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Searching for Analogues of the Natural Compound, Caffeic Acid Phenethyl Ester, with Chemoprotective Activity

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1. Introduction

Cancer is a disease characterized by uncontrolled growth and division of genetically altered cells and its emergence requires several elements, including self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of apoptosis, limitless replicative potential, tissue invasion and metastasis, and sustained angiogenesis (Hanahan and Weinberg, 2011). Cancer is thought to evolve along a multi-step process, cancer cells are the descendants of a normal cell in which some kind of internal or external stress causes a change in its genetic code. This event is said to “initiate” the cell to a precancerous state. In a second stage, this precancerous cell divides in response to a promoting agent to produce daughter cells, and these daughter cells divide to produce more daughter cells, and so on. The genetic instabilities passed down through the generations finally result in one cell that no longer requires the promoting agent to stimulate its proliferation, and a cancer cell is born with the ability to make proteins such as growth factors that stimulate proliferation. Finally in the third stage of carcinogenesis, progression, this cancer cells divides to produce daughter cells, these cells also divide, and soon there is a population of cancer cells with the ability to invade and metastasize (Vincent & Gatenby, 2008).

The study of liver cancer has been intensified in recent years. Hepatocellular carcinoma (HCC) is the most common hepatic cancer responsible for over one million deaths annually worldwide, the percentage of affected men compared to affected women varies between 2:1 and 4:1, depending on the geographic region (Naugler & Schwartz, 2008). The cause of HCC in most cases is the ongoing liver cell damage, so HCC occurs in persons with chronic liver disease, most often in the setting of cirrhosis but other risk factors are the infection with hepatitis type C and type B, consumption of mycotoxins such as aflatoxin, genetic and hormonal factors, obesity, and exposure to chemical carcinogens such as nitrosamines.

Early detection of HCC plays an important role to have more treatment options and chances of survival. Survival of lately diagnosed individuals is poor, surgical resection provides the only chance of cure, but it is not suitable for the majority of patients. For most patients, nonsurgical treatment is the only option. Therefore, the study of the origin of the disease

and at the early stages of HCC is still of great interest to find methods of prevention, early diagnosis and treatment.

Chemoprotection involves the use of synthetic or natural compounds to inhibit slow or reverse carcinogenesis, is based on the hypothesis that the disruption of biological events involved in carcinogenesis will inhibit this process and can be applied to any stage of carcinogenesis. Chemoprotective compounds can act at any of the various stages of carcinogenesis. Those that block mutagenesis prior to tumor development can be considered antimutagens and by its mechanism are classified in four groups: **Bioantimutagens** are naturally occurring substances that reduce mutant yield by acting on the DNA repair or replicative processes. These compounds act after a DNA adduct has formed but before the DNA lesion is fixed into a mutation. An example of a bioantimutagen is vanillin, present in vanilla beans, which appears to enhance post-replication recombinational repair under certain conditions. **Desmutagens** encompass all agents that affect mutagenicity through mechanisms other than DNA repair or replication. These mechanisms include enzyme induction, mutagen scavenging, and blocking of mutagen activation. **Chemical Inactivators and Enzymatic Modulators** are agents that prevent the formation of mutagens or their activation to more potent forms. And a final group, **Antioxidants and Free Radical Scavengers**, scavengers bond with mutagens to render the mutagen incapable of reacting with DNA, one class of chemicals that forms complexes with mutagenic compounds is the porphyrins, including chlorophyllin. Chlorophyllin inhibits the mutagenicity of a variety of dietary mixtures as well as individual large planar mutagens as aflatoxin B1 and benzo[a]pyrene. Antioxidants exert their effect by donating electrons to unstable oxygen species generated from endogenous processes or formed as a result of radiation or chemical exposure. Ascorbic acid (vitamin C) is an example of a water-soluble extracellular antioxidant with intriguing dual properties. On one hand, it quenches singlet oxygen and assorted free radicals; on the other hand, it preserves the function of reducing them back to active form after they have quenched free radicals. Carotenoids such as 5-carotene and lycopene illustrate lipid-based antioxidants (Kohlmeier et al., 1995).

2. Natural products with biological activities

Medicinal herbs have been used to treat diseases and this practice continues today worldwide. Chemoprotectors have often been detected when studying the effect of substances purified from extracts of natural products to which folk medicine has attributed therapeutic properties.

