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The Immunomodulatory and Anticancer Properties of Propolis

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Abstract Propolis, a waxy substance produced by the honeybee, has been adopted as a form of folk medicine since ancient times. It has a wide spectrum of alleged applications including potential anti-infection and anticancer effects. Many of the therapeutic effects can be attributed to its immunomodulatory functions. The composition of propolis can vary according to the geographic locations from where the bees obtained the ingredients. Two main immunopotent chemicals have been identified as caffeic acid phenethyl ester (CAPE) and artepillin C. Propolis, CAPE, and artepillin C have been shown to exert summative immunosuppressive function on T lymphocyte subsets but paradoxically activate macrophage function. On the other hand, they also have potential antitumor properties by different postulated mechanisms such as suppressing cancer cells proliferation via its anti-inflammatory effects; decreasing the cancer stem cell populations; blocking specific oncogene signaling pathways; exerting antiangiogenic effects; and modulating the tumor microenvironment. The good bioavailability by the oral route and good historical safety profile makes propolis an ideal adjuvant agent for future immunomodulatory or anticancer regimens. However, standardized quality controls and good design clinical trials are essential before either propolis or its active ingredients can be adopted routinely in our future therapeutic armamentarium.

Keywords Immune · Anticancer · Propolis

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The Pharmacological Background and General Properties of Propolis

Propolis is a resinous product collected by honeybees from plants, and its use as a folk medicine can be traced back to more than 2,000 years ago. Propolis was mainly used by bees for building their hives and for protection against intruders. Therefore, the original Greek word "Propolis" means "defense of the city." The basic composition of propolis consists mainly of extracts collected from plant exudates mixed with the metabolites from bees. Therefore, the constituents of propolis from different sources also differ from each other depending on the species of the plants and flowers from which they are derived [1–3].

Propolis has been adopted by a wide range of ethnic groups since ancient times. Egyptian used propolis to embalm cadavers; Greek and Roman physicians employed propolis as an antiseptic; and Incas used propolis as an antipyretic. In Chinese culture, propolis has been used as an anti-infection and anticancer remedy. In the modern era, propolis has been purported to exert antiviral, antibacterial, antifungal, anti-inflammatory, and hepatoprotective properties [1, 4, 5]. It has been proposed that such diverse effects are in part due to its immunomodulating properties [4, 6].

Propolis is one of the most popular forms of alternative medicine. In 1997, the annual global consumption of propolis was estimated to be about 700–800 tons/year [7].

Propolis has been used as an ingredient for toothpaste, mouthwash [8], and cosmetic products such as face cream and lotions. Propolis is marketed in many different forms such as capsules, hydroalcoholic extract, creams, powder, or chewing gum and is available over the counter. It has also been applied as varnishing material for musical instruments [9] and as a chemical preservative for meat products [10].

There have been more than 300 compounds identified from propolis so far [11]. The compounds identified in propolis are mainly polyphenols [2]. The major polyphenols found in propolis are flavonoids, accompanied by phenolic acid and aldehydes, phenolic aldehydes, ketones, etc. Other compounds are volatile oils and aromatic acids (5–10 %) and waxes (30–40 %), and the rest are minor miscellaneous chemicals. Some essential elements like magnesium, calcium, iron, nickel, and zinc as well as vitamins have also been found in propolis [12]. Recently, new compounds have been isolated from propolis, such as 3,5-diprenyl-4-hydroxycinnammic acid (artepillin C) from Brazilian green propolis, and it may be one of the key immunomodulatory components [13].

The chemical composition of propolis can be highly variable depending on the geographic location. For example, Brazilian propolis, which is a form of tropical propolis, is significantly different from propolis found in the temperate zone because of the differences in the vegetal sources [2, 11]. Furthermore, the propolis from different regions of Brazil still displays significant variation in their chemical compositions [14]. Among the Brazilian propolis, the green propolis in southern Brazil, which has *Baccharis dracunculifolia* as its major plant source, is the most popular and well studied. The other famous type is the Brazilian red propolis which is collected in northern Brazil.

General Immunological Properties of Propolis

Propolis has been found to have a wide spectrum of biological and pharmaceutical properties and has been demonstrated to have direct antimicrobial effects in vitro [15], without the presence of an immunological response. Aga et al. found in 1994 that Brazilian propolis is effective against microbes such as *Bacillus cereus*, *Enterobacter erogenous*, and *Arthroderma benhamiae*. However, some recent studies suggested that Brazilian green propolis also has immune-enhancing effects both in vitro and in vivo [16]. Propolis has been found to exert cytotoxic effects on malignant cancer cell lines indirectly through activation of macrophages [5, 17]. Its anti-inflammatory role and wound healing-promoting properties have also been reported [18, 19]. In general, recent studies suggested that the immunological properties of different propolis are mostly mediated via direct immunosuppression and result in a decrease in the release of immunostimulatory cytokines (Table 1). Due to the difference in study design, the exact underlying mechanisms of these actions remain uncertain at this time. Propolis is relatively safe for human use, except for inducing allergic dermatitis in some individuals [1, 4].

