REVIEW ARTICLE

Baccharin from Green Propolis: A Review of its Antiproliferative Effects

Gabriel S. De Sicco[1], Gabriela de O. Almeida[2], Débora M. Rodrigues[3], Jairo K. Bastos[3], Vanessa L. Campo[4*]

Abstract

Baccharin (3-prenyl-4-dihydrocinnamoyloxy-cinnamic acid) is a prenylated phenolic compound found in the extract of the *Baccharis dracunculifolia* plant and green propolis. It is one of the main components of this resinous material, which results from the interaction between bees and plants. This substance has been extensively studied for its biological effects, mainly its anti-inflammatory and antitumor activities. In this literature review, we have compiled several articles available between 1999 and 2022 discussing scientific research on baccharin. The objective is to explore this compound's already evaluated beneficial effects in different types of tumors. The cancer begins with a genetic mutation that transforms a normal cell into a mutated cell. Subsequently, important mechanisms for its development and progression occur, including increased proliferation, evasion of apoptosis, angiogenesis, invasion, and metastasis. In this regard, numerous studies are being conducted to identify natural compounds that can act on these stages of carcinogenesis, including the components of green propolis. It has already been demonstrated that baccharin can act at different stages of tumor development, exhibiting antimitotic, antiangiogenic, pro-apoptotic, and immunomodulatory activities. Additionally, baccharin has been found to interfere with carcinogenic metabolic pathways and exhibit genotoxic effects. Baccharin has demonstrated significant antitumor activity when applied to melanoma cells, colon cancer, gastric cancer, hepatocarcinoma, sarcoma, prostate cancer, breast cancer, and leukemia tumor cells. Therefore, the studies cited in this review provide evidence of baccharin's ability to inhibit relevant steps in the carcinogenic process of different types of tumors.

Keywords: Baccharin; Green propolis; Antitumor activity; Tumors; Carcinogenesis.

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Introduction

Propolis is a resinous material composed by a complex mixture of substances. It is formed with the help of honeybees, as these insects collect resinous, gummy, and balsamic substances present in plant secretions and add salivary secretions to this mixture.[1] Since ancient times, propolis has been used due to its numerous pharmacological properties. The Greeks, Egyptians, and Arabs extensively applied propolis in its natural form for the treatment of wounds and illnesses.[2]

Crude propolis is a lipophilic material that is typically found in a solid state. At 15°C, it is hard and brittle, while at temperatures above 30°C, it becomes malleable and sticky. The color of propolis can vary, ranging from green and red to brown. This color variation is directly associated with the geographic location and the vegetation from which it originated.[3]

Considering that bees visit multiple plant species, which vary depending on the region, a significant variation can be observed in the composition of propolis, including its active principles and their concentrations in the mixture. *Baccharis dracunculifolia*, commonly known as "*alecrim-do-campo*", is one of the primary plant sources of propolis in southeastern Brazil, and it is also widely found in the southern region of the country.[4] During the collection of plant resin, *Apis mellifera* bees break off vegetative apexes of *B. dracunculifolia* and transfer the resin to the corbicula, where it is stored and transported back to the hive. This resin is then used in the production of green propolis. As a result, the resin mixture obtained will contain the active principles naturally present in the plant species, with a particular emphasis on baccharin (3-prenyl-4-dihydrocinnamoyloxy-cinnamic acid),

[1]Faculty of Medicine, Barão de Mauá University Center, Ribeirão Preto, São Paulo, Brazil

[2]School of Pharmaceutical Sciences of Ribeirão Preto, Department of Clinical, Toxicological and Bromatological Analysis, University of São Paulo, Ribeirão Preto, São Paulo, Brazil.

[3]School of Pharmaceutical Sciences of Ribeirão Preto, Department of Pharmaceutical Sciences, University of São Paulo, Ribeirão Preto, São Paulo, Brazil.

[4]Faculty of Medicine, Barão de Mauá University Center, Ribeirão Preto, São Paulo, Brazil. University of São Paulo School of Pharmaceutical Sciences of Ribeirão Preto, Department of Pharmaceutical Sciences, Ribeirão Preto, São Paulo, Brazil.

Corresponding Author: Vanessa L. Campo, Faculty of Medicine, Barão de Mauá University Center, Ribeirão Preto, São Paulo, Brazil. University of São Paulo School of Pharmaceutical Sciences of Ribeirão Preto, Department of Pharmaceutical Sciences, Ribeirão Preto, São Paulo, Brazil, Email: vanessa.campo@baraodemaua.br

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a prenylated phenolic compound.

Baccharin, along with other components of green propolis, has been the subject of extensive research due to its biological effects. Among the various actions attributed to this cinnamic acid derivative, its notable antitumor activity will be the focus of this literature review article.

Methodology

A literature review was conducted to explore the antitumor effects of baccharin in various types of cancer. Recognizing the importance of expanding and deepening scientific knowledge regarding alternative therapies targeting such effects, we thoroughly searched the literature using databases such as the MedLine Center for Biotechnology Information (PubMed), Google Scholar, and SciFinder. The search was performed using the following keywords: "Green propolis," "Baccharin", "*B. dracunculifolia*", "Cancer", "Tumor" and "Antitumor activity." In accordance with inclusion and exclusion criteria, and after analyzing the abstracts, a total of 56 articles published between 1999 and 2022 were selected for further analysis and review.

Discussion

The cancer results from an initial genetic alteration or mutation resulting from intrinsic factors, such as random errors at the time of cell replication, or extrinsic, with induction by radiation and viral infections being important examples in this case. However, it is worth noting that the formation of the tumor does not only result from an initial genetic alteration but also from other associated genetic modifications that function as propagators of this mutation.[5] In this sense, various changes in cell physiology lead to the transformation of a normal cell into a cancer cell, including self-sufficiency in proliferative signals, insensitivity to growth-inhibitory signals, evasion of apoptosis, unlimited replicative potential, sustained angiogenesis, tissue invasion, and metastasis.[6]

The components of green propolis have been extensively studied for their various biological actions, with antitumor activity being one of the main focuses. As a result, this compound has been found to exhibit antimitotic, pro-apoptotic, and antiangiogenic activities and immunomodulatory capacity. Additionally, it has been observed that the compound has genotoxic effects and can interfere with metabolic pathways. Specifically, effective action has been demonstrated against various types of tumors, including melanoma, colon cancer, gastric cancer, leukemia, hepatocarcinoma, sarcoma, prostate cancer, and breast cancer.[7-11]

Baccharin mechanisms of action

The proposed mechanisms of baccharin against tumors are summarized in Figure 1 and described in the following sections.