An example is *Rhoeo discolor* is a plant with extended use for treatment of commonly used to treat cancer, venereal diseases and superficial mycoses in Mexican traditional medicine. *Rhoeo* extract is antimutagenic for *S. typhimurium* strain TA102 pretreated with ROS generating mutagen norfloxacin in the Ames test, and protects liver cell cultures against N-diethylnitrosamine (DEN) induction of unscheduled DNA synthesis. *Rhoeo* extract showed similar radical scavenging effect to that of α -tocopherol and more than ascorbic acid. It is important to note that this extract was neither mutagenic in *S. typhimurium* nor genotoxic in liver cell culture, even at concentrations as high as 4 and 166 fold of those needed for maximal antimutagenic or chemoprotective activities (Gonzalez-Avila et al., 2003; Rosales-Reyes et al., 2008).

Sprague Dawley rats injected with methylnitrosourea MNU produced a variety of alterations ranging from severe inflammatory reaction in lung and skin to colon adenocarcinoma. There are an elevation of malondialdehyde (MDA) and nitric oxide (NO) in serum obtained from these rats. When is given to this rats a daily dose of *Nigella* grains with honey from bees after one week of MNU administration and for six months, this compounds protected 100% against MNU-induced oxidative stress, carcinogenesis and abolished the NO and MDA elevations (Mabrouk et al., 2002).

As in the case of honey, propolis has been known to mankind from the remotest of ancient times and has been widely used by many cultures for different purposes, among which its long history of use in herbal medicine traditions is included. Propolis is a complex resinous mixture gathered from plants and used by honeybees in their hives as a general-purpose sealer and antibiotic. This is a product of interest as much in the field of medicine as the pharmaceutical industry. It is attributed with numerous properties: it is an anti-inflammatory agent, an immunostimulant, a hepatoprotector, a carcinostatic, it has anti-microbial, anti-viral, anti-fungal, antiprotozoan properties, and it is an anesthetic and a tissue regenerator. It has been demonstrated that Korean propolis, like the commercial type, induces apoptosis of human hepatoma cell lines. The ethanolic extract of propolis is a good inhibitor of mutagenicity and the methanolic extract presents cytotoxicity against murine colon 26-L5 carcinoma and human HT-1080 fibrosarcoma. It is suggested that propolis exerts a protective effect in colonic carcinogenesis, preventing the development of preneoplastic lesions, given that ethanolic extract administered after exposure to a cancerous agent (1,2 dimethylhydrazine), is strongly associated with a reduction in the number of aberrant crypts in the distal colon (Farré, 2004).

2.1 Active compounds in propolis

Patients are experimenting with natural compounds in their efforts to heal themselves of cancer, in different regions of the world is estimated that from 10 to 80 percent of cancer patients use some form of complementary medicine as part of their overall therapy. For many of these patients, a part of the complementary approach is the use of natural compounds, without the guidance of their oncologist or any real guidance from scientific studies. This is the reason to study natural compounds that can be used properly in the treatment of cancer (Boik, 2001).

Polyphenolic compounds are widely distributed in the plant kingdom and display a variety of biological activities, including chemoprevention and tumor growth inhibition. Propolis is made up of a variety of polyphenolic compounds, several of its isolated compounds have shown anti-carcinogenic activity, associated with the inhibition of the cellular cycle and the induction of apoptosis, as in the case of 3-2 acid (2-dimethyl 8.3 methyl 2-butenyl) benzopyran-6-propenoic or induced apoptosis without affecting the cellular cycle of cancerous cells, such as prenyl flavanone propolin A, which also shows antioxidant activity. It has been shown that the carbon prenylates of p-cummaric acid in Brazilian propolis act against hepatocarcinoma. Caffeic acid (CA) and caffeic acid phenethyl ester (CAPE), members of the polyphenolic compounds, are present in high concentrations in medicinal plants and propolis. CA and CAPE have been investigated for direct antitumor activity in vivo and in vitro. Orsolic et al, found that the local presence of CA and CAPE, by subcutaneous injection in the tumoral tissue, caused a significant delay in tumor formation

and increased life span 29.3 to 51.73%, respectively. CA and CAPE, significantly suppressed human HeLa cervical carcinoma cell proliferation in vitro (Orsolich et al., 2005).