Table 1 Recent studies on the immunological effects of propolis

Propolis source	Extraction method	Species tested	Effects	References
Brazil	70 % EtOH	Mouse	Stress-induced suppression of IL-6, IL-1B, TLR2, and TLR4 expression in mice. Propolis normalizes the TLR4 but not IL-6, IL-1B, and TLR2.	[47]
Brazil	70 % EtOH	Mouse	Stress-induced suppression of IFN-γ, IL-10, and IL-4 secretion in mice. Propolis normalizes the IL-4 but not IFN-γ and IL-10.	[79]
Brazil	70 % EtOH	Mouse	Propolis inhibits lymphocytes proliferation, with or without Con-A stimulation. It also decreased the production of IFN-γ.	[80]
North China	80 % EtOH and water	Mouse	Both ethanol extract and water-soluble fraction inhibit the activation and differentiation of monocytes/macrophages. Propolis suppresses IL-6 but not IFN-γ or IL-2.	[81]
Poland	EtOH/chloroform and water	Human	Propolis suppresses cytokines produced by monocytes/macrophages (IL-1β, IL-12), Th1 (IL-2), and Th2 (IL-4) cells. But there is a corresponding increase in TGF-β1 secretion by T reg cells.	[46]
Turkey	96 % EtOH	Human	Propolis inhibits the production of proinflammatory cytokines such as IFN-γ and TNFα under PHA stimulation.	[82]
Brazilian or Bulgarian	70 % EtOH	Human	Propolis increases bactericidal activity of macrophages by enhancing H_2O_2 and NO production.	[83]
Korea	95 % EtOH	Mouse cell line	Propolis inhibits LPS- and IFN-γ-induced NO production through inhibiting NFκB pathway.	[84]
Africanized honeybee and Bulgarian	Ethanol	Rats	Propolis, but not its purified compounds caffeic acid and quercetin, can increase antibody production.	[85]

Propolis from Different Geographic Sites has Different Immunomodulatory Effects

Variations in the biological effects of propolis obtained from different geographic locations have been reported and it was suggested that they have different constituents depending upon the plant species and the local climate and environment [20, 21]. We compared the immunological effect of propolis ethanol extracts obtained from four different locations, namely, Brazil, New Zealand, China, and Tasmania on resting human peripheral blood mononuclear cells. We studied the proliferation by using XTT colorimetric assay (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide). This is similar to MTT assay but with a higher sensitivity and dynamic range. We then confirmed our findings by trypan blue assay. Of the four types of propolis studied, there were no significant proliferative effects on peripheral blood mononuclear cells, with the exception of Brazilian green propolis. Interestingly, we found that the modest "stimulatory" effect of Brazilian green propolis was not because of an increase in cell proliferation but rather an increase in cellular mitochondrial activities (i.e., elevated XTT activity but no increase in trypan blue assay). The inhibitory effect of propolis from New Zealand, China, and Tasmania were due to their cytotoxic effect on human peripheral blood mononuclear cells, and all of them induced cell death in a dose- and time-dependent manner. These findings highlight that propolis from various origins have different biological effects and the use of a single biological assay can be misleading in the interpretation of the results.

This unique difference between Brazilian green propolis and the other three extracts could be explained by the variation in their composition, since Brazilian green propolis is the only propolis collected from a tropical region. The similar cytotoxic effect of propolis from New Zealand, China, and Tasmania might be due to their similar chemical profile, as a previous report suggested that the constituents of propolis from temperate regions are more or less the same [22]. The extraction method for the preparation of extract is another factor that can influence the effect of propolis [23]. Therefore, future standardization of the extraction methods and appropriate selection of specific propolis will be vital for comparison of the results in the literature. In addition, whether other fractions of propolis such as the water fraction can exert different immune effects deserve further investigation.

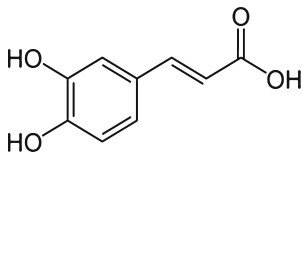
Caffeic Acid Phenethyl Ester and Artepillin C are the Two Immunopotent Chemicals of Propolis

Among the large number of chemical compounds identified from propolis [22], caffeic acid phenethyl ester (CAPE) is mainly found in propolis from the temperate regions but has only trace amounts in most tropical propolis such as Brazilian green propolis. CAPE is a caffeic acid derivative (Fig. 1) and is one of the most potent bioactive ingredients of propolis that can be synthesized in the laboratory [24]. The anti-inflammatory and antitumor properties of CAPE have been investigated by many scientists [25–27]. CAPE exerts its antitumor effect through the inhibition of DNA synthesis [28]; interruption of growth signal transduction [29]; induction of apoptosis via the intrinsic apoptotic pathway [30]; and promotion of antiangiogenic effects [31, 32].