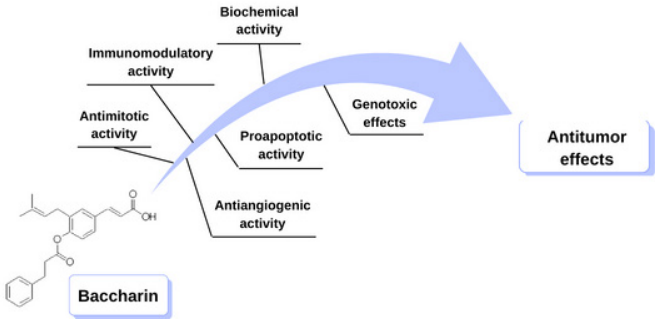

Figure 1: Flow chart of the proposed mechanisms of Baccharin against tumors

Antimitotic activity

The cell cycle is a process that involves the duplication of genetic material and nuclear division (mitosis). It naturally occurs in all tissues and is regulated by growth factors, steroid hormones, and cytokines produced by the cells themselves, which stimulate the resting cells. One of the factors that predisposes the occurrence of cancer is the mutation in proto-oncogenes involved in the positive control of the cell cycle. In the case of cells with mutations in these genes, there is an overstimulation of cell replication events, leading to uncontrolled proliferation.[12] Therefore, mitotic activity is considered a crucial factor in the growth and progression of cancer, and substances that act as antimitotics hold promise for the treatment of tumors. The antimitotic property of baccharin was mentioned in a study conducted by Gastaldello et al. (2021), where its effects were evaluated in animals with induced melanoma.[7]

Antiangiogenic activity

Angiogenesis is another crucial factor in the development of tumors and is promoted by the expression of endogenous angiogenic factors and cytokines. Solid tumors often have a disorganized vasculature, leading to local variations in blood flow and oxygen levels. In response to decreased oxygen tension, the body produces angiogenic factors such as vascular endothelial growth factor (VEGF) and increases the expression of certain cytokines, the main one being interleukin-8 (IL-8). This results in the formation of new blood vessels in the region, providing continuous nourishment and oxygenation to the growing and proliferating cells. Moreover, it facilitates the occurrence of metastases as tumor cells can easily migrate to other regions of the body.[13] Therefore, the inhibition of angiogenesis is an interesting mechanism for preventing the development and progression of tumors. This is one of the actions elucidated for baccharin in the study conducted by Gastaldello et al. (2021). In the study, the number of blood vessels was quantified using optical microscopy in different fields of melanoma tumors in both treated and untreated animals. The results showed a reduction in the formation of new blood vessels in animals treated with baccharin.[7]

Pro-apoptotic activity

Apoptosis is a programmed cell death mechanism that eliminates cells that are no longer needed by the body or that have suffered severe stress or damage. The signaling for a cell to undergo apoptosis can be intrinsic, activated by pro-apoptotic receptors on the cell surface, or extrinsic, activated by signals originating from intracellular mitochondria. In both cases, cell death is mediated by caspases.[14] Caspases are proteases that are found in their zymogenic (inactive) form within cells and are activated only when they receive signals from pro-apoptotic receptors or through the release of cytochrome C by mitochondria.[15]

The apoptotic process is a form of physiological cell death. However, in tumor cells, this mechanism becomes deregulated. Cancer cells can acquire the ability to resist apoptosis through various mechanisms, with one of the most common involving the p53 tumor suppressor gene, which is a pro-apoptotic regulator.[6] The p53 protein indeed plays a central role in inducing cellular responses to stress signals and cellular damage. It functions by binding to DNA and regulating the transcription of genes involved in various cellular processes, including the repair of genetic material, cell cycle arrest, senescence, and apoptosis.[16] In many types of cancer, mutations in the p53 gene occur, which confer resistance to apoptosis induction and allow the mutant cells to evade cell death. Apart from p53, there are numerous other anti- and pro-apoptotic proteins that can be involved in carcinogenesis. In general, this is observed when mutations occur involving the genes responsible for the synthesis of these proteins, leading to a change in the balance between them and thus causing a reduction in cellular apoptosis.[17]

In this context, compounds that restore the activation of apoptotic mechanisms have the potential to eliminate cancer cells that depend on these defects for survival.[17] In vitro studies have already revealed the pro-apoptotic action of baccharin in different tumor cell lines. The research conducted by Akao and collaborators (2003) demonstrated that compounds isolated from Brazilian propolis baccharin and drupanin were capable of inducing apoptosis in colon cancer (SW480) and leukemia (HL60) cell lines at doses exceeding 30 µM. Another study has also confirmed the pro-apoptotic effect of baccharin on colon cancer cells (DLD-1).[8] Kumazaki and collaborators (2014) showed that the combined treatment with baccharin and drupanin inhibited the growth of the cancer strains through the extrinsic apoptotic signaling pathway, by increasing the expression of TRAIL and FasL and by increasing the activation of caspase-8, as well as through the intrinsic pathway, through upregulation of mi-R143 levels.[18]

Immunomodulatory activity

A fascinating correlation has also been established between the tumor and the tissue's inflammatory environment. Initially, immune system cells contribute to the transformation of a normal cell into a cancerous one, and subsequently, they directly interfere with its development and progression as they begin to constitute the tumor microenvironment. In this context, the activation of neutrophils is cited as one of the main causes of tumor progression, being responsible for the stimulation of angiogenesis and, consequently, facilitating metastasis.[19] The experimental study carried out by Gastaldello and collaborators (2021) demonstrated an interesting action of baccharin in this protumorigenic mechanism.[7] It was reported that animals with melanoma treated with this compound showed a significant reduction in the number of circulating neutrophils in the bloodstream when compared to untreated animals.