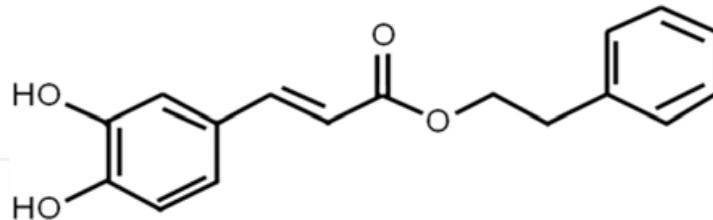


Fig. 1. CAPE

The CAPE [2-propenoic acid, 3-(3,4-dihydroxyphenyl)-, 2-phenethyl ester] is an active component of propolis with a wide variety of biological activities at non-toxic concentrations in mammals organisms. It has shown activities as antibacterial, anti-inflammatory, antioxidant, antitumor and antiproliferative. CAPE is chemopreventive against intestinal, colon and skin cancer, and has shown to decrease the formation of preneoplastic hepatic lesions when administered on a rat model of liver carcinogenesis (Carrasco-Legleu et al., 2004; Carrasco-Legleu et al., 2006) but the mechanism for these properties is not completely known. Besides CAPE, other caffeic acid esters in propolis may have biological effects; here we focus on CAPE and structurally related compounds.

2.2 Analogues and derivatives

As well as CAPE, its analogues are widely distributed in the plant kingdom as in coffee, fruit and propolis. Analogue is a drug whose structure is related to another, but its chemical and biological properties may be different. The term "analogue" refers to chemical compounds with a close structural relationship to the parent compound. It includes compounds having a structural similarity, but one or more atoms in its structure have been replaced by others (Fischer & Ganellin, 2006; Nill, 2002; Wermuth, 2006).

Crude propolis itself is not an ideal source of CAPE because the concentration can vary greatly depending on the source of the propolis, CAPE is commonly present at 1 to 5 percent, but some propolis samples appear to contain none and such a standardized extract is not yet available commercially. Propolis can cause allergic dermatitis after topical contact in sensitive individuals, and oral administration may sensitize a person to this (Boik, 2001). One impediment to the widespread use of CAPE is that its extraction from natural products, is complex and with very low yields. In a similar way, by chemical synthesis, reaction together with purification methods require prolonged purification procedures and the yields range between 35 and 50%. And currently is commercially available only at high cost.

For this reason, molecules with structure related to the CAPE have been studied, seeking to obtain compounds that retain biological activity, as well as showing advantages of being cheap, and obtained quickly and easily. Currently the research for compounds with biological activity is supported on methodologies such as quantitative structure- activity relationship (QSAR) that aims to predict and optimize the biological activity, suggest a mode of action, classified according to biological activity, determine structural features of the molecule important for biological activity and reduce the experimental part. It is based in that biological activity is a function of chemical structure, chemical structure implies given properties that can be quantified using physicochemical parameters and there is

always a function relating biological activity with changes in the properties. The QSAR methodology starts with a compound showing activity in relation to the desired therapeutic goal. Later in the learning phase is necessary to test an exploration series, which consists of a set of products with similar structure to the original compound but with variable substituents or fragments, which allows observation of the changes produced in their biological activity depending on the substituents. In the optimization phase, from data collected it is possible get a function that allows design the best combination of substituents to achieve optimal biological activity (Kubinyi, 1990).