CAPE is a potent inhibitor of T cell receptor-mediated T cell proliferation. This inhibition is via suppression of both IL-2 gene transcription and IL-2 synthesis in stimulated T cells [33]. CAPE inhibits the binding and DNA transcriptional activity of NFκB and NFAT which are two key transcription factors for T cell activation. CAPE can also inhibit dendritic cell maturation after in vitro lipopolysaccharide (LPS) stimulation [34, 35].

Another important and specific component of propolis is artepillin C (3,5-diprenyl-4-hydroxycinnamic acid), which is a low-molecular-weight phenolic compound (Fig. 1). It is uniquely found in Brazilian green propolis and is one of its main constituents. Artepillin C can also be synthesized in the laboratory [18]. It was first isolated by a Japanese group [36] and was found to have anticancer properties [37, 38]. In contrast, artepillin C can protect the nontumorigenic liver cell line, HepG2, from oxidative damage [39]. The bioavailability of artepillin C has been studied in vivo using mouse models [40]. So far, no significant toxicity has been observed.

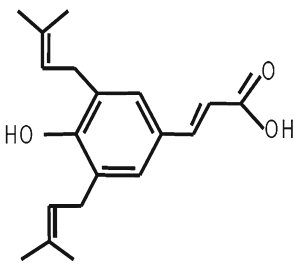
The in vitro and in vivo antitumor effects of artepillin C on different types of cancer have been studied by different groups. Artepillin C can induce apoptosis of leukemic cells in vitro and in vivo [37] and it can also suppress human



CAPE
Caffeic acid phenyl ester
Molecular Formula: $C_{17}H_{16}O_4$

Molar mass :284.31g/mol

Appearance: yellow / light yellow powder
Solubility:organic solvent



Artepillin C
3,5-diprenyl-4-hydroxycinnamic acid
Molecular Formula: $C_{19}H_{24}O_3$

Molar mass: 300.39g/mol

Appearance: white powder

Solubility: organic solvent

Fig. 1 The chemical structure and physical and chemical properties of CAPE and artepillin C

colonic cell growth by inducing G_0/G_1 cell cycle arrest through upregulating Cip1/p21, a cyclin-dependent kinase inhibitor [41]. In addition, it blocks the PAK1 signaling so as to inhibit the growth of neurofibromatosis-associated tumor [42]. Oral administration of artepillin C has also been reported to prevent the development of adenoma into adenocarcinoma in a mouse model [43].

However, studies on the immunological role of artepillin C have been far less than that of CAPE, probably due to its lower prevalence in Brazilian green propolis alone. Artepillin C can indirectly kill cancer cells by increasing the T cell-mediated cytotoxicity by increasing the CD4/CD8 ratio and the total number of T cells in vivo [37]. It can also inhibit NFκB activity in macrophages so as to inhibit the exacerbation of carrageenan-induced paw edema in a mouse model [40]. Artepillin C can inhibit the neutrophil mobilization into the inflammatory area. Therefore, artepillin C can serve as antiallergic agent [18]. A summary of recent findings on the immunological properties of both CAPE and artepillin C are listed in Table 2.

Brazilian Propolis but not Artepillin C can Stimulate the Proliferation and Survival of Human Monocytes

Peripheral blood mononuclear cells include different cell populations, mainly monocytes, T cells, B cells, and natural killer cells. Among these cell populations, we found that Brazilian green propolis mainly acted on stimulated CD14+ monocytes. The percentage of CD3 + CD4+ T cells was also increased; however, the effect of propolis on the proliferation of these cell types was quite variable. This may be due to individual variation depending on the existing immune status of the donors. Our findings related to the stimulatory effect of Brazilian green propolis on human monocytes were consistent with previous in vitro and in vivo studies which suggested that propolis activates resting macrophages, a cell type derived from monocytes. Table 3 summarized some of the previous findings about the effect of different propolis on macrophages.

Brazilian green propolis exerted a stimulatory effect on CD14+ monocytes and also enhanced the survival of these monocytes (Fig. 2). In our previous experiments, only a negligible amount of LPS was detected in our samples of Brazilian green propolis. Therefore, we can rule out the possibility of contamination by LPS, since it has been reported that LPS can stimulate monocyte proliferation and survival in vitro [44]. Artepillin C, which is a major and unique component of Brazilian green propolis, does not account for the property of Brazilian green propolis on monocytes.

The antiapoptotic effect of Brazilian green propolis raised the question as to what happens to monocytes after they are stimulated by Brazilian green propolis. Surprisingly, we found that Brazilian green propolis induced monocytes to differentiate into CD14lowHLA-DRhigh monocytes (Fig. 2). The effect of Brazilian green propolis might be also due to its summative effects on a panel of cytokines [45, 46]. But this proposal remains controversial [47]. We found that TNFα, a monocyte-stimulating factor, was not involved in the antiapoptotic property of Brazilian green propolis. However, more studies are required to further delineate the mechanism(s) behind the antiapoptotic effect of Brazilian green propolis on monocytes.