However, the immune system's role isn't solely facilitating tumor progression. Certain cells contribute positively to its elimination, with macrophages playing an important role in this mechanism. These defense cells act by producing nitric oxide (NO) and tumor necrosis factor (TNF-α), which have the capacity to destroy cancer cells.[20] In this sense, another important finding of the study conducted by Gastaldello and collaborators (2021) was the increase in the number of macrophages in the tumor region in animals with melanoma treated with baccharin, indicating another possible mechanism of antitumor action of this compound.[7]

Biochemical activity

Another important mechanism involved in the antitumor action of propolis components involves their interference in carcinogenic metabolic pathways.[7] The uncontrolled growth characteristic of malignant transformation requires adaptive changes in various metabolic processes to satisfy the energy and biochemical needs of the tumor cell.[21] Thus, interference in such processes can be the target of compounds with antitumor action, such as baccharin.

A very relevant example of metabolic alteration is associated with the contribution of sex steroid hormones to the growth and progression of prostate and breast cancer. It has been observed that in hormone-dependent breast and prostate tumors, to enhance the synthesis of the most potent endogenous steroid (testosterone and 5α-dihydrotestosterone in the case of men and 17β-estradiol in women), cancer cells begin to overexpress aldo-keto reductase 1C3 (AKR1C3), the enzyme responsible for this conversion.[22,23]

In terms of baccharin's antitumor activity against these types of tumors, several studies have already demonstrated its impact on this metabolic pathway. Endo and collaborators (2012) revealed the capacity of baccharin to inhibit AKR1C3 potently and selectively, which could potentially suppress the proliferation of hormone-dependent cancer cells.[9]

Genotoxic effects

The repair mechanisms associated with DNA damage play a very important role in carcinogenesis, since most oncogenic changes in humans are caused by inefficient repair of damaged DNA.[24] Cells constantly suffer damage to their DNA. Cells with the damaged genetic material can carry out its repair with precision, restoring it to normality.

If the cell is unable to repair the damage, the DNA damage response (DDR) pathway is activated, leading to senescence or death. However, if the cell activates its repair machinery but the damage is not repaired properly, cells with genomic alterations can survive and replicate, contributing to tumor development.[24,25]

Thus, tumor cells typically exhibit abnormalities in the DNA damage response machinery that differentiate them from normal cells, rendering them unable to repair the damage caused. These properties of cancer cells can be useful for targeting a therapy to cancer cells, leading to their specific death without causing damage to healthy cells. Noteworthy examples widely used in clinical settings include chemotherapy and radiotherapy, both of which induce DNA damage and cause tumor cell death.[25]

In this context, compounds that can induce DNA damage in tumor cells may be useful for the destruction of tumor cells in a specific way. The study by Mishima and collaborators (2005) revealed that baccharin can induce DNA damage in S-180 sarcoma cells in a specific and dose-dependent manner, thereby revealing yet another mechanism of this compound's antitumor activity.[10]

Effects of Baccharin on some cancers

The proposed effects of baccharin towards different types of tumors are summarized in Figure 2 and described in the following sections.

Melanoma

Melanoma is a highly malignant type of skin cancer that originates in melanocytes and can manifest anywhere in the body, including mucous membranes. Melanocytes are specialized skin cells derived from pluripotent neural crest stem cells responsible for producing melanin pigment.[26] A drastic increase in the incidence of this tumor type has been observed worldwide, accounting for 7% of all cancer deaths and 80% of fatalities from skin neoplasms.[7] At its earliest stage, melanoma can be cured; however, if not properly treated in a timely manner, it has a high probability of metastasizing to other regions of the body, thereby complicating treatment.[27] The high malignancy of the tumor is attributed to its intratumoral heterogeneity, as it's established that tumor-initiating cells possess self-renewal and multipotency capacities. Thus, they are responsible for

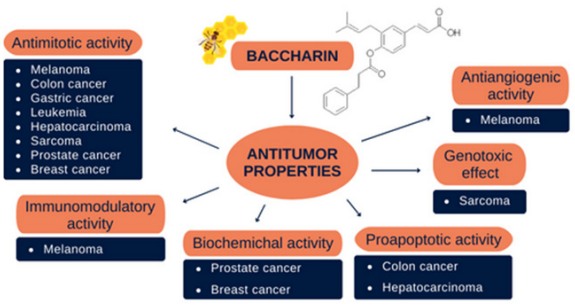

Figure 2: Flow chart of the proposed antitumor effects of baccharin towards different tumor cell lines

the tumor's initiation, promotion and progression, as well as for its metastasis and recurrence.[28]

Established risk factors for cutaneous melanoma include ultraviolet radiation from sun exposure - the main etiological agent related to the disease -, artificial tanning (especially for individuals who had their first exposure before 30 years of age), family history of melanoma, in addition to some phenotypic characteristics such as light skin and eyes color, and tendency to freckles, and individual susceptibility.[29,30] Regarding this last factor, it is essential to point out the variability of pigment genes, being the melanocortin 1 receptor (MC1R) the main one related to melanoma, for which some variants related to the development of the disease have already been mentioned. There is also a very strong risk factor related to the development of melanoma: the presence of melanocytic nevi (regular brown spots protruding or not) or dysplastic (larger spots with irregular shape and multiform coloration). For this risk factor, it is worth mentioning the study described by Chang and collaborators (2009), which found an important association between the number of nevi and increased risk of developing the disease.[31]

The pathophysiology of the disease is characterized by the transformation of melanocytes into metastatic melanoma, which requires the occurrence of genetic mutations and an increase in the proliferative capacity of these cells. Cutaneous melanomas have a particularly high base mutation rate compared to other solid tumors, these being extensively characterized by ultraviolet signature mutations, such as the C → T switch, caused by UVB transitions, or G → T caused by UVA transitions.[32]

In the experimental analysis described by Gastaldello and collaborators (2021), the tumor behavior of melanoma in mice inoculated with B16F10 cells after treatment with the compounds baccharin and p-coumaric acid was evaluated.[7] As a result of the study, a significant modulation in the number of inflammatory cells was observed in the groups treated with the compounds, including an important reduction in the number of circulating neutrophils, as well as an increase in the number of macrophages in the tumor region in animals with melanoma treated with the baccharin. The results of the study showed that animals inoculated with B16F10 cells that received the baccharin treatment during the experiment period presented a significant reduction (919.2 ± 0.002) in relation to the number of neutrophils when compared to the Melanoma group (1851 ± 0.002) ($p = 0.0372$). Regarding the presence of macrophages, it was observed that the mean number of macrophages per non-coincident field in the Melanoma group (2.42 ± 0.39) was considerably lower when compared to the Melanoma + baccharin group ($3.59 \pm 0, 39$), resulting in a statistically significant difference with $p < 0.05$.

