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Review of the anticancer activities of bee products

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PEER REVIEW

Peer reviewer

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Comments

Having read through the present manuscript, I could interpret that the authors dealt with the bee products from the honeybees species belonging to the subfamily Apinae, which contains less than 10 species. There are two types of beekeeping in the world. One is called Apiculture which keeps Apinae bees. The other is Meliponiculture where bees belonging to the subfamily Meliponinae, which contains about 400 species. Meliponinae bees also produce honey and propolis which have been utilized for people widely in tropical and subtropical areas.
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ABSTRACT

Bee products have long been used in traditional medicine. The raw materials, crude extracts and purified active compounds from them have been found to exhibit interesting bioactivities, such as antimicrobial, anti-inflammatory and antioxidant activities. In addition, they have been widely used in the treatment of many immune-related diseases, as well as in recent times in the treatment of tumors. Bee product peptides induce apoptotic cell death *in vitro* in several transformed (cancer) human cell lines, including those derived from renal, lung, liver, prostate, bladder and lymphoid cancers. These bioactive natural products may, therefore, prove to be useful as part of a novel targeted therapy for some types of cancer, such as prostate and breast cancer. This review summarizes the current knowledge regarding the *in vivo* and *in vitro* potential of selective bee products against tumor cells.

KEYWORDS

Bee products, Cancer cells, Chrysin, Flavonoid, Inflammatory, Propolis

1. Introduction

Bees are flying insects in the order Hymenoptera and are closely related to wasps and ants. Asides their important ecological and economic role in the pollination of natural and commercial plant species[1], respectively, they are also known commercially for their role in producing natural products. In addition to honey, commercial bee products also include beeswax, bee pollen, royal jelly and propolis[2]. Each of these different bee products are, or are becoming, economically important and additionally are known to have several potent bioactivities. Indeed, bee products have been used in traditional medicine throughout society. For instance, bee pollen is reported to boost energy and

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stamina[3], propolis to help maintain good health[4], royal jelly to support the immune system and increase energy[5], whilst honey, mainly used as a natural sweetener in every food culture, is also used traditionally used for treatment of burns, sore throats and as an antiseptic[6]. More recent studies have found that several bee products have a potential anticancer activity *in vitro* and *in vivo*[7].

Several recent studies have reported that some natural bee products inhibit tumour cell growth and metastasis and induce apoptosis of cancer cells[8], suggesting the potential application of these natural compounds (or their active components) as part of an alternative medical treatment of human tumours[7]. When chemo- and radio-therapy are used systemically or over a broad directed tissue area to kill cancerous cells, they also typically harm healthy cells in the process causing undesirable side effects that limit the treatment (duration and/or dose) and effectiveness, or in the worst cases can kill the patient faster than the cancer would have done. Accordingly, research for alternative anticancer drugs has become a popular topic, especially for natural products. Natural products are viewed as generally being weaker but much safer. There is some element of validity in this approach in that, although some natural products are as toxic to normal cells, others do not directly interact with the cells but rather activate the immune system and so rely on the natural immune discrimination between healthy and infected or transformed cells. Alternatively, some natural products differentially act upon transformed and normal cells. Moreover, natural sources are a rich and largely untapped resource of potentially new agents against such diseases. For example, the origin of mostly current anticancer drugs is in one way while the rest are from natural sources[9], yet the diversity of such natural compounds remains relatively unknown. Although relatively few of the actual isolated compounds advance to become clinically effective drugs in their own right, they may serve as a template for the preparation of more efficacious analogues using total or combinatorial synthesis, or manipulation of biosynthetic pathways[9].

During the past two decades, the simple and polyphenolics plus peptides from bee products have started to attract more attention for their potential use in cancer therapy. This review aims to summarize the anticancer activity of bee products.