Table 2 Recent studies on the immunological properties of CAPE and Art-C

Chemicals	Species	Effects	References
CAPE	Human	Presence of CAPE in the system inhibited SEB- and PHA-induced T cells proliferation, downregulated the expression of CD69, CD25, and ICAM-1, prevented the entry of the cells in the S-phase of the cell cycle, and inhibited IL-2 transcription and synthesis and NFκB and NFAT transcription.	Marquez et al. [33]
CAPE	Human	Inhibited cytokine production in mature monocytes-derived dendritic cells stimulated by LPS and by crude mite extract. No effect on the upregulation of costimulatory molecules and antigen-presenting ability. The suppression of cytokine is mainly due to the inhibition of IκBα phosphorylation and NFκB activation.	Wang et al. [34]
CAPE	Mouse	Splenocytes from CAPE-treated mice were activated in vitro in the absence of CAPE. Con-A induced T cell proliferation, increased IL-4 and IL-2, increased anti-CD3 activated splenocytes, and increased CD4/CD8 ratio.	Park et al. [27]
Artepillin C	Mouse	Increased CD4/CD8 ratio.	Kimoto et al. [37]
Artepillin C	Mouse	Inhibited the growth of pokeweed mitogen-stimulated normal blood lymphocytes, but not cytocidal to normal unstimulated lymphocytes.	Kimoto et al. [38, 43]
Artepillin C	Mouse	Downregulated PGE2 production in vivo and nitric oxide by RAW 264.7 cells in vitro.	Paulino et al. [40]

Table 3 The recent findings related to the effect of propolis on macrophages

In vitro/in vivo	General effect on macrophage	Reference
In vitro	Activation	[74]
Mouse	Activation	[17]
Mouse	Activation	[86]
In vitro	Antiapoptosis	[87]
Mouse	Activation	[83]
Mouse	Activation	[88]

Brazilian green propolis alone does not induce TNFα production by monocytes. However, TNFα production was significantly upregulated by Brazilian green propolis-primed monocytes after LPS stimulation (Fig. 2). This implies that the immunomodulatory effect of Brazilian green propolis is indirectly induced by priming monocytes to become more sensitive to bacterial endotoxin challenges. Further studies are required to confirm whether the increase in TNFα production in Brazilian green propolis-primed monocytes after LPS stimulation is due to the enhanced survival or differentiation of proinflammatory monocytes. However, the limitation of all current studies is that they have not been done on human subjects. There are almost no reports about which factors can affect the differentiation of proinflammatory monocytes in vivo. Further studies addressing the effect of Brazilian green propolis on proinflammatory monocyte differentiation might help us understand the immunomodulatory properties of Brazilian green propolis on monocytic lineage. It will provide us with new insights on identifying novel immune enhancement therapy for such diseases of severe infections or cancers.

Suppressive Effects of Brazilian Green Propolis and Artepillin C on Both Activated T Cells and Leukemic Cells

T cells are another major cell population of peripheral blood mononuclear cells and play an important role in immune defense. The immune system plays a pivotal role in the human body's defense against infection. There is a delicate balance in the immune system so that the immune defense will not target self-antigens. However, a defect in such immune tolerance towards self-antigens will lead to deleterious autoimmune diseases. The ability for immune cells to distinguish self from nonself allows an individual to clear exogenous antigens. However, this self-preservation characteristic becomes a barrier during allogeneic bone marrow transplantations. Undesired recipient's T cell activation can lead to graft rejection.

The immunomodulatory effect of Brazilian green propolis in resting human peripheral blood mononuclear cells is mainly due to their action on CD14+ monocytes. However, T cells are another major component of peripheral blood mononuclear cells that may be affected. Surprisingly, we found that Brazilian green propolis suppressed T cell proliferation and activation in our mixed leukocyte reaction model [13]. We further identified that artepillin C, which is reported to be a unique compound present in Brazilian green propolis, can be an active ingredient responsible for the immune effect of Brazilian green propolis on activated T cells.

We did not observe any significant immunostimulatory effects on T cells by Brazilian green propolis. However, on the contrary, we found that it could suppress peripheral blood lymphocyte proliferation in a dose-dependent manner in our mixed leukocytes reaction model [13]. This observation demonstrated the possible differential role of Brazilian green propolis on resting T cells and activated T cells. In addition, the immunosuppressive property of Brazilian green propolis on T cells suggests a new potential anti-inflammatory property.

