
SCIENTIFIC EVIDENCES TO PHARMACOLOGICAL ANTICANCER ACTION OF *Baccharis dracunculifolia* BRAZILIAN PROPOLIS

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Abstract

Propolis is a traditional nutritional supplement produced by bees and widely used as a folk remedy around the world. Its chemical composition and biological activities varies depending on the geographical location, botanical origin, season and the type of bees. In Brazil, the most popular and the most researched grade is “green” propolis, largely produced in the State of Minas Gerais, from the plant *Baccharis dracunculifolia*. This specific grade of propolis when properly processed shows remarkable activities as an antitumoral (cytotoxic, anti-proliferative and antimetastatic), analgesic and anti-inflammatory, immune-modulator, antioxidant, antimicrobial and healing agent. It has been safely used either by doctors or as a folk remedy in Brazil, Japan and many other countries as supportive nutrition along with orthodox cancer treatment, or supporting biological therapy of cancer. Paulino et al., here reviews his own propolis studies and many other peer reviewed publications on its pharmacological inter-and intracellular pathways. He will demonstrate that *Baccharis dracunculifolia* propolis can modulate interesting cellular targets in different cancer cells, such as nuclear transcription factor (NFκB); post translational prenylation in Ras-GTPase signalling; p38-MAPK, PI3K/Akt/PKB pathway; COX-2 and prostaglandin E₂ pathway; and iNOS or e-NOS expression and respective nitric oxide production. In addition, it modulates DNA fragmentation induced by cytochrome-C pathway; p53-protein signalling; releasing of pro-apoptotic proteins Bax and Bak; inhibition of neoangiogenesis by modulation of matrix metalloproteinases (MMPs) and vascular endothelial growth factor (VEGF) expression; control of the cell differentiation by modulating p21(Waf1/Cip1) protein in association with CDK2 and cyclin E. Finally, *Baccharis dracunculifolia* propolis has been used in associative therapy to improve the efficacy of chemotherapy drugs (e.g. combination with paclitaxel, resveratrol, vinorelbine, etc) while shortening treatment time and reducing treatment side-effects (with both synthetic chemotherapy and radiotherapy). Recently published results suggest that *Baccharis dracunculifolia* propolis can be used for supporting biological therapy or in association with chemotherapy drugs or radiotherapy, as a natural food supplement to help to treat or prevent cancer. Clinical and pharmacokinetics trials with animals and humans will be mentioned.

Key-Words: Propolis

INTRODUCTION

Propolis is a natural product produced by bees and widely used to treat several diseases. Bees collect from some plants resins and balsamic substances, these substances are transported to beehives, and used to close the apertures and to protect the beehives against the bacterial and fungal contamination. This resinous substance is called propolis on beehive. This propolis is re-collected, clean, and triturated and percolated in standard conditions. This propolis extract is used to prepare various pharmaceutical formulations: capsules, creams, oral spray, shampoos, etc.

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In Central and East Europe main botanical source to produce propolis is *Populus nigra*. This propolis is brown and its chemical composition is based main in caffeic acid derivatives such as CAPE and aryl derivatives (Allergenic components in European *Populus* propolis).

In Brazil we have many regions and many types of forests. Main regions are: in North - Amazonian Forest where propolis is unknown yet. In Central - Pantanal is a great plan with a long time under water and the propolis is unknown yet too. In South of Brazil we have two different types of forest, in mountain, the Araucarian forest, and in the cost, the Atlantic forest (there the propolis is Brown, and we have many studies about it. In Northeast we have two regions near to cost of Atlantic Ocean, mangue and the propolis is red under new investigation now, because the first trial shown great cytotoxicity in cancer cell lines. And the second is the cerrado and propolis is unknown yet with few pharmacological studies. The last and more studied propolis become from Minas Gerais State in the Southeast of Brazil. In this region the mountain and forests containing a lot of *Baccharis dracunculifolia*, main botanical source to produce Green propolis.