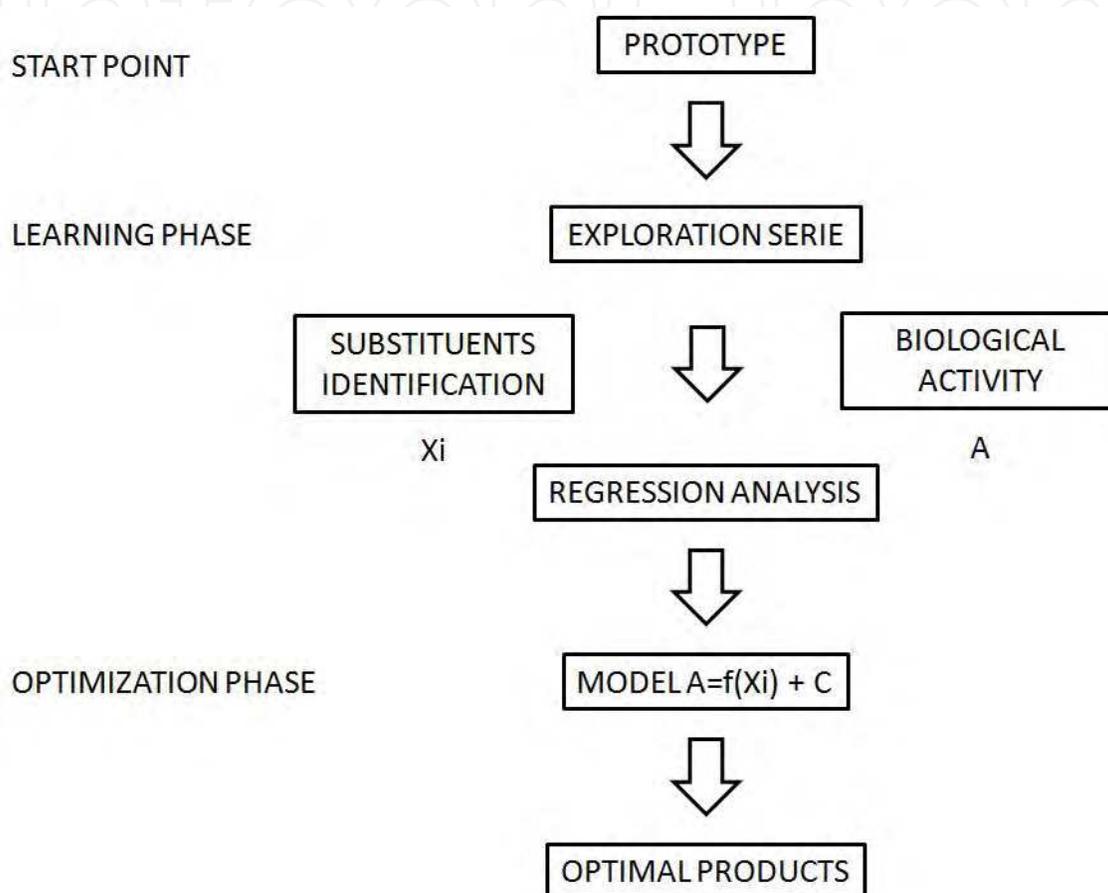


Fig. 2. QSAR methodology diagram

Several compounds structurally related to CAPE are simple derivatives including cinnamic acid amides, sugar esters and glycosides, or in more complex forms such as rosmarinic acid (caffeic acid dimer), litospermic acid (caffeic acid trimer), verbascoside (ester and glycoside heterosidic of dihydroxyphenetylethanol and caffeic acid) and derivatives linked to flavonoids (Jiang et al., 2005; Lin et al., 2005). Several publications have shown that many compounds structurally related to CAPE, preserve in different degree its biological activity, and these compounds are known by properties like antibacterial, anti-inflammatory, immunostimulatory, anti-atherosclerotic, neuroprotective, antiproliferative, antiviral and antioxidative (Chang et al., 2007; Natarajan et al., 1996; Son & Lewis, 2002; Uwai et al., 2008).

To obtain information about the molecular mechanism of CAPE chemoprevention, Natarajan et al. test the effect of CAPE on this transcription factor. U-937 cells were stimulated with Tumor Necrosis Factor- α (TNF- α), to induce Nuclear Factor Kappa B (NF-

κ B) activation and by Electrophoretic Mobility-Shift Assays (EMSA) of nuclear proteins they found that the activation of NF- κ B by TNF- α is completely blocked by 2h preincubation with CAPE (25 μ g/ml), and this effect was similar in an *in vivo* rat model (Carrasco-Legleu et al., 2004; García-Román et al., 2007; García-Román et al., 2008). It's worth mentioning that the role of the NF- κ B in activities as antibacterial, anti-inflammatory, antioxidant, antitumor and antiproliferative, has been documented. Normally NF- κ B is found in the cytoplasm in an inactive state held by an inhibitory subunit called NF- κ B Inhibitor (I κ B), with a steric impediment which stop the translocation to the nucleus. Several stimuli as cytokines IL-1 and TNF- α act over the membrane receptors, and activate a series of enzymes and proteins kynasa that fosforilate I κ B. Fosforilated I κ B and its subsequent degradation allow translocation of NF- κ B to the nucleus, and triggers the transcription of widely gamma of genes. Following this line of thought, also were examined structural analogues of CAPE, these analogues have been previously characterized for their ability to inhibit human HIV integrase and cell growth (Burke et al., 1995), and although all the compounds were active in inhibiting NF- κ B activation, there were marked variations in their inhibitory ability, they found that compounds 1 and 6 (Fig. 3) inhibited NF- κ B translocation to the nucleus more efficiently than CAPE (line P) (Natarajan et al., 1996).

As result of structure activity relationship analysis they found that alteration of the hydroxyl group placement from 3,4-dihydroxy (CAPE) to 2,5-dihydroxy (compound 1) increased the potency of inhibition over that resulting from replacement of the hydroxyl groups of CAPE with two methyl ethers (compound 2) and the addition of a third hydroxyl group (compound 3) resulted in a loss of potency, with these analysis they suggest that the number and the placement of hydroxyl groups is a critical determinant of the extent of inhibition. In the ester group of analogues, the caffeic acid portion was held constant and the phenylethyl side chain was varied. