and MDA 231 cells. These results indicated that CAPE, which is a selective agonist to hERβ, but does not show any estrogenic effect on estrogen receptor-positive breast cancer cells and in immature rat uterine tissue, is a potential selective estrogen receptor modulator.

CAPE induces cell cycle arrest and antiproliferative effects on C6 glioma cells *in vitro* and *in vivo*. C6 glioma cells treated with CAPE resulted in morphological changes to an astrocytic phenotype, increased the expression of glial differentiation marker proteins, including glial fibrillary acidic protein and S-100β, and exhibited inhibitory effects on the invasion of C6 glioma cells.[49]

Tumour necrosis factor related apoptosis-inducing ligand (TRAIL/APO2L) is a naturally occurring anticancer agent that preferentially induces apoptosis in cancer cells and is not toxic to normal cells. The cytotoxic and apoptotic effects of EEP and phenolic compounds isolated from propolis were investigated in combination with TRAIL on two prostate cancer cell lines: hormone-sensitivity LNCaP (human prostate adenocarcinoma) and hormone-refractory DU-145 (human prostate cancer).[67] The strongest cytotoxic effect on LNCaP cells was exhibited by apigenin, kaempferid, galangin and CAPE in combination with TRAIL. These authors also showed that propolis extract markedly augmented TRAIL-mediated apoptosis in prostate cancer cells and suggested the significant role of propolis in chemoprevention of prostate cancer.

Propolis and Experimental Carcinogenesis

The use of initiation-promotion protocols has allowed a better understanding of the different stages of the chemically induced carcinogenesis. There are three principal stages in carcinogenesis: (1) initiation: mutations occur and cells are initiated; (2) promotion: clonal expansion of initiated cells takes place and forms preneoplastic lesions; and (3) progression: a preneoplastic lesion becomes a tumour due to genetic and metabolic changes. Chemoprevention may occur in all these stages and is based on the hypothesis that disruption of the biological events involved in any stage of carcinogenesis can decrease cancer incidence.[68]

The protective action of Brazilian EEP was evaluated on 1,2 dimethylhydrazine (DMH) induced colon carcinogenesis in rats.[69] EEP administration after DMH initiation led to a smaller number of aberrant crypts in the distal colon, suppressing the development of preneoplastic lesions. It has been reported that Brazilian AEP significantly reduced DMH-induced DNA damage in colon cells, but it did not suppress the development of aberrant crypt foci in rats.[70]

Diethylnitrosamine is a hepatotoxic and a carcinogenic substance used as an initiating agent in some two-stage (initiation-promotion) protocols for hepatocarcinogenesis studies in rats.[71] Hepatocyte proliferation is an important parameter in the development of liver cancer induced by genotoxic carcinogens in rats. During cancer initiation, regenerative hepatocyte proliferation is presumably required to fix the mutational event. Proliferation leads to the clonal expansion of initiated cells that form hepatocyte nodules during cancer promotion.[72]

The effects of Brazilian AEP and EEP on azoxymethane induced aberrant crypt foci in male Wistar Hannover (GALAS) rats were investigated, suggesting that both extracts possessed a chemopreventive ability in the early phase of colon carcinogenesis, modulating cell proliferation.[73]

The chemopreventive influence of a Brazilian AEP in a two-stage (initiation-promotion) medium-term bioassay for chemical liver carcinogenesis was evaluated.[74] Male Wistar rats were sequentially initiated with diethylnitrosamine and exposed to a diet containing hexachlorobenzene and to AEP. Data showed that propolis 0.1% did not protect against the development of any of the differentially identified putative preneoplastic foci in diethylnitrosamine-initiated animals, exerting no chemopreventive effect on the chemically induced hepatocarcionogenesis in rats.

The effect of dietary supplementation with chrysin was investigated on proliferation and apoptosis in diethylnitrosamine-induced carcinogenesis.[75] Chrysin administration significantly reduced the number and size of preneoplasic nodules and the expression of COX-2 and nuclear factor-κB and p65 in rats, whereas increased p53, Bax and caspase3 mRNA and protein levels were seen. Likewise, a decrease in levels of β-arrestin and the anti-apoptotic marker Bcl-xL was also noticed. Thus, it was suggested that chrysin exerts hepatoprotective effects and its chemopreventive activity is associated with p53-mediated apoptosis during early hepatocarcinogenesis.