2. Honey and cancer

For centuries, honey has been known for its medicinal and health promoting properties. Honey is a complex produced by various species of honey bees (*Apis* sp.) from the nectar of plant blossoms or the exudates of plant phloem feeding insects (honeydew), or a mixture of both. These differences in direct and indirect (via phytophagous insect exudates) botanical sources, as well as the different foraging strategies of different bee isolates/species give rise to the seasonal, biogeographic (regional) and species specific variations in different honeys[10]. Although honey is principally a concentrated aqueous solution of inverted sugars (glucose and fructose), it aslo containes other saccharides, amino acids, organic acids, vitamins, minerals, antioxidants, flavonoids, phenolic acids and carotenoids [11-13]. Of the various kinds of phytochemicals present in honey, the phenolic and flavonoid content are relatively high and are comprised of simple and polyphenols, such as acacetin, apigenin, caffeic acid, caffeic acid phenethyl ester (CAPE), chrysin, galangin, kaemfereol, pinocembrin, pinobanksin and quercetin[14] that contribute to its antioxidant activity[12,15,16]. Flavanoids typically have anticancer properties[17] because of their antioxidant activity and also their related ability to alter many signalling pathways, including stimulation of tumor necrosis factor-alpha (TNF-α), inhibition of cell proliferation, induction of apoptosis, and cell cycle arrest[18-22]. Honey is thought to exhibit a broad spectrum of therapeutic properties in addition to an antioxidant activity, including an antimicrobial activity[19,23-25], cytostatic and anti-inflamatory activity[24]. Of interest here, however, is that honey can provide the basis for the development of novel therapeutics for patients with soft and hard (tumour) tissue cancers, especially jungle honey (wild honey collected from forest regions). In addition to affecting the chemotactic induction of neutrophils and reactive oxygen species[26], jungle honey has been shown to posses a significant antitumor activity *in vitro* against human breast, cervical, oral and osteosarcoma cancer derived cell lines[27,28]. However, the *in vivo* or *in vitro* effect of honey on hormone-dependent human cancers, such as breast, endometrial and prostate cancers, as well as solid tumour cancers *in vivo*, remains largely unknown. Honey has moderate anti-tumour activity and anti-metastatic effects against renal cell carcinoma and rat and murine tumours[22,29-31], and potentiated the effect of standard chemotherapy with 5-flourouracil or cyclophosphamide[32]. Some of the principal phytochemicals in honey (epigallocatechin-gallate, lycopene, genistein and resveratrol) have been used for treatment of prostate cancer [33,34], although the exact relative composition will vary between different regional, season and botanical source of the honey. There is increasing evidence to support that honey is a natural anti-inflammatory, antimicrobial, anticancer agents and potential for healing chronic ulcers and wounds[35]. Whilst the antibacterial effects of neat or high concentration honey are likely to be largely due to its osmotic potential, the *in vitro* and *in vivo* anti-cancer effects are usually seen at much lower concentrations (e.g. IC_{50} values of 100-200 μg/mL) even before systemic dilution in the tissue and so are more likely to reflect the actual bioactivities of its trace components. To this end, honey is known to contain caffeic acid, CAPE and flavonoid aglycones that downregulate many cellular enzymatic pathways, including protein tyrosine kinases, cyclooxygeneses and ornithine decarboxylase[36]. However, it should be born in mind that in addition to the above anti-proliferation and anti-metastic effects plus the induction of apoptosis in tumour cells, honey has, in contrast, been reported to induce the proliferation of malignant cells, albeit that this was

under possible nutrient limited conditions[19,37,38].

The mechanisms of action of honey in reducing tumour proliferation have been reported to broadly be via enhancing the immune response aginst the tumour cells. For example, honey increased the production of interleukin (IL)-1B, IL-6 and TNF-α in the human monocytic cell line, MM6, as well as primary human monocytes[19,39], increased secondary immune response antibody production[40], and enhanced neutrophil curculation and chemotaxis to the tumour[26]. Other diverse mechanisms are reported to include the modulation of signalling pathways[18] including TNF-α[19], inhibition of cell proliferation and induction of apoptosis[20], cell cycle arrest[21] and inhibition of lipoprotein oxidation[41]. However, the ability of honey to induce a reduced tumor growth or to inhibit metastasis was reported to be dependent upon the time of treatment, being an effective prophylactic but somewhat ineffective treatment agent [31].

It is of note that honey and cancer have a potentially sustainable inverse relationship in developing countries, where resources for cancer prevention and treatment are limited but honey can be plentiful[42].

3. Anticancer activity of propolis

Propolis (bee putty or bee glue) is produced by bees from the resin collected from trees and shrubs[43], which is combined with beeswax and secretions from the bee's salivary glands (rich in enzymes) plus some pollen. The color varies from yellow, brown or black, depending on the plants that the resinous substance was collected from, and so will vary with the local flora (geographical location and season) and foraging preferences (bee species). There is a long history of the recorded use of propolis by humans, dating back at least as far as the Egyptians who used it for embalming the body as an antibacterial tool[44]. Propolis, just like honey, has been the subject of many studies due to its antimicrobial, antifungal, antiviral and hepatoprotective activities[45]. More recently, propolis has been investigated for its potential anticancer activities[20-22].