Brazilian propolis resin or crude extract productions are better organized in the South and Southeast and the best Brazilian propolis is exported to Japan and Europe. Therefore, recently the Brazilian industry improves the pharmaceutical technology process to produce formulations and increase the value of the Brazilian propolis. Main Brazilian propolis is Brown from two botanical sources *Araucaria angustifolia* and *Eucalipto citriodora*, and the Green from *Baccharis dracunculifolia*. Brazilian Green propolis is produced by mean of interesting mechanism: *Baccharis dracunculifolia* during specific time in the year produce a pathological process similar to cancer. During this time honey bees collect the resin from buds of *Baccharis dracunculifolia* in order to repair the hive and to protect it from bacteria and various diseases.

Chemical composition is variable in accord with each region which honey bees collect the resin. General chemical information includes: 55% resins and balsams, 30% wax, 10% pollen, and secondary metabolics including: phenolic acids and its esters, flavonoids, terpenes, beta-steroids, aromatic acids and alcohols, sesquiterpenes, derivatives from estilbene, etc...

The chemical composition of *Baccharis dracunculifolia* Premium Brazilian Propolis was determined by high performance liquid chromatography using a Merck-Hitachi apparatus (from Germany), equipped with a pump (model L-7100, Merck-Hitachi) and a diode array detector (model L-7455, Merck-Hitachi). Please, note here the high concentration of ARTEPILLIN C (87.97 mg/g) in the sample. Therefore, the interesting family of compounds found main in the Brazilian propolis is the prenylated cinnamic acid derivatives. These compounds are potent antioxidant, anti-inflammatory, analgesic, hepatoprotective etc like described in the literature.

BIOLOGICAL ACTIVITIES IN CANCER

The most spectacular growth in the biological area of propolis is the antitumoral activity. Since 2003 several papers were published with focus in intracellular of transducing signaling. Most recent publication show important targets in cancer cells, such as pro-apoptotic proteins (Bax, Bak, Caspase 3, cytochromo C) on regulation of cell differentiation (p38, p56, p21 and CDKs) or in inflammation associated to cancer, nuclear factor kappa B, inhibitory protein kappa B, cyclooxygenase two, nitric oxide two.

Here, we have shown some pharmacological mechanisms of nutrition supplement with propolis: suggesting potential action on cancer.

Induction of apoptosis

The first interesting approach is to induction of apoptosis into cancer cells. This purpose is reviewed by Reed and Pellecchia (2005) into hematologic malignancies and shown several pharmacological targets in research on pharmaceutical industry in preclinical, or in phase one, two or three. The main of chemical leads addressing apoptosis-relevant targets including BCL2, death receptors, p53, Akt and IKK or NFκB.

Normal cell change to cancer cell phenotypic because of some external or internal stimulus. After stimulus the cell produce the apoptotic protein on cytosol, like BAX/BAK, that act on mitochondria. Mitochondria is the source of energy on cell, and under BAX and Bak can induce to release of three proteins: Smac-diablo, cytochromo C and AIF. Smac Diablo protein can module the apoptotic response; Cytochromo C bind to APAF1 protein and produce a complex that activate the caspase 9; AIF protein has the function of nuclear transcription factor on apoptosis. In the APAF1 and cytochromo C complex formation, Cytochromo C reductase inhibits it. The complex Cytochromo C plus APAF1 stimulate the action of Caspase 9 that is an apoptotic protein that activate the caspase 3 by enzymatic cleavage of pro-caspase 3 on cytosol. Caspase 3 is a protease extremely potent, and inhibits the cell differentiation on G1-S and also translocate to nucleus where promote DNA damage by nuclease enzymatic action. This effect on DNA promote the break down of the cancer cell reducing the size of tumor and reducing the metastasis possibility.

One of the first studies showing the anticancer effect of propolis was published by Scheller et al. (1989). They analyzed the effect of ethanolic extract of propolis (EEP) on mature mice bearing Ehrlich carcinoma. They concluded that propolis reduced the tumor cell viability *in vivo* by means of cytochrome-C-reductase inhibitor. Kimoto et al. (1996) showed for the first time that Artepillin C extracted from Brazilian propolis produces a change in the cell cycle and apoptosis of cancer cells.

Kimoto et al. (1998) showed that when Artepillin C was applied to human and murine malignant tumor cells *in vitro* and *in vivo*, it produced a cytotoxic effect and the growth of tumor cells was clearly inhibited. Artepillin C was found to cause significant damage to solid tumor and leukemic cells by an MTT assay, a DNA synthesis assay, and morphological observations *in vitro*. The cytotoxicity of green propolis may be related to DNA fragmentation and the consequent apoptosis induced by Artepillin C. Another hypothesis suggests that propolis may reduce lipid peroxidation and invigorate the immune system by means of lymphocyte activation. An elevation in the TCD4/CD8 cell rate and in total number of defense cells was observed. This effect was demonstrated in renal carcinogenesis induced by ferric nitrilotriacetate in mice (Kimoto 2000), in apoptosis of human leukemia cells (Kimoto et al., 2001a) and in pulmonary carcinogenesis induced by ferric nitrilotriacetate in mice (Kimoto et al., 2001b)

Other prenylated compounds found in propolis have a cytotoxic effect on three cancer cell lines through DNA fragmentation and efficiently induced apoptosis in the cancer cell lines, but had no effect on the cell cycle program (Chen et al., 2003). A similar effect was found for drupanin and baccharin in human cancer cell lines, though their effects were less potent than that of Artepillin C (Akao et al., 2003).

Regulation of Cell Differentiation

Radiation, chemotherapy or other stimulus can produce a cell response, normally mediated by increase of phosphorylative activity of p38 MAP Kinase and by expression of p53 protein. This protein regulates several genomic pathways, including transcription of the p21 Waf1/Cip1, a recognized cyclin dependent kinase (CDKs) inhibitor, can regulate the cell differentiation. It has been shown that the over expression of p21 Waf1/Cip1 induce the G1-S arrest in cancer cell lines and reduce de growth of the tumor.

Several reports in the literature prove that the cyclin-dependet kinase inhibitor p21^{WAF1CIP1} is a major player in the cell cycle and is mainly regulated at the transcriptional level (see Gartel and Radhakrishnan 2005 to recent review (Hershenson 2004).

In addition, Cheng et al. (2005), demonstrated the expression of the cyclin-dependent kinase inhibitor p21(Waf1/Cip1) was significantly increased in chrysin-treated cells. Both cyclin-

dependent kinase 2 (CDK2) and 4 (CDK4) kinase activities were reduced by chrysin in a dose-dependent manner. The authors suggest that chrysin exerts its growth-inhibitory effects either through activating p38-MAPK, leading to the accumulation of p21(Waf1/Cip1) protein. Similarly, Artepillin C was effective on colon carcinogenesis. Shimizu et al (2005) had found that Artepillin C inhibited cell growth, inducing G(0)/G(1) arrest. The events involved a decrease in the kinase activity of a complex of cyclin D/cyclin-dependent kinase 4. Artepillin C appears to prevent colon cancer through the induction of cell-cycle arrest by stimulating the expression of Cip1/p21, a useful chemoprevention factor in colon carcinogenesis.

Reduction of the inflammation and angiogenesis around tumor

We have shown our own results involving the reduction of the inflammation that combined with decrease of the angiogenesis, produce a good effect against tumor growth or metastasis. Tumor cells induce an inflammatory response during the first step of their growth. This effect is important because it leads to an increase in the local blood flow and other factors such as vasorelaxation, increase of local temperature and the induction of Vascular Growth Endothelial Factor or Matrix Metaloproteinase which induce neoangiogenesis and additional nutritional support to cancer cells. An important approach to decreasing the size of tumors and inhibiting metastasis is to decrease the inflammatory events associated with the onset of cancer

Inflammation is produced by mean of several pathways such as LPS or other inflammatory mediator that interact with a specific receptor like Tool Like Receptor (TLR) in membrane of the cells. This receptor starts a phosphorilative intracellular cascade. This effect activates the kinase of inhibitory kappa B (IKK) and the phosphorilation on inhibitory of NFκB protein (IκB). After this, NFκB is activates and start the nuclear translocation and gene expression, specially to inflammatory enzymes and proteins, including, cytokines: IL1, 12 e TNF-α; cyclooxygenase 2, and the consequent increase of production of PGE2. All cascades together produce inflammation: increase of vascular permeability, vasorelaxation, cell migration and established the perfect conditions to growth of tumor and metastasis.