CD4+ T cell activation orchestrates the immune responses and is responsible for initiating T cell proliferation. By using the same mixed leukocytes reaction model, Brazilian green propolis was not only found to suppress the initiation of CD4 T cell proliferation, but could also inhibit activated T cells (Fig. 3). The inhibitory effect of Brazilian green propolis on CD4 T cells was partly due to selectively inducing apoptosis in the proliferating T cells and not by the

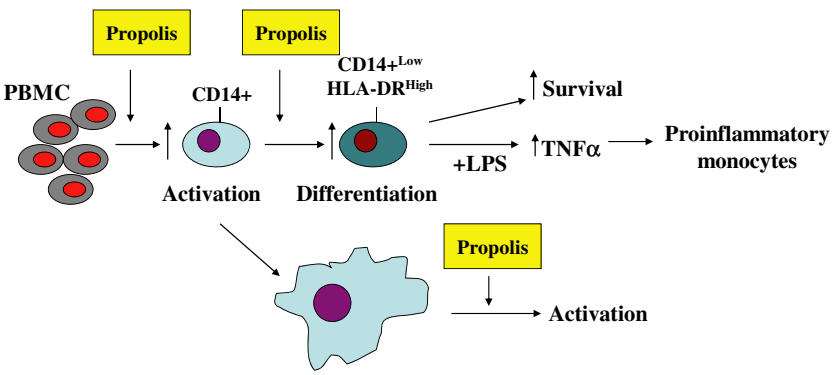


Fig. 2 Propolis but not artepillin C can induce monocyte and macrophage activation and differentiation into proinflammatory phenotypes

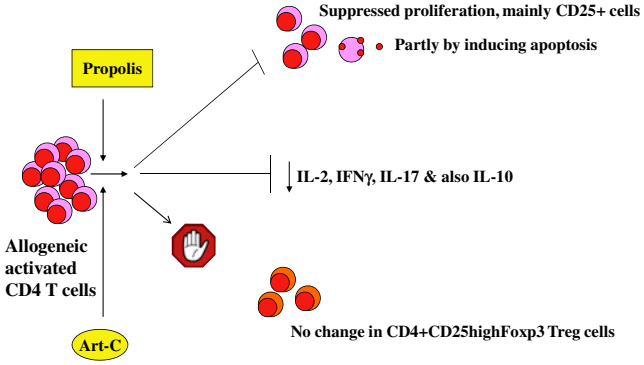


Fig. 3 Both propolis and artepillin C can suppress allogeneic activated CD4 T cells

induction of regulatory T cells. However, the suppressive effect of Brazilian green propolis was reversible, after either restimulation by the same irradiated stimulator cells or by IL-2. T cell activation and cytokine production were also inhibited in the presence of Brazilian green propolis.

Despite the promising results of the suppressive effect of Brazilian green propolis on T cells, it may be difficult to apply it clinically to patients. Propolis is a mixture of chemicals and their constituents vary with many different environmental factors such as climate and plant sources[48, 49]. Standardization for propolis is difficult even for Brazilian green propolis, which has relatively stable chemical components when using standard extraction protocols. In addition, the clinical outcome of using Brazilian green propolis as an immunosuppressant for treatment of graft versus host disease (GVHD) remains to be shown clinically.

On the other hand, artepillin C is uniquely found in Brazilian green propolis and has not been found to cause allergy. Artepillin C is also one of the active ingredients responsible for the immunosuppressive property of Brazilian green propolis. We measured its effect on peripheral blood lymphocytes and CD4 T cells using the mixed leukocytes reaction model. As expected, artepillin C suppressed T cell proliferation and activation by the same mechanisms as that of Brazilian green propolis. The major difference found between artepillin C and Brazilian green propolis was related to the inhibitory effect on cytokines produced by artepillin C which did not last as long as that exerted by Brazilian green propolis. The suppressive effects of artepillin C were mediated through direct cytotoxic effects on the early stage of responder T cell activation. Although we do not know whether artepillin C can activate other immune cells, the findings of Paulino et al. in 2008 supported ours by demonstrating that artepillin C suppressed macrophage activation.

Graft versus leukemia is an important response to help eradicate leukemia after hematopoietic stem cell transplantation. However, this response is always inhibited by the immunosuppressants targeted at treating GVHD. Hence, a good immunosuppressant for the treatment of GVHD should be able to inhibit T cell activation and at the same time suppress the cancer cells. Artepillin C at concentrations that can suppress T cell proliferation showed a significant inhibitory effect on leukemic cell growth, especially for T lineage leukemia. On the other hand, it had no effect on other normal blood cells. Thus, we postulate that artepillin C can serve as a novel immunosuppressive drug for the treatment of GVHD.

In fact, mixed leukocytes reaction has provided a functional read out for the immunosuppressive effect of artepillin C. The actual cell–cell interaction of GVHD is extremely complicated in vivo. The most optimal routes of delivery and the pharmacokinetics of artepillin C for effective therapy remain undetermined. Further studies on the effects of artepillin C on T cell activation, differentiation, and effector functions in GVHD clinical setting is mandatory. We are currently studying a murine in vivo bone marrow transplantation GVHD model, and hopefully, this will verify our in vitro findings. The mechanisms of how artepillin C suppresses T cell expansion and function should be further explored. It would be interesting to explore the effect of artepillin C on T cell leukemia in vivo and whether or not artepillin C can reverse common leukemic drug-resistant mechanisms.