Propolis is a rich mixture of polyphenols, flavonoid aglycones, phenolic acids and their esters and phenolic aldehydes and ketones. As with all bee products, the exact composition will vary with the plants sampled and so also shows a biogeographic, seasonal and bee-species specificiy[46-49]. Polyphenolic compounds are known to have anticarcinogenic activity on murine tumor models[31,50-54]. In addition, caffeic acid, CAPE and quercitin can inhibit cancer cell growth[55-57]. Artepillin C, isolated from propolis, was reported to induce cytotoxicity of carcinomas and malignant melanoma cells by apoptosis, abortive mitosis and mass necrosis. The tumor growth suppression was likely to be due to its own direct cytotoxicity as well as enhanced immunity[51] and inhibition of lipid peroxidation[52]. Other studies have shown that three different propolins (A-C) induce apoptosis in human melanoma cells[58], whilst another compound from propolis (PM3) inhibits the *in vitro* growth of MCF-7 breast cancer cells and induces apoptosis[59].

One positive effect of anticancer therapy is the ability to initate apoptosis (regulated cell death) in cancer cells[60], and especially if specifically in cancer cells. Apoptosis is a natural mechanisim to regulate cell death in various developmental and functional stages. There are two main pathways of apoptosis. The first is induced by an external signal stimulated by TNF receptors, TNF-related apoptosis-inducing ligand (TRAIL)-R1 or death receptor 4 (DR4), and TRAIL-R2 (DR5). The second pathway is mediated by mitochondria and pro-apoptotic proteins, including cytochrome c[61]. The interest in propolis for anticancer therapeutics is due to its apparent ability to induce apoptosis, although the mechanism induced seems to be dependent on the type and concentration of the propolis extract[62]. Recent studies have suggested that the astaxanthin and flavonoids in propolis can protect SH-SY5Y cells (an adenergic cell line derived from a human neuroblastomic bone marrow) from beta-amyloid induced apoptotic death[63].

In vitro exposure to the water-soluble extracts of propolis (WSP) increased was more effective against tumour growth and metastasis when given before tumor innoculation (prophylatic)[63-65] and had a strong antimetastatic effect upon the percentage of apoptotic rat hepatoma MCA cells from 20% (control) to 25% after exposure to 50 μg/mL of WSP for 15 h, but the percentage of apoptotic Chinese hamster lung fibroblast carcinoma V79 cells treated with the same WSP was much smaller at 10%, indicating the potential different degrees of sensitivity to propolis amoung cancer cells and normal fibroblasts[64,65]. Other studies have shown that propolis may induce apoptosis through activating the caspase-dependent pathway[66]. The caspase inhibitor Z-Asp-CH_2-DCB could completely prevent the *in vitro* DNA fragmentation stimulated by propolis in the U937, J447.1, Ps88, HL-60 and Jurkat leukemia cell lines, suggesting that the effect is not cell-specific[66].

The mechanism of propolis-induced apoptosis appears to be independent of the kind of cancer cells studied, but dependent on the concentration of the propolis extract. Thus, several studies have reported that propolis induces apoptosis through the release of cytochrome c from the mitochondria to the cytosol, through the caspase cascade and TRAIL signals[67].

Of the active compounds found so far in propolis, CAPE and chrysin appear to play a key role. CAPE exhibits strong antitumor effects in oral cancer cells, including the neck and tongue, and many proteins involved in the apoptotic process are affected by CAPE[68]. The mechanisms of inhibition of the activity of p53, p21, p38 mitogen-activated protein kinase (p38 MAPK) and c-Jun N-terminal kinase in tumour cells by CAPE[69,70], appear to result from the inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) that is associated with the down regulation of the inhibitors of apoptotic proteins (IAPs), such as cIAP-1 and cIAP-2 expression[71].

Chrysin, another bioactive component of honey that is also found at higher concentrations in propolis, has been shown

to have significant biological and pharmacological properties that include antioxidant and anti-inflammatory effects[72], as well as an anticancer property[73]. Chrysin influences the apoptotic process in many types of cell lines, especially leukemia, and induces apoptosis in these cells by activation of caspases, suppression of anti-apoptotic proteins, such as IAPs, cellular FLICE-like inhibitory protein, phosphoinositide 3-kinase (PI3K)/Akt signal pathway, and the inhibition of IκB kinase and NF-κB[73].

In conclusion, the induction of apoptosis in various cancer cells by propolis extracts (WSP) and its enriched active compounds, such as CAPE and chrysin, are dependent on the concentration of the products used. Propolis appears to induce apoptosis through the release of cytochrome c from the mitochondria to the cytosol, mediated through the caspase cascade and activation of pro-apoptotic proteins.