Another important observation from the study described by Gastaldello and collaborators (2021) was the significant reduction in the number of blood vessels and mitosis in the neoplastic area, indicating an antiangiogenic and antimitotic activity of these components of green propolis.[7] From the

data obtained by the study, the mean number of vessels per field (4.8 ± 0.347) under light microscopy observed in the animals in the melanoma group was greater than in the animals in the Melanoma + baccharin group (3.1 ± 0.347) ($p < 0.0001$), which demonstrates a significant difference between vessel formation in the baccharin-treated groups. Regarding the results obtained for the evaluation of antimitotic activity, the group that received treatment with baccharin (500 µg/kg p.o.) during the study period had a significant reduction in the number of cells in mitotic activity in the tumor region (1.567 ± 0.2554) compared to the Melanoma group (3,486 ± 0.2554) ($p < 0.0001$).

Colon Cancer

Colon cancer, also known as colorectal cancer, includes tumors that start in the colon or rectum. It is the third most common cancer, ranking 4th as the cancer most related to death.[33] The main factors related to increased risk of development include age over 50 years, overweight, unbalanced diet or excessive consumption of red and processed meat. In addition, family history, smoking, alcohol consumption or the presence of inflammatory bowel diseases such as chronic ulcerative colitis and Crohn's disease, as well as hereditary diseases such as familial adenomatous polyposis, are factors related to increased chance of tumor development.[34]

The signs and symptoms most often associated with colorectal cancer include abdominal pain, change in bowel habits (change in stools, constipation, and diarrhea), unexplained weight loss, fatigue, mucus in stools, nausea or vomiting, bleeding, and rectal pain.[35]

In the experimental study described by Akao and collaborators (2003), the compounds artepilin C, baccharin and drupanin, extracted from Brazilian propolis, were tested in different human tumor lines to evaluate their cell growth inhibition effect.[8] Fistly, the study revealed that for the colon cancer cell line SW480, extracts composed by artepilin C and baccharin or drupanin had an inhibitory effect on cell growth for 72 hours at a dose of 50 mg/ ml. In this experimental analysis, a total of 3 colon cancer cell lines were evaluated: SW480, DLD-1 and COLO201. Subsequently, two concentrations (30 and 150 µM) of the three compounds extracted from propolis were tested. The results showed that baccharin, at a concentration of 30 µM, showed cytotoxicity only for the SW480 strain, with an effect in the order of 0-50%, while at a concentration of 150 µM, this compound was able to induce cytotoxicity in the 3 strains, being 75-100% for the SW480 strain and 0-50% for the DLD-1 and COLO201 strains. Akao and collaborators also demonstrated by assays with the SW480 strain that the antitumor activity of baccharin is due to its pro-apoptotic activity.[8]

Another study proved the action of baccharin on the apoptosis of colon cancer cells, revealing that this function is due to the activation of the intrinsic and extrinsic pathway. Kumazaki and collaborators (2014) demonstrated that co-treatment with baccharin and drupanin has a synergistic effect on the activation of apoptosis in DLD-1 cells by increasing the expression levels of TRAIL and FasL mRNAs, activating caspase-8 and increasing the expression level of FADD.[18] Furthermore, it was observed that apoptosis is favored by the increase in the expression of miR-143, an anti onchogenesis miRNA that represses the expression of the Erk5 gene, resulting in cell cycle arrest and dose-dependently inhibiting cell growth.

Gastric cancer

Stomach cancer includes adenocarcinomas, which account for about 95% of gastric tumor types, as well as lymphomas (approximately 3%) and sarcomas (rare tumors). It is an important health problem, being the fourth most common cancer and the second leading cause of cancer death worldwide.[36] The main risk factors cited for this disease are overweight, smoking, alcohol consumption, excessive salt intake, family history of stomach cancer, pre-existing diseases such as pernicious anemia, atrophic gastritis, intestinal metaplasia, gastroesophageal reflux disease and infections by the *Helicobacter pylori* bacteria.[37]

H. pylori infection is the most common cause of sporadic distal gastric cancer,[38] as it induces persistent inflammation in the gastric mucosa. This inflammatory process leads to the occurrence of oxidative damage that may be related to pre-neoplastic conditions.[39]

There are no specific symptoms of gastric cancer, but some signs may suggest a benign disease such as gastritis, ulcers, or a stomach tumor. These signs and symptoms include loss of weight and appetite, dysphagia, abdominal pain, vomiting with or without blood, and abdominal swelling because of the primary tumor mass.[40]

In the experimental analysis described by Akao and collaborators (2003), three gastric cancer cell lines (MKN1, MKN28 and MUGC4) were evaluated for growth suppression after exposure to Brazilian propolis components - artepilin C, baccharin and drupanin - for 72 hours.[8] Regarding exposure to the baccharin component, induction of cytotoxicity was observed in the three strains at a concentration of 150 µM, and for MKN1 this concentration induced cytotoxicity in the order of 50–75%, while for the strains MKN28 and MUGC4, the observed effect was in the range of 0–50%. At a concentration of 30 µM, baccharin had no effect on tumor growth suppression for any of the three evaluated strains.

Leukemia

Leukemia is a malignant disease of white blood cells whose main characteristic is the accumulation of young abnormal cells in the bone marrow that replace normal blood cells. This is because in leukemia, a blood cell that has not yet reached maturity undergoes a genetic mutation that turns it into a cancer cell. This abnormal cell does not function properly and multiplies in an exacerbated and uncontrolled manner, while its death rate is reduced.[41]

Leukemia can be divided into chronic or acute based on the speed of disease evolution, the former being characterized by onset with mild symptoms that slowly

worsen, while acute leukemia, in turn, is characterized by a rapid worsening in a short time span. Leukemias can also be grouped based on the type of cell lineage they affect, that is, lymphoid (T and B lymphocytes) or myeloid (red blood cells, platelets, and leukocytes such as neutrophils, basophils, eosinophils, and monocytes) cells. Therefore, based on these two classifications, four most common types of leukemia are established: acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL) and chronic lymphocytic leukemia (CLL).[41]

The experimental evaluation by Akao and collaborators (2003) also evaluated the tumor growth suppression action of four leukemia strains (HL60, NB4, K562 and U937) when exposed for 72 hours to the components artepilin C, baccharin and drupanin.[8] The study revealed that, at a concentration of 30 µM, baccharin had cytotoxic activity only in the HL60 strain, while at a concentration of 150 µM, the compound showed an effect in the four strains, inducing cytotoxicity in the order of 0-50% for the strains K562 and U937, and 50-75% for strains HL60 and NB4.