In this case, Mirzoeva and Calder, studied the effect of propolis and its components on eicosanoid production during the inflammatory response and shown that propolis reduce the prostaglandins and leucotrienes production during the experimental inflammation. Ansoorge et al. (2003), described that propolis and some of its constituents down-regulate DNA synthesis and inflammatory cytokine production but induced TGFβ1 production of human immune cells.

But the most important results were shown by our research group. In the recent publication we have evaluated the analgesic and anti-Inflammatory effects of *Baccharis dracunculifolia* Brazilian propolis. Propolis reduced the oedema induced by carragenin, reduce the cell migration, and decrease the release of inflammatory cell mediators such as nitric oxide, prostaglandin E2, cytokine (IL1 e TNFα). We have shown that Brazilian green propolis and Artepillin C are potent NFκB inhibitor in HEK cells and can modulate COX2 and nitric oxide production in RAW 264.7 (Paulino et al., 2006).

Clinical pharmacokinetics assay with propolis

An interesting question is, how much and what chemical markers are absorbed to blood after intake of Green propolis? To answer this question we elaborate a pharmacokinetic experiment in volunteer human. In this experiments in clinical pharmacology we have assayed with a formulation containing *Baccharis dracunculifolia* Green propolis, named Cytopropolis®. All experimental procedures were realized in SOCIMED Hospital, Tubarão, SC, Brazil, under previous recommendation by Local ethical committee. Briefly, twenty volunteers (18-25 years both sexes) intake six capsules of Cytopropolis® (Pharmanectar Lote PADE1105-BIO, fab. in 2005/11) (700mg each)/volunteers, and after 1, 2, 4 and 24h the blood was collected, treated and the plasmatic concentrations of phenolic compounds was evaluated by HPLC assay. The results was present by mean of plasma total phenolic compounds concentrations (Artepillin C and derivatives, cinnamic acid and derivatives, pinobanksin, caffeoyl quinic and derivatives) after the consumption of Cytopropolis® supplement in 20 healthy subject volunteers.

We have shown that 16.8% of ingested Artepillin C was present in the plasma showing the apparent bioavailability of this compound in human in amounts that was pharmacological effective such as antioxidant and anti-inflammatory.

The last and most important question was: the preclinical effects are reproducible in human with cancer, during the nutrition supplement with propolis? Then we accompanied several patients supplemented with *Baccharis dracunculifolia* green propolis during their cancer treatment and evaluate the case reports in this Brazilian patients.

Case Report of Breast Cancer

An interesting case of breast cancer as reported in follow by clinical history is reported by Dr. Ellen Silveira (Hospital das Clínicas de Minas Gerais):

Female, aged 60 years, aprx. 70kgs, married, 3 children, social drinker, non-smoker, family history negative for breast cancer (mother) alive currently at 82 years; sisters alive and well). Had a benign lump in the left breast at age 25 years following the birth of her first child. Had excisional biopsy; the result was negative for breast Ca. Extranumerary breasts (both sides). Menopause at age 49 years following elective hysterectomy (reports heavy bleeding and premenopausal symptoms which started at age 42 years (anxiety, hot flushes, headache and anemia of 6g/dl). Had 2 extranumerary breasts surgically removed in 1998. Started annual check-up mammograms at age 40. Started HRT at age 44 years, irregularly and mainly as skin patches (could not take orally due to low tolerance).