4. Royal jelly inhibition of N-acetyltransferase (NAT) activity in tumor cells

Arylamine carcinogens can induce some tumors in humans, but these carcinogens require further metabolic activation to be able to exert genotoxicity within the target organs. N-acetylation is one of the metabolic pathways that activate arylamine carcinogens and is also believed to be an important step in arylamine metabolism. N-acetylation catalyzed by NAT requires acetyl coenzyme A as its cofactor, and is an important enzyme in the biotransformation and metabolism of various drugs and compounds that may play an important role in the etiology of bladder, breast and colorectal cancers[74,75]. Indeed, the genes coding for NAT (NAT1 and NAT2) are polymorphic and specific variants may be related to an increased risk of cancer in individuals.

Royal jelly, sectreted from the salivary glands of worker bees, is a special food that influences the development of female bee larvae, where a diet low in royal jelly allows the development of larvae into worker bee adults, but larvae feed sufficient royal jelly instead develop into queen bees[76]. It is comprised of free amino acids, polypeptides, sugars, fatty acids (mostly 10-hydroxy-2-decanoic acid), minerals and vitamins. In humans, the oral consumption of royal jelly is known to decrease the total serum cholesterol level, but it can cause an IgE anaphylactic reaction in atopic women[77]. Lyophilized royal jelly has been reported to prevent hyperlipidermia and improve the coagulaation status of blood in rats[78]. Moreover, it has been reported that royal jelly has a potential antitumor activity in mice[79]. The relationship between royal jelly and N-acetylation in the metabolism of 2-aminofluorene (2-AF) was evaluated in human liver tumor cells, where cytochrome P450 was found to be important in the metabolism of N-acetylated AF (2-AAF) by converting N-hydroxy-2-AAF to the mutagenic and potentially carcinogenic product R16[80]. Whether royal jelly can inhibit cP450 activity in the human hepatocellular carcinoma derived J5 cell line is not known, but it decreased NAT activity and the N-acetylation of 2-AF cells[80]. In another study the administration of royal jelly before or after a tumor transplant was found to be ineffective against either preventing the growth and development or the metastasis of the tumor, but when coadminsitered with the tumour cells it effectively inhibited metastasis[31].

5. Bee venom in cancer therapy

Bee venom, a complex mixture of substances, is used to defend the bee colony against a broad diversity of predators from other arthropods to vertebrates. Bee venom, produced in the venom gland located in the abdominal cavity, contains several biologically active peptides, including melittin, apamin, adolapin, mast cell degranulating peptide and many enzymes, plus also non-peptide components, such as histamine, dopamine, phospholipase A_2 (PLA_2) and norephnephrine[81,82]. Bee venom has traditionally been used as a non-steroidal anti-inflammatory drug for the relief of pain and the treatment of chronic inflammatory diseases, such as rheumatoid arthritis and multiple sclerosis, as well as in the treatment of tumors[83-86].

Bee venom inhibits the proliferation of carcinoma cells and tumor growth *in vivo* due to the stimulation of the local cellular immune responses in lymph nodes[83,87-89]. The mechanism of action of bee venom involves apoptosis, necrosis and lysis of the tumor cells[87-89]. Current research shows that bee venom induces apoptosis in human leukemic cells, but not in murine bone marrow cells, via the induction of Bcl-2 and caspase-3 expression through the downregulation of mitogen-activated signal pathways[90]. Bee venom has also been reported to induce apoptosis through caspase-3 activation in synovial fibroblasts[91] and to inhibit cyclooxygeneses-2 expression in human lung cancer cells[87].

Melittin is the major protein component in bee venom, comprising some 40%-60% of the venom, and is the principal toxin causing inflammation, pain and sensitivity[85], but has also attracted considerable attention for its potential use in cancer therapy[92]. Melittin is a water soluble, cationic, amphiphilic α-helical peptide of 26 amino acid residues that is known to exert a variety of membrane-pertubing effects, such as hemolytic and antimicrobial activity[93]. Melittin can also induce structural alterations of membranes, including pore formation, fusion and vesiculation, which ultimately lead to hormone secretion, aggregation of membrane proteins, and a change in the membrane potential, but this action is equally effective against normal cells[94-97]. Moreover, melittin is a potent inhibitor of calmodulin (and so cell growth) and can stimulate a diverse array of signal transduction enzymes, including G-proteins, protein kinase C, adenylate cyclase, PLA_2 and phospholipase C[95-98]. Within G-protein mediated signal transduction, melittin directly stimulates nucleotide exchange by heterotrimeric GTP activity by reducing the affinity of both GTP and GDP to Gs[99]. These diverse effects suggest that melittin exerts multiple effects on cellular functions. Although it was of interest that melittin binds to some cancer cells at a higher affinity than to normal cells, and so potentially allowing a low dose

selectivity for action against the transformed cells than normal cells[100,101], the current use of melittin is based upon target specific nanoparticle delivery.