Hepatocarcinoma

Hepatocellular carcinoma (HCC) is a malignant tumor of great epidemiological relevance, since it affects more than half a million people worldwide, being the fifth most common type of cancer in men and the seventh in women.[42] This type of cancer develops when mutations occur in primary liver cells, due to internal or external factors, causing them to multiply in an uncontrolled way. The main events related to the carcinogenesis of HCC are linked to conditions that interfere with DNA synthesis, with emphasis on cirrhosis and infection by hepatitis B (HBV) and C (HCV) viruses.[11] The first and most important risk factor for the development of HCC is liver cirrhosis, a condition characterized by the replacement of the normal liver structure by abnormal nodules surrounded by fibrosis.[43] In cirrhosis, there is marked cell proliferation and high DNA synthesis in the regeneration nodules, increasing the risk of errors in gene replication, thus favoring the occurrence of mutations.[11] Another important clinical condition for the development of HCC is infection by HBV and HCV, which can occur indirectly or directly. In the first situation, the carcinogenesis process is favored by the recurrent cycles of necrosis and regeneration of hepatocytes, which increases the probability of spontaneous mutations. In the second case, malignant transformation occurs by the integration of viral DNA to host cell DNA in regions responsible for cell cycle contro.[44]

HCC is a highly aggressive tumor with a high death rate. In cases where the diagnosis is made in the symptomatic phase, typically characterized by jaundice and ascites, the average life expectancy is less than one month if untreated. Even so, what is observed in practice is that the treatments available are not so effective.[42]

A study carried out by Filardi (2010) evaluated the cytotoxic activity of green propolis extract (containing, among others, artepilin C and baccharin as main constituents) against HepG2 hepatocellular carcinoma cells.[45] For this, cells of the HepG2 lineage were exposed to different concentrations of green propolis extract (50, 100, 200 and 400 µg/ml) in periods of 24, 48 and 72 hours and monitored for cell viability and occurrence of apoptosis. The data obtained by Filardi revealed that green propolis has a significant cytotoxic effect mainly at concentrations of 400 µg/ml. In addition, exposure of cells to propolis extract led to changes in cell architecture and monolayer organization with expressive presence of apoptotic forms.

Sarcoma

The term sarcoma refers to the types of cancers originating in connective tissues, such as bones, muscles or cartilage. Sarcomas are rare solid tumors that arise from mesenchymal cells and represent 1 to 2% of all malignancies in adults and about 15% of cases in children, affecting about 200,000 individuals per year.[46,47] Sarcomas can be divided into soft tissue sarcomas - including cancers originating in adipose tissue, muscle, nerve or blood vessel - or bone tumors - such as osteosarcoma, chondrosarcoma and Ewing's sarcoma.[47]

Although uncommon, sarcomas represent a therapeutic challenge, since they can be highly aggressive, invasive and capable of leading to metastases.[46] In order to evaluate the biology of cancer and the efficacy of new compounds regarding their antitumor activity, several mouse and cell lines are used as experimental models. In addition to being cultivated in-vitro, some tumor strains can also be evaluated in vivo through their inoculation in animal models.[48] In this context, *in-vivo* models of sarcomas are widely used for testing new therapeutic candidates to evaluate the effects of compounds in more complex biological systems.

The study published by Mishima and collaborators (2005) evaluated the tumoricidal activity of baccharin in vivo, through its oral administration (30 or 100 mg/kg/d in 5% gum arabic solution for 28 days) in allograft mice with S-180 sarcoma.[10] The results revealed that baccharin is able to effectively suppress tumor growth at both concentrations tested in the time period evaluated. In addition, its genotoxic effects (comet assay) on normal splenocytes were also observed, revealing that the compound in question induces dose-dependent DNA damage in tumor cells and does not induce DNA damage in normal splenocytes. Thus, the results suggest that baccharin is a preferential inducer of cytotoxicity and genotoxicity in tumor cells, which is an important factor to be considered regarding the safety of a candidate compound for therapeutic application.

Prostate cancer

Prostate cancer is the fourth most diagnosed cancer worldwide, being the second most frequent cancer and the fifth leading cause of cancer death among men in 2020.[49] The etiology of the disease is still unknown, however, it is already known that it is closely related to genetic factors, and a family history of prostate and breast cancer are known risk factors.[50] In addition to genetic inheritance, many environmental

factors are also involved in pathogenesis, including diet and inflammation.[51]

The development of prostate cancer is androgen-dependent, and androgen deprivation therapy has still been the main treatment for the disease, through surgical or chemical castration. However, despite the reduction in circulating androgen levels, in many cases, tumor recurrence is observed, which has a higher potential for metastasis. This recurrence is due to the intratumoral conversion of weak adrenal androgens, such as dehydroepiandrosterone (DHEA) and 4-androstene-3,17-dione, to the potent androgen receptor ligands: testosterone and 5α-dihydrotestosterone (DHT). In this context, it is important to emphasize the role of the enzyme aldo-keto reductase 1C3 (AKR1C3), which is responsible for converting 4-androstene-3,17-dione into testosterone and 5α-androstane-3,17-dione into DHT. In addition, AKR1C3 is also able to act as a coactivator of androgen receptors, promoting the growth of prostate tumors through this alternative pathway. In many cases of prostate tumors, especially in relapsing ones, the AKR1C3 enzyme is overexpressed, leading to an exacerbated tumor growth, even after castration. Thus, the reduction of expression levels or the inhibition of the activity of this enzyme are important mechanisms for the inhibition of tumor growth and treatment of the disease.[23]

Endo and collaborators (2012) study evaluated the inhibition of AKR1C3 by cinnamic acid derivatives from propolis, including baccharin.[9] The researchers found that baccharin is a potent competitive inhibitor (Ki 56 nM) with high selectivity for AKR1C3, showing no significant inhibition against other AKR1C isoforms (AKR1C1, AKR1C2 and AKR1C4). The study revealed that baccharin (50 μM) was able to suppress the proliferation of PC3 prostate carcinoma cells that express AKR1C3 in a 72 h culture. In addition, the researchers evaluated the effect of baccharin on vector-only transfected control cells and on PC3 lines in which AKR1C3 was expressed 5-fold more than control cells. The result showed that the treatment of cells that overexpress AKR1C3 with baccharin (50 μM) for 72 h resulted in a more significant reduction in proliferation (43%) than in control cells in control cells (35%), proving the involvement of AKR1C3 in the proliferation of PC3 cells, which is effectively inhibited by baccharin.