Diagnosed with (bifocal invasive) breast cancer, i.e. 2 nodules in the right superior quadrant (RSQ) of the right breast following a mammogram (found inconclusive) and US (on 06/09/04). Had core biopsy of the right breast (plus PAAF of the left breast on the same occasion). Had quadrantectomy plus axilar dissection with removal of one metastatic linfonode (and a further 15 with reactional/inflammatory changes) on 13/09/04. Recommended radiotherapy and chemotherapy by oncologist. Refused chemo. Had a total of 31 sessions of radiotherapy daily, from Nov to Dec 2004. From January 2005 started on Tamoxifen (one tablet daily, recommended 5 years) and propolis extract (aqueous and alcoholic) initially for 3 months, then as capsules until Dec 2005.

Since January 2006 has been taking a total of 4 capsules daily of cytopropolis. Reports increase in level of energy with propolis. History of pneumonia and chronic sinusitis which also improved following use. Follow-up every 6 months by a breast specialist.

Other info: current medication: antihypertensive (oral). A US of abdomen (2004) showed liver steatosis and hemangioma. High cholesterol (320mg/dl). Use of Sinvastatin and Fluoxetine (PROZAC) for 6 mths only.

The patients are until now very well and she is in continuous propolis consumption daily.

Finally, we have summarized the pharmacological evidences to action of propolis in cancer by mean of table 1 in attach. In conclusion we can suppose that there are some pharmacological evidences to use of *Baccharis dracunculifolia* green propolis can use of safety and effective nutritional supplement during the treatment of cancer.

REVIEW OF PHARMACOLOGICAL MECHANISMS OF ACTION TO ANTICANCER ACTIVITIES OF *BACCHARIS DRACUNCULIFOLIA* PROPOLIS AND ITS CHEMICAL CONSTITUTION

GENERAL MECHANISMS	ESPECIFIC MECHANISM	EFFECT	CÉLL TARGET	PROPOLIS OR COMPOUND	REFERENCE
INDUCTION OF APOPTOSIS	Cytochromo C reductase inhibition	Inhibition of cytochromo C reductase preserve the complex Cytochromo C and APAF1 active to long time	<i>In vivo</i> in Ehrlich tumor	Ethanollic extract of propolis	<u>Scheller S, Krol W, Swiacik J, Owczarek S, Gabrys J, Shani J.</u> Antitumoral property of ethanolic extract of propolis in mice-bearing Ehrlich carcinoma, as compared to bleomycin. Z Naturforsch [C]. 1989 Nov-Dec;44(11-12):1063-5.
	DNA Fragmentation	DNA programmed destruction and lost of protein synthesis capacity. Increase of immune cell response and change of radio CD4/CD8.	Solid tumor and leukemia cell	Artepillin C extracted from <i>Baccharis dracunculifolia</i> propolis	Kimoto T, Arai S, Aga M, Hanaya T, Kohguchi M, Nomura Y, Kurimoto M. Cell cycle and apoptosis in cancer induced by the artepillin C extracted from Brazilian propolis. Gan To Kagaku Ryoho. 1996 Nov;23(13):1855-9 Kimoto T, Arai S, Kohguchi M, Aga M, Nomura Y, Micallef MJ, Kurimoto M, Mito K. Apoptosis and suppression of tumor growth by artepillin C extracted from Brazilian propolis. Cancer Detect Prev. 1998;22(6):506-