Propolis has also been investigated for protective effects against photocarcinogenesis in a hairless mouse experimental model. Sydney propolis was able to reduce cutaneous inflammation, immunosuppression and lipid peroxidation induced by UV exposure.[76] Propolis significantly and dose-dependently protected against both sunburn oedema and the suppression of contact hypersensitivity, and against lipid peroxidation. The overexpression of interleukin-10 and the depletion of interleukin-12 characteristic of photoimmune suppression were markedly reduced by propolis. Further, the upregulation of interleukin-6 was decreased, and the associated induction of heme oxygenase was shown to play a role in propolis skin protection.

Possible Mechanism of Action of Propolis and its Compounds Exerting Antitumour Effects

The main mechanism of action of propolis and its compounds, regarding their antitumoural action, are related to apoptosis, cell cycle arrest and interference on metabolic pathways.

Considering the antiproliferative activity of propolis on certain neoplastic cells, an attempt has been made to evaluate its growth inhibitory activity on cancer cell lines and to provide new information about cancer therapy. It has been proposed that the inhibitory effect of propolis on human prostate cancer proliferation was achieved by regulation of cyclin D1, B1 and cyclin dependent kinase as well as by p21 expression.[21]

Cuban propolis exerted a significant antiproliferative activity on the MCF-7 human breast cancer cell line (estrogen receptor positive, ER+) rather than MDA-MB 231 (ER–).[77] This effect was concentration- and time-dependent and partially attributed to apoptosis. Indeed, the data showed that propolis caused a significant inhibition of MCF-7 cell growth in the G1 phase of cell cycle, in a dose- and time-dependent manner. Thus, it was hypothesized that propolis possesses an estrogen-like activity. Low concentrations of nemorosone, a polycyclic polyisoprenylated benzophenone isolated from floral resins of *Clusia rosea* Jacq. and Cuban propolis samples, elicited growth-inhibitory effects on MCF-7 cells, but not on MDA-MB-231 and LNCaP, suggesting that nemorosone could be a promising adjuvant to ER antagonists in ER+ breast cancer prevention or treatment.[78]

Both artepillin and green propolis extract selectively blocked PAK1 signalling, without affecting another kinase known as AKT.[79] Propolin G was isolated from Taiwanese propolis, which exerted a broad spectrum of biological activities.[34] Its chemical structure has been identified by nuclear magnetic resonance and fast atom bombardment-mass spectrometry spectra and was found to be identical to a known compound, nymphaeol C. Propolin G could efficiently induce apoptosis in brain cancer cell lines (glioma and glioblastoma) and the apoptosis might have occurred through a mitochondrial- and caspase-dependent pathway. Propolin G also possessed free radical scavenging activity.

Propolis markedly augmented TRAIL mediated apoptosis in cancer cells, confirming the importance of propolis in chemoprevention of malignant tumours.[32,80]

Seven different propolis extracts from Turkey were investigated on the human breast cell line MCF-7, and it was concluded that propolis may exert antitumour effects by increasing apoptosis through the caspase pathway.[33]

It is known that polyphenols play an important role in cancer chemoprevention. Molecules of TRAIL take part in immune surveillance and defence mechanisms against tumour cells. Some cancer cells are resistant to TRAIL-mediated cytotoxicity. There is a great deal of experimental evidence showing that polyphenols sensitize TRAIL-resistant cancer cells and markedly augment TRAIL-induced programmed death in cancer cells. Szliszka and Krol have described dietary polyphenols targeting the TRAIL mediated apoptotic pathway.[80]

Chrysin reduced melanoma cell proliferation in both human and murine melanoma cells due to intracellular accumulation of protoporphirin IX.[41] Chrysin also induced cell death in human and murine melanoma cells through caspase-dependent mechanisms, involving down-regulation of ERK 1/2 and activation of p38 MAP kinases.