Another study that evaluated the action of baccharin on prostate cancer lines was that of Mishima and collaborators (2005).[10] In this work, several cancer cell lines were exposed to different concentrations of baccharin and drupanin for 48 hours to evaluate the cytotoxic action of these compounds and the "dose x response" effect on cell growth. One of the strains evaluated was LNCaP, a lymph node metastasis strain derived from human prostate adenocarcinoma cells, for which baccharin was shown to be cytotoxic and tumor growth inhibitory (GI50 338.8 μM, TGI 812.8 μM, LC50 1548.8 μM). In this study, the response in the PC3 strain was also evaluated, for which baccharin was also shown to inhibit growth (GI50 501.2 μM, TGI 2290.9 μM).

Breast cancer

Female breast cancer is the leading cause of global cancer incidence (2020), accounting for 11.7% of all cases. It is the fifth leading cause of cancer mortality worldwide, with 685,000 deaths. Among women, breast cancer is responsible for 1 in 4 cancer cases and 1 in 6 cancer deaths.[49] Several factors have already been determined to be at risk for the development of breast cancer. The first and most important of them is sex, and women are much more affected by this disease, mainly due to greater hormonal stimulation. In addition to being female, factors such as age, family history, ethnicity, genetic mutations, reproductive history, obesity, smoking, alcohol consumption and others are already known as risk factors.[52] In general, breast tumor pathogenesis is initiated with genetic mutations in the terminal duct-lobular units that lead to an uncontrolled proliferation of mutant cells.[53]

Breast cancer is often hormone-dependent, that is, it depends on hormonal stimuli to develop and proliferate.[54] Thus, as in prostate cancer, the overexpression of AKR1C3 is also related to the development and growth of breast tumors, because the enzyme is directly involved in estrogen biosynthesis.[55] Reducing 17β-hydroxysteroid dehydrogenases (17β-HSDs) are essential to produce 17β-estradiol (the most potent endogenous estrogen) from Δ4-androstene-3,17-dione (androstenedione), with testosterone being the intermediate in this reaction. AKR1C3 is a 17β-HSD involved in several reactions that can lead to the development of breast cancer. AKR1C3 catalyzes the reduction of androstenedione to testosterone, progesterone to 20α-hydroxyprogesterone and, to a lesser extent, estrone to 17β-estradiol.[56] The combined effect of these activities on breast cancer cells would be to increase the ratio between 17β-estradiol and progesterone and therefore increase estrogen receptor (ER) activation and reduce progesterone receptor (PR) signaling, which is directly related to the development and proliferation of breast cancer.[22]

As discussed earlier, Endo and collaborators (2012) work proved that baccharin is a potent and highly selective inhibitor for AKR1C3.[9] Therefore, it may be an interesting compound for reducing the proliferation and treatment of breast cancer.

Another important study that reveals the cytotoxic action of baccharin on breast cancer cells is that of Mishima and collaborators (2005).[10] In this work, the authors tested the exposure of the MCF-7 strain to different concentrations of baccharin, aiming to evaluate the interference of the compound on cell growth through the "dose x response" profile. The results obtained showed that baccharin is a potent inhibitor of tumor growth for the MCF-7 strain (GI50 141.3 μM, TGI 588.8 μM).

Conclusion

The increasing incidence of cancer worldwide increasingly leads to the need to search for new therapies. The studies presented in this review show that baccharin, a natural

component extracted from green propolis, has relevant properties for the treatment of some types of tumors, including melanoma, colon cancer, gastric cancer, leukemia, hepatocarcinoma, sarcoma, prostate cancer and breast cancer. These properties are attributed to different mechanisms in the carcinogenic process, including antiproliferative, pro-apoptotic, anti-angiogenic, immunomodulatory and biochemical activities and genotoxic effects, resulting in an antitumor effect toward the evaluated cell lines.

Furthermore, it is important to highlight that there are other ongoing studies aimed at evaluating the activity of baccharin against additional tumor cell lines and fully understanding the mechanisms by which baccharin exerts its antitumor actions.

Thus, according to what was exposed, it is possible to conclude that baccharin may represent a prototype for the development of novel anticancer drugs, or even to be used as adjuvant therapy alongside existing treatments for cancer such as chemotherapy and radiotherapy.

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Conflict of interest

The authors don't have conflict of interest.