Anticancer Properties of Propolis

After analyzing the current knowledge on the immunological effects of propolis, we explored another common clinical application of propolis—as an anticancer therapeutic agent. As we elaborated in the immune effect section, we also found that some of the crude or fractionated propolis obtained from different countries exerted modest cytotoxic effects on a panel of leukemia or solid tumor cell lines (Chan, unpublished data). However, the in vitro model may not reflect the bioavailability issue correctly, and the effects of intermediate active chemical metabolites after liver enzymatic modifications may also be missed. We, therefore, reviewed the currently available in vivo data including results from both animal studies and human clinical trials. There is much more available information on the in vivo anticancer effect of propolis when compared to that of immune effects. Collectively, the potential anticancer effects of propolis can be summarized into the following possible mechanisms: (1) suppressing cancer/precancerous cells proliferation via its immunomodulatory effect; (2) decreasing the cancer stem cell populations; (3) blocking specific oncogene signaling pathways; (4) exerting antiangiogenic effects; (5) modulating the tumor microenvironment; and lastly, (6) as an adjunct or complementary treatment to existing mainstream anticancer therapies (Fig. 4).

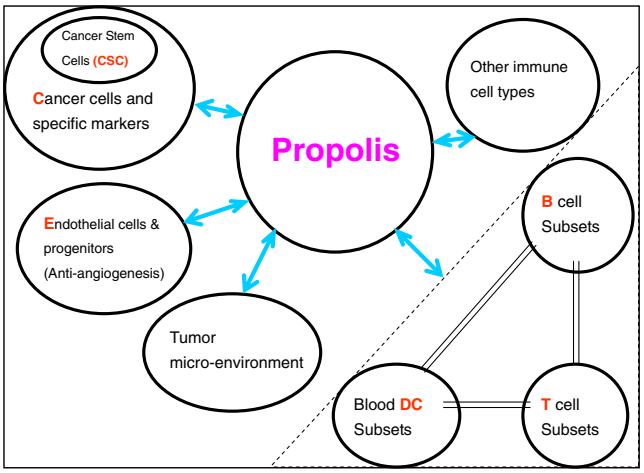


Fig. 4 The multidirectional anticancer action of propolis

Propolis can Suppress Cancer or Precancerous Cells via its Immunomodulatory Effect

Propolis has been studied as a cancer preventive medication in the setting of chronic skin inflammation. By using a UV-induced inflammatory hairless mouse model with an increased risk of photocarcinogenesis, ethanol crude extract from Australian propolis could minimize the skin inflammation by exerting its immunosuppressive effect and reducing lipid peroxidation [50]. It could also protect against sunburn edema and contact hypersensitivity in a dose-dependent manner. Propolis could suppress the typical photoimmune response by suppressing the proinflammatory cytokines IL-6 and IL-12 but overexpressing the immune-tolerant cytokine IL-10.

However, for solid organ precancerous conditions, the protective effect of propolis remains controversial. Brazilian propolis was unable to render any hepatoprotective effect in rats exposed to diethylnitrosamine (DEN), a liver cancer initiation agent [51]. However, studies on the effect of oral chrysin (5,7-dihydroxyflavone), a bioflavonoid found in propolis that has potent aromatase inhibitory effect on similar DEN-initiated hepatocarcinoma in a rat model, found that the number and size of cancer nodules formed were significantly reduced [38, 52]. This hepatoprotective effect was also reflected in the reduction of serum levels of liver parenchymal and ductal enzymes [53]. Summing up these studies together, it is possible that propolis has a mixture of compounds and their summative effects do not have any hepatoprotective effect. However, the individual compound may behave differently from that of the mixture. We should, therefore, interpret the results cautiously before jumping to premature conclusions.

In 2011, Badr et al. reported on the ability of water-soluble Egyptian propolis derivatives to inhibit the proliferation of Ehrlich ascites carcinoma (EAC) cells transplanted intraperitoneally in mice. Mice were given propolis by the enteral route through gastric gavaging prior to intraperitoneal injection of EAC cells. Then the mice were given daily propolis for 11 successive days. It was found that the volume of ascites fluid and the total number of EAC cells were reduced in the propolis-treated group. There was increased phagocytic activity and increased lymphocyte transformation rate and granulocytosis/monocytosis found in the mice treated with propolis suggestive of a possible immunostimulatory effect exerted by propolis [54]. However, there was no appropriate control group in this study and it would be difficult to conclude whether or not the beneficial effects were derived solely from propolis administration.

Propolis may Decrease Cancer Stem Cells Population

Other than using propolis to target cancer or precancerous cell populations, there are reports that propolis may be useful in targeting cancer stem cells. Using the putative breast cancer stem cells with the characteristic CD44(+)/CD24(−/low) phenotype and the capability to generate mammospheres from single cells, it was found that CAPE, a propolis derivative, caused a dose-dependent inhibition of all three properties of cancer stem cells including self-renewal, progenitor formation, and clonal growth [55]. The cancer stem cells population also dropped significantly.