The in-vitro anticarcinogenic and antimitotic effects of propolis and mitomycin-C on transitional carcinoma cell cultures have been investigated.[25] Using tissue samples from patients with bladder carcinomas, it was verified that the exposure to propolis can decrease cell division, suggesting its use as an antimitotic and anticarcinogenic agent.

Mucronulatol is one of the most cytotoxic substances present in Caribbean propolis. Mucronulatol exerts cytotoxicity in cancer cell lines by targeting the control of cell cycle progression, indicating that the mechanism of action of this compound involves interference with the cell cycle machinery.[35]

CAPE-induced oxidative stress may influence the radiosensitivity and proliferation of medulloblastoma cells.[46] The results indicated that CAPE inhibited the growth of Daoy (human medulloblastoma) cells, demonstrating that the antiproliferative and radiosensitizing effects of CAPE on medulloblastoma cells may be achieved by depleting glutathione, increasing reactive oxygen species activity and inhibiting nuclear factor-κB activity.

The effects of Chinese propolis and its constituents (chrysin, galangin, pinocembrin, caffeic acid and CAPE) were evaluated against tunicamycin-induced neuronal cell death in SH-SY5Y cells.[36] Chinese propolis and chrysin inhibited staurosporine-induced cell death. These findings indicate that the inhibitory effects of Chinese propolis against neuronal cell death induced by endoplasmic reticulum stress or staurosporine may be exerted primarily by chrysin.

Although recent advances have been made in tumour detection and treatment, the development of metastasis remains a significant cause of morbidity and mortality from the disease.[49] Thus, blocking the tumour progression is a crucial goal for chemoprevention. The mechanisms involved in the chemoprevention by propolis are not fully understood, but one may suppose that the interference by one or more propolis components in mutagenic/carcinogenic metabolic pathways or its putative antioxidant activity could explain its effects on DMH genotoxicity.[69]

Although some studies have demonstrated the mechanisms of action of propolis and its constituents, further investigations are needed to understand how they exert antitumour effects in humans and animals.

Conclusions

There is a great deal of literature dealing with the cytotoxic action of propolis *in vitro*. Although propolis may exert a direct effect on different tumour cells *in vitro*, the administration of propolis to animals or humans is followed by its solubility and systemic bioavailability. Thus, the action of propolis *in vivo* may occur mainly due to its immunomodulatory action, exerting either chemopreventive or therapeutic effects.

Cellular immune responses mediated primarily by activated T lymphocytes play an important role in eliminating malignant tumour cells. Accordingly, high frequencies of tumour-infiltrating lymphocytes are associated with a lower risk of relapse, reduced tumour progression as well as improved overall survival in cancer patients (e.g. melanoma and colorectal cancer). Over the past 20 years, different strategies to stimulate antitumour immunity have been extensively studied in humans. However, consistently effective immunotherapy has not yet been developed for any type of malignancy. Moreover, tumours have developed numerous mechanisms to evade both innate and adaptive immunity.[81] Thus, propolis and its compounds need to be further explored regarding antitumoural and immunomodulatory action *in vivo*.

Some isolated compounds have also been investigated and could be responsible for the antitumour action of propolis. However, since the composition of propolis is very complex, more compounds should be investigated in tumour assays both *in vitro* and *in vivo*, as well as the synergistic effects between them. The main mechanism of action of propolis involves apoptosis, cell cycle arrest and interference in metabolic pathways. Apoptosis, a mechanism by which cells undergo death to control cell proliferation or in response to DNA damage, can provide novel potential drug targets that are able to induce death in cancer cells.[82]

Taken together, these observations point to the development of new antitumour drugs based on propolis or its compounds, as discussed elsewhere.[2] As an example of this approach, Kim *et al*.[31] have synthesized a polymeric nanoparticle encapsulated formulation of propolis that is easily dispersed in aqueous media and with a therapeutic efficacy against human pancreatic cancer cell lines. New investigations using preclinical models might benefit from the effects of propolis.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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