References

1. Rios N, Yánez C, Rojas L, Mora F, Usubillaga A, Vit P. Chemical composition of essential oil of Apis mellifera propolis from Falcón State, Venezuela. Emirates Journal of Food and Agriculture [Online]. 2014;639-642.
2. Paula LAL, Santos MFC, Pagotti MC, Faleiros R, Ramos HP, Veneziani RCS, Bastos JK, Caffrey CR, Ambrosio SR, Magalhães LG. Uncovering Biological Application of Brazilian Green Propolis: A Phenotypic Screening against *Schistosoma mansoni*. Chemistry & Biodiversity [Online]. 2020; 17(9):e2000277.
3. Salgueiro, FB, Castro, RN. Comparação entre a composição química e capacidade antioxidante de diferentes extratos de própolis verde. Química Nova [Online]. 2016; 39:1192-1199.
4. Filho AAS. Fitoquímica da *Baccharis dracunculifolia*. In: Gutierre JHB, Ceccantini JL, Ikeda AT, Busetto A, Tolentino CAF, Góes EM, Maniglia E, Urbinati EC, Almeida IM, Baldan MLOG, Ghirardello N, Pleitez V, Nobara A, Filho JP, Rodrigues L, editors. *Baccharis dracunculifolia*: Uma das principais fontes vegetais da própolis brasileira. São Paulo: Unesp; 2012. p. 37-43.
5. Onuchic AC, Chammas R. Câncer e o microambiente tumoral. Revista de medicina [Online]. 2010; 89(1):21-31.
6. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell [Online]. 2000; 100(1):57-70.
7. Gastaldello GH, Cazeloto ACV, Ferreira JC, Rodrigues DM, Bastos JK, Campo VL, Zoccal KF, Tefé-Silva C. Green Propolis Compounds (Baccarin and p-Coumaric Acid) Show Beneficial Effects in Mice for Melanoma Induced by B16f10. Medicines [Online]. 2021; 8(5):20.
8. 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. Biological and Pharmaceutical Bulletin [Online]. 2003; 26(7):1057-1059.
9. Endo S, Matsunaga T, Kanamori A, Otsuji Y, Nagai H, Sundaram K, El-Kabbani O, Toyooka N, Ohta S, Hara A. Selective inhibition of human type-5 17β-hydroxysteroid dehydrogenase (AKR1C3) by baccharin, a component of Brazilian propolis. Journal of natural products [Online]. 2012; 75(4):716-721.
10. Mishima S, Ono Y, Araki Y, Akao Y, Nozawa Y. Two related cinnamic acid derivatives from Brazilian honey bee propolis, baccharin and drupanin, induce growth inhibition in allografted sarcoma S-180 in mice. Biological and Pharmaceutical Bulletin [Online]. 2005; 28(6):1025-1030.
11. Pimenta JR, Massabki PS. Carcinoma hepatocelular: um panorama clínico. Rev Bras Clin Med [Online]. 2010; 8:59-67.
12. Malebary SJ, Khan R, Khan YD. ProtoPred: advancing oncological research through identification of proto-oncogene proteins. IEEE Access [Online]. 2021; 9:68788-68797.
13. Xie K. Interleukin-8 and human cancer biology. Cytokine & growth factor reviews [Online]. 2001; 12(4):375-391.
14. Ashkenazi A, Herbst RS. To kill a tumor cell: the potential of pro-apoptotic receptor agonists. The Journal of clinical investigation [Online]. 2008; 118(6):1979-1990.
15. Kaufmann SH, Vaux DL. Alterations in the apoptotic machinery and their potential role in anticancer drug resistance. Oncogene [Online]. 2003; 22(47):7414-7430.
16. Vazquez A, Bond EE, Levine AJ, Bond GL. The genetics of the p53 pathway, apoptosis and cancer therapy. Nature reviews Drug discovery [Online]. 2008; 7(12):979-987.
17. Wong RSY. Apoptosis in cancer: from pathogenesis to treatment. Journal of experimental & clinical cancer research [Online]. 2011; 30(1):1-14.
18. Kumazaki M, Shinohara H, Taniguchi K, Yamada N, Ohta S, Ichihara K, Akao Y. Propolis cinnamic acid derivatives induce apoptosis through both extrinsic and intrinsic apoptosis signaling pathways and modulate of miRNA expression. Phytomedicine [Online]. 2014; 21(8-9):1070-1077.
19. Singel KL, Segal BH. Neutrophils in the tumor microenvironment: trying to heal the wound that cannot heal. Immunological reviews [Online]. 2016; 273(1):329-343.
20. Corthay A, Skovseth DK, Lundin KU, Røsjø E, Omholt H, Hofgaard PO, Haraldsen G, Bogen B. Primary antitumor immune response mediated by CD4+ T cells. Immunity