Our laboratory has also previously reported that Brazilian propolis that contains only minute amounts of CAPE could also reduce the number of putative cancer stem cells (Ahmed and Sze, unpublished data). Using the bioprospecting concept for novel drug discovery, we incubated a myeloma cell line (RPMI 8226 cells) with crude Brazilian propolis extracts for 24 h. Half a million multiple myeloma cells were then incubated with Hoechst 33342 at 5 μg/mL in a 37 °C water bath for 90 min and examined with flow cytometry using UV excitation to determine the proportion of side population (SP), which is one way to identify putative cancer stem cells. Propidium iodide (PI) was added to detect viable cells. Solvent, PI, and culture medium were separately used as negative controls. We found that propolis crude extracts used at concentrations that killed half of the myeloma populations (range, 15–50 μg/mL) demonstrated a significant reduction in the SP of myeloma cells. The percentage of SP in the Brazilian propolis-treated group was decreased dramatically to 0.14 %, while the percentage of SP in the negative controls remained at around 17 %. The live cell populations in the three negative controls were similar. However, both our and Omene's studies were in vitro laboratory studies and further in vivo animal studies are needed before we can draw any definite conclusions.

Artepillin C or CAPE from Propolis can Block Specific Oncogene Signaling Pathway

It was reported that artepillin C, a major component in Brazilian green propolis extract, can completely suppress the growth of human neurofibromatosis-associated tumor xenografts in mice through the blocking of oncogenic PAK1 signaling [42]. Interestingly, a similar inhibitory effect can be induced by CAPE as well. It is known that propolis with CAPE are different from those with artepillin C. It was reported that using CAPE-rich water-miscible extract "Bio-30" of New Zealand propolis [56] could completely suppress the growth of a human NF1 cancer known as malignant peripheral nerve sheath tumor in mice. Bio-30 also induced nearly complete regression of human NF2 tumor (schwannoma) in mice. It was noted that the poor bioavailability and water solubility of CAPE rendered it unusable clinically. An attempt to make CAPE soluble was done by using Bio-30 that contained high amounts of lipids that could dissolve CAPE. The good animal results seen may also be due to the synergistic interactions of other propolis ingredients with CAPE. Interestingly, CAPE was also found to be a potential selective estrogen receptor modulator by its actions as a selective agonist to hERbeta, but it does not show any estrogenic effect on estrogen receptor-positive breast cancer cells [57].

Propolis can Exert Antiangiogenic Effects

Since cancer tissues require the support of both oxygen and nutrients to sustain rapid uncontrolled proliferation, it was interesting to find out that propolis has antiangiogenic effects. It was found that the ethanol extract of Brazilian propolis could significantly reduce the number of newly formed vessels in in vitro tube formation assays [58]. Such an effect was mainly mediated through inducing apoptosis in tube-forming endothelial cells through the inactivation of the survival signal ERK1/2 [59]. Furthermore, the author demonstrated that it was the artepillin C in the propolis which accounted for such action.

Another group focused on the effects of propolis and its related products on vascular endothelial growth factor (VEGF)-induced human umbilical vein endothelial cells (HUVEC) proliferation and migration [60, 61]. They found that, while royal jelly and Chinese red propolis suppressed both VEGF-induced HUVEC proliferation and migration, bee pollen and CAPE suppressed only proliferation. The order of suppression of VEGF-induced in vitro tube formation was, in descending order, CAPE > Chinese red propolis > bee pollen > royal jelly. The effect of CAPE on angiogenesis was not mentioned in the previous studies, but it is possible that a different form of propolis was studied. It is known that Brazilian propolis contains more artepillin C but not CAPE.

Propolis can Modulate the Tumor Microenvironment

It has been known that the cancer microenvironment consists of stromal, endothelial, immune, and cancer cells. This environment is an integral part of the life cycle of a tumor. Clinically, intervening in the symbiosis of cancer microenvironments can be achieved by using thalidomide in multiple myeloma [62] or vincristine in leukemia [63]. Interestingly, both thalidomide and vincristine are both potent immunomodulator and antiangiogenesis agents. They modulate the tumor microenvironment as one of the "multidirectional" activities. Other than directly inducing cancer cells apoptosis, they also act via their antiangiogenesis and immunomodulation effects. It was found that CAPE from propolis could effectively suppress the adhesion and invasion potential of human hepatocellular carcinoma cells (SK-Hep1) by totally abolishing the expression of MMP-2 and MMP-9. It was postulated that such action was related to the inhibition of the NFκB pathway [64].

Recently, a Japanese group used an array of laboratory technologies to examine various compounds derived from Brazilian green propolis and found that some compounds significantly inhibited the expression of the HIF-1α protein and HIF-1 downstream target genes such as glucose transporter 1, hexokinase 2, and VEGF-A [65]. This study demonstrates to us that cancers and their tumor microenvironment can be tackled via novel approaches such as combining modern technology and knowledge of traditional herbal medicine (i.e., integrative medicine).

Propolis can Potentiate the Anticancer Effect or Reduce the Therapy-Induced Toxicity of Various Chemotherapeutic Agents

Both Western and traditional Chinese medicine treat cancers with a combination of agents. Therefore, it is natural to find that there is an emerging trend of research exploring the potential of propolis extracts as an adjunct or complementary therapy to mainstream anticancer chemotherapies or radiotherapies.

A few studies investigated the additive or synergistic effects of propolis extracts in mainstream anticancer chemotherapies by using an in vivo murine model. Padmavathi et al. studied the synergistic effects of propolis ethanol extract with paclitaxel by using a DMBA-induced breast cancer model in the rat. They found that the group with combined usage of paclitaxel and propolis achieved the lowest tumor weight compared to those with paclitaxel alone, propolis alone, or untreated controls [66].

Aside from potentiating the anticancer effect, propolis can also help to reduce therapy-induced toxicity of some cytotoxic agents. Water-soluble Croatian or Brazilian

propolis extracts and related flavonoids were found to decrease irinotecan-induced toxic effects on normal blood, liver, and kidney cells without affecting the irinotecan cytotoxicity in the Ehrlich ascites tumors cell in vivo model [67]. A similar protective effect of propolis can be found in tamoxifen-induced hepatotoxicity in another rat cancer model. Pretreatment with CAPE starting 10 days before tamoxifen injection significantly prevented the tamoxifen-induced liver toxicity as shown by the reduction in the elevation of hepatic parenchymal enzymes [68].

In another in vivo murine study evaluating the rate of induced lung metastases by infusing transplantable mammary carcinoma cells intravenously [69], water-soluble Croatian or Brazilian propolis extracts were given either intraperitoneally or orally (50 or 150 mg/kg) in combination with the chemotherapeutic agent epirubicin or radiotherapy. Cancer cells were injected intravenously and then the numbers of metastases to the lung were assessed. The propolis plus chemotherapy regimen significantly reduced the lung metastatic rate compared to the treatment arms containing either epirubicin or propolis alone ($P<0.001$). Moreover, the life span in propolis-treated mice was significantly longer after radiotherapy with gamma rays compared to those without propolis.

These in vivo animal studies support the use of propolis in human clinical trials in future precancerous or cancer scenarios. Indeed, there have been some preliminary human clinical trials using propolis mainly as an anti-infection agent [70, 71]. A Brazilian study investigated the efficacy of orally administered Brazilian propolis in treating *Helicobacter pylori* infection[72]. *H. pylori* infection is a known predisposing factor for gastric cancer development. The treatment regimen used 20 drops of Brazilian green propolis ethanol extract three times daily for 7 days. Except for two participants who suffered from mild nausea and one participant who complained of epigastric pain, no other adverse effects were noted. There was a 20 % decline in the observed values of Urease Breath Test, which is a surrogate marker for intragastric bacterial load, in half of the 18 participants.

Conclusions

The current in vitro and some preliminary in vivo data suggest that propolis has immunomodulatory, antitumor, and antimicrobial properties. Propolis from different geographic locations may have different active ingredients. Artepillin C is one of the major compounds uniquely found in Brazilian green propolis [13], and CAPE is one of the major components of many propolis found in the temperate zones. This wide spectrum of therapeutic effects makes propolis a potential candidate in several clinical scenarios. Propolis and its active ingredients artepillin C or CAPE have all been shown to have immunomodulatory effects on a wide spectrum of immune cells including those of lymphoid or monocytic lineages. We found that both propolis and artepillin C can kill leukemic cells and at the same time induce immunosuppression on T cells; therefore, it is an ideal compound for future clinical trials in this setting. Propolis and its active ingredients artepillin C and CAPE also have potential antitumor properties and they exert such action by different mechanisms including suppressing cancer cells proliferation via its immunomodulatory effects; decreasing the cancer stem cell populations; blocking specific oncogene signaling pathways; exerting antiangiogenic effects; and modulating the tumor microenvironment. The relatively easy administration by the oral route and good safety profile as reflected by its long history of usage since ancient times makes propolis an ideal adjuvant agent for future anticancer regimen. However, robust manufacturing processes, standardized quality controls, and good design clinical trials are all critical steps in verifying these claims.

Propolis is still a treatment with unknown efficacy and safety for many diseases. While it has been extensively studied in cancer, potential uses may include treatment of immunological diseases such as allergy, asthma, or atopic dermatitis, as indicated by its immune effects [73–76]. Herbal medications [77] and dietary manipulation and supplements [78] have been used for centuries for the treatment of autoimmune and allergic diseases and are now just beginning to be studied in a scientific manner. Along with propolis, many of these therapies may be used more prominently as more information about their components and mechanism of action is elucidated.

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