The activation of PLA_2 can have a cytotoxic effect on cancer cells through several subsequent cellular changes. Melittin-induced cell necrosis was ameliorated by a calpain protease inhibitor, which suggests that PLA_2-mediated calpain activation might be a therapeutic strategy for inhibiting cancer cell growth by melittin, since the TNF-α-induced activation of cytosolic PLA_2 is an important component of the signaling pathway leading to cell death[102]. Moreover, melittin increased the membrane permeability of L1210 cells and so perturbed the membrane integrity.

Tumor metastasis is a complex process involving extensive interactions between the tumor cells and host tisues, but it is the major cause of death in cancer patients as well as in the limitation of relatively simple treatments (localised chemo- and/or radio-therapy or surgical tissue removal) compared to systemic treatment. It can be roughtly divided into the four steps of (i) tumor cell dissociation, (ii) intravasation and crudation, (iii) arrest and extravasation and (iii) adhesion, angiogenesis and proliferation[103]. Bee venom has been shown to directly inhibit the invasive and migratory ability of human breast (epithelial) cancer MCF-7 cells via the suppression of MMP-9 expression, and this could be mimicked by melittin, but not by apamin and PLA_2. Thus, the specific inhibition of MMP-9 by bee venom is likely to be mediated by melittin alone or perhaps in conjunction with other less common compounds[98]. These results indicate that bee venom is a potential anti-metastatic and anti-invasive agent that may merit future clinical research on its potential anti-cancer properties.

6. Conclusions

Several bee products have been found to have anticancer activity *in vitro* on a range of tumor cell lines, including renal, lung, prostate, bladder, melanoma, osteosarcoma, mammary and lymphoid cancer derived cell lines. In addition, most of the reports on the mechanism of action of bee products in inhibiting tumor growth *in vitro* and *in vivo* suggest it is mediated via apoptosis, necrosis, and lysis of the tumor cells.

Honey and cancer have a sustainable inverse relationship in the setting of developing nations, where resources for the production of honey (and to a certain extent other bee products) are plentiful but resources for standard cancer prevention are limited. The mechanism on how bee products induce apoptosis and cell-cycle arrest is still of great interest for future research.

Propolis induces apoptosis pathways in cancer cells, with CAPE and chrysin being identified as the two main agents that are the cause of the antiproliferative effect by changingthe expression of cancer relating genes.

Royal jelly affected the N-acetylation and inhibited the metabolism of 2-AF in the human liver tumor cell line in a dose-dependent manner and also decreased the profile of 2-AF metabolites in J5 cells.

Bee venom has been widely used in the treatment of some immune-related diseases as well as tumor treatments in modern days. Several cancers cells can be the potential targets of bee venom peptides, mediated through PLA_2 inhibitors, such as melittin. The cell cytotoxic effects mediated through the activation of PLA_2 by melittin have been suggested to be the critical mechanism for the anti-cancer activity of bee venom.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Some bee products such as honey and propolis have been used as medicines against human diseases in the world. In the present research, authors have summarized the recent various knowledge concerning the bioactivities of the products, especially anticancer activity.

Research frontiers

This research is dealing with the relationship between honeybee products and the cancer being regarded as one of the most serious diseases nowadays, from the point of view of anticancer activity of honey, propolis, royal jelly, and bee-venom, respectively.

Related reports

Some plant species produce characteristic antibiotics to protect themselves from various enemies. Some honeybee species collect the antibiotics for protecting themselves and their nest, that is, propolis. Therefore there have been many reports concerning propolis, however, with very few reports regarding the relation between propolis and tumor cells so far.

Innovations and breakthroughs

One of the honeybee products is propolis which has been well known to have various effective bioactivities. In the present study, authors have indicated enlighteningly that a

certain propolis should have the anticancer activities.

Applications

This valuable research survey shows that some of the honey products such as propolis have notable anticancer activity for men, and would encourage further actual applications.

Peer review

Having read through the present manuscript, I could interpret that the authors dealt with the bee products from the honeybees species belonging to the subfamily Apinae, which contains less than 10 species. There are two types of beekeeping in the world. One is called Apiculture which keeps Apinae bees. The other is Meliponiculture where bees belonging to the subfamily Meliponinae, which contains about 400 species. Meliponinae bees also produce honey and propolis which have been utilized for people widely in tropical and subtropical areas.

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