[Online]. 2005; 22(3):371-383.
21. Wu X, Daniels G, Lee P, Monaco ME. Lipid metabolism in prostate cancer. American journal of clinical and experimental urology [Online]. 2014; 2(2):111.
22. Byrns MC, Penning TM. Type 5 17β-hydroxysteroid dehydrogenase/prostaglandin F synthase (AKR1C3): role in breast cancer and inhibition by non-steroidal anti-inflammatory drug analogs. Chemico-biological interactions [Online]. 2009; 178(1-3):221-227.
23. Zang T, Verma K, Chen M, Jin Y, Trippier PC, Penning TM. Screening baccharin analogs as selective inhibitors against type 5 17β-hydroxysteroid dehydrogenase (AKR1C3). Chemico-biological interactions [Online]. 2015; 234:339-348.
24. Khanna A. DNA Damage in Cancer Therapeutics: A Boon or a Curse? Targeting DNA Damage in Cancer. Cancer research [Online]. 2015; 75(11):2133-2138.
25. Hosoya N, Miyagawa K. Targeting DNA damage response in cancer therapy. Cancer science [Online]. 2014; 105(4):370-388.
26. Panda S, Dash S, Besra K, Samantaray S; Pathy PC, Rout N. Clinicopathological study of malignant melanoma in a regional cancer center. Indian journal of cancer [Online]. 2018; 55(3):292.
27. Labani S, Asthana S, Rathore K, Sardana K. Incidence of melanoma and nonmelanoma skin cancers in Indian and the global regions. Journal of Cancer Research and Therapeutics [Online]. 2021; 17(4):906.
28. Palacios-Ferrer JL, García-Ortega MB, Gallardo-Gómez M, García MA, Díaz C, Boulaiz H, Valdivia J, Jurado JM, Almazan-Fernandez FM, Arias-Santiago S, Amezcua V, Peinado H, Vicente F, Palacio JP, Marchal JA. Metabolomic profile of cancer stem cell-derived exosomes from patients with malignant melanoma. Molecular oncology [Online]. 2021; 15(2):407-428.
29. Gandini S, Autier P, Boniol M. Reviews on sun exposure and artificial light and melanoma. Progress in biophysics and molecular biology [Online]. 2011; 107(3):362-366.
30. Berwick M, Erdei E, Hay J. Melanoma epidemiology and public health. Dermatologic clinics [Online]. 2009; 27(2):205-214.
31. Chang Y, Newton-Bishop JA, Bishop DT, Armstrong BK, Bataille V, Bergman W, Berwick M, Bracci PM, Elwood JM, Ernstoff MS, Green AC, Gruis NA, Holly EA, Ingvar C, Kanetsky PA, Karagas MR, Marchand LL, Mackie RM, Olsson H, Østerlind A, Rebbeck TR, Reich K, Sasieni P, Siskind V, Swerdlow AJ, Titus-Ernstoff L, Zens MS, Ziegler A, Barrett JH. A pooled analysis of melanocytic nevus phenotype and the risk of cutaneous melanoma at different latitudes. International journal of cancer [Online]. 2009; 124(2):420-428.
32. Mármol I, Sánchez-de-Diego C, Dieste AP, Cerrada E, Yoldi MJR. Colorectal carcinoma: a general overview and future perspectives in colorectal cancer. International journal of molecular sciences [Online]. 2017; 18(1):197.
33. Schadendorf D, Akkooi ACJV, Berking C, Griewank KG, Gutzmer R, Hauschild A, Stang A, Roesch A, Ugurel S. Melanoma. The Lancet. 2018; 392(10151):971-984.
34. Terzić J, Grivennikov S, Karin E, Karin M. Inflammation and colon cancer. Gastroenterology [Online]. 2010; 138(6):2101-2114.
35. Majumdar SR, Fletcher RH, Evans AT. How does colorectal cancer present? Symptoms, duration, and clues to location. The American journal of gastroenterology [Online]. 1999; 94(10):3039-3045.
36. Van Cutsem E, Sagaert X, Topal B, Haustermans K, Prenen H. Gastric cancer. The Lancet [Online]. 2016; 388 (10060):2654-2664.
37. Karimi P, Islami F, Anandasabapathy S, Freedman ND, Kamangar F. Gastric Cancer: Descriptive Epidemiology, Risk Factors, Screening, and Prevention Gastric Cancer. Cancer epidemiology, biomarkers & prevention [Online]. 2014; 23(5):700-713.
38. Bornschein J, Selgrad M, Warnecke M, Kuester D, Wex T, Malfertheiner P. *H. pylori* infection is a key risk factor for proximal gastric cancer. Digestive diseases and sciences [Online]. 2010; 55(11):3124-3131.
39. Ladeira MSP, Salvadori DMF, Rodrigues MAM. Biopatologia do *Helicobacter pylori*. Jornal Brasileiro de Patologia e Medicina Laboratorial [Online]. 2003; 39(4):335-342.
40. Axon A. Symptoms and diagnosis of gastric cancer at early curable stage. Best practice & research Clinical gastroenterology [Online]. 2006; 20(4):697-708.
41. Puggina, DAB. Um Panorama Geral sobre as Leucemias. Ciência News [Online]. 2020.
42. Gomes MA, Priolli DG, Tralhão JG, Botelho MF. Hepatocellular carcinoma: epidemiology, biology, diagnosis, and therapies. Revista da Associação Médica Brasileira (English Edition) [Online]. 2013; 59(5):514-524.
43. Iida VH, Silva TJA, Silva ASF, Silva LFF, Alves VAF. Cirrose hepática: aspectos morfológicos relacionados às suas possíveis complicações. Um estudo centrado em necropsias. Jornal Brasileiro de Patologia e Medicina Laboratorial [Online]. 2005; 41:29-36.
44. Michielsen PP, Francque SM, Van Dongen JL. Viral hepatitis and hepatocellular carcinoma. World Journal of Surgical Oncology [Online]. 2005; 3(1):1-18.
45. Filardi MA. Potencial antitumoral de extratos da própolis Brasileira e de folhas de graviola (*Annona muricata*): efeito citotóxico sobre células hepatocarcinogênicas HEPG2 [dissertation]. Viçosa (MG): Federal University of Viçosa; 2010.
46. Taylor BS, Barretina J, Maki RG, Antonescu CR, Singer S, Ladanyi M. Advances in sarcoma genomics and new therapeutic targets. Nature Reviews Cancer [Online]. 2011; 11(8):541-557.
47. Moreira SBR, Oliveira RFF, Baptista TS, Lima LC, Forte ECN. Sarcoma-características e resultados em um centro de referência oncológica no sul do Brasil. Brazilian Journal of Health Review [Online]. 2022; 5(2):4277-4292.

48. Cruz M, Enes M, Pereira M, Dourado M, Ribeiro ABS. Modelos experimentais em oncologia: O contributo da cultura de células para o conhecimento da biologia do cancro. Revista portuguesa de pneumologia [Online]. 2009; 15(4):669-682.
49. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians [Online]. 2020; 71(3):209-249.
50. Fournier G, Valeri A, Mangin P, Cussenot O. Cancer de la prostate. Épidémiologie. Facteurs de risques. Anatomopathologie. In: Annales d'urologie. Elsevier Masson [Online]. 2004; 38(5):187-206.
51. Hughes C, Murphy A, Martin C, Sheils O, O'Leary J. Molecular pathology of prostate cancer. Journal of clinical pathology [Online]. 2005; 58(7):673-684.
52. Łukasiewicz S, Czeczelewski M, Forma A, Baj J, Sitarz R, Stanisławek A. Breast Cancer - Epidemiology, Risk Factors, Classification, Prognostic Markers, and Current Treatment Strategies - An Updated Review. Cancers [Online]. 2021; 13(17):4287.
53. Bombonati A, Sgroi DC. The molecular pathology of breast cancer progression. The Journal of Pathology [Online]. 2011; 223(2):308-318.
54. Bai Z, Gust R. Breast cancer, estrogen receptor and ligands. Archiv der Pharmazie: An International Journal Pharmaceutical and Medicinal Chemistry [Online]. 2009; 342(3):133-149.
55. Rodrigues DM, Portapilla GB, Silva GM, Duarte A, Rotta CG, Silva CHTP, Albuquerque S, Bastos JK, Campo VL. Synthesis, antitumor activity and in silico analyses of amino acid derivatives of artepillin C, drupanin and baccharin from green propolis. Bioorganic & Medicinal Chemistry [Online]. 2021; 47:116372.
56. Penning TM, Burczynski ME, Jez JM, Hung CF, Lin HK, Ma H, Moore M, Palackal N, Ratnam K. Human 3α-hydroxysteroid dehydrogenase isoforms (AKR1C1–AKR1C4) of the aldo-keto reductase superfamily: functional plasticity and tissue distribution reveals roles in the inactivation and formation of male and female sex hormones. Biochemical journal [Online]. 2000; 351(1):67-77.