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		Nucleosomal DNA fragmentation	HL60	Baccharin e Drupanin isolated from <i>Baccharis dracunculifolia</i> propolis	Akao Y, Maruyama H, Matsumoto K, Ohguchi K, Nishizawa K, Sakamoto T, Araki Y, Mishima S, Nozawa Y. Cell growth inhibitory effect of cinnamic acid derivatives from propolis on human tumor cell lines. <i>Biol Pharm Bull.</i> 2003 Jul;26(7):1057-9
	Direct activation of Caspase 3	Increase of nuclease activity and DNA fragmentation	Human promonocytic leukemic cell line U937	Chrysin, found in <i>Baccharis dracunculifolia</i> propolis	Woo KJ, Jeong YJ, Park JW, Kwon TK. Chrysin-induced apoptosis is mediated through caspase activation and Akt inactivation in U937 leukemia cells. <i>Biochem Biophys Res Commun.</i> 2004 Dec 24;325(4):1215-22
		Aumento direto da expressão de p53 com efeito pró-apotótico nas células tumorais	Linhagem de células de cancer de próstata resistente à androgeno DU145	Extrato etanólico de própolis	Scifo C, Milasi A, Guarnera A, Sinatra F, Renis M. Resveratrol and propolis extract: an insight into the morphological and molecular changes induced in DU145 cells. <i>Oncol Res.</i> 2006;15(9):409-21
		Ativação da p38-MAPK levando ao acúmulo de	Célula de glioma C6	Crisina (5-Hidroxi-flavona),	Weng MS, Ho YS, Lin JK. Chrysin induces G1

Regulação da diferenciação celular	Aumento da atividade da p21, p27 e p16	p21(Waf1/Cip1) ou por inibição da atividade proteosoma		comum na própolis verde de Bacharis	phase cell cycle arrest in C6 glioma cells through inducing p21Waf1/Cip1 expression: involvement of p38 mitogen-activated protein kinase. <i>Biochem Pharmacol.</i> 2005 Jun 15;69(12):1815-27
		Aumento da expressão da p21(Waf1/Cip1)	Células isogênicas de carcinoma de colo retal	Artepillin C	Shimizu K, Das SK, Hashimoto T, Sowa Y, Yoshida T, Sakai T, Matsuura Y, Kanazawa K. Artepillin C in Brazilian propolis induces G(0)/G(1) arrest via stimulation of Cip1/p21 expression in human colon cancer cells. <i>Mol Carcinog.</i> 2005 Dec;44(4):293-9
Redução da inflamação associada ao tumor	Inibição da via do NFκB (Fator de Transcrição Nuclear tipo kappa B)	Inibição direta da atividade do NFκB (p50-p65), reduz a expressão de moléculas pró-inflamatórias como COX2, iNOS e citocinas	Células embrionária de rim humano transfectada com pLuc-NFκB	Extrato etanólico de própolis Pharmanectar e Artepillin C	Paulino N, Teixeira C, Martins R, Scremin A, Dirsch VM, Vollmar AM, Abreu SR, de Castro SL, Marcucci MC. Evaluation of the Analgesic and Anti-Inflammatory Effects of a Brazilian Green Propolis. <i>Planta Med.</i> 2006 Aug 10; [Epub ahead of print]
		Redução da expressão de COX2 limita a	Células de macrófagos murinos	Crisina	Woo KJ, Jeong YJ, Inoue H, Park JW,

		<p>produção de prostanóides inflamatórios na fase aguda da inflamação, diminuindo a vasodilatação e a nutrição do tumor. A redução da produção de IL8 e TNFα evita a cronificação da inflamação</p>			<p>Kwon TK. Chrysin suppresses lipopolysaccharide-induced cyclooxygenase-2 expression through the inhibition of nuclear factor for IL-6 (NF-IL6) DNA-binding activity. FEBS Lett. 2005 Jan 31;579(3):705-11</p>
<p>Redução da produção de mediadores pró-inflamatórios</p>					
	<p>Redução da peroxidação lipídica</p>	<p>Redução do dano oxidativo às membranas celulares.</p>	<p>Tumor sólido e células leucêmicas</p>	<p>Artepillin C</p>	<p>Kimoto T, Arai S, Aga M, Hanaya T, Kohguchi M, Nomura Y, Kurimoto M. Cell cycle and apoptosis in cancer induced by the artepillin C extracted from Brazilian propolis. Gan To Kagaku Ryoho. 1996 Nov;23(13):1855-9.</p> <p>Kimoto T, Arai S, Kohguchi M, Aga M, Nomura Y, Micallef MJ, Kurimoto M, Mito K. Apoptosis and suppression of tumor growth by artepillin C extracted from Brazilian propolis. Cancer Detect Prev. 1998;22(6):506-15</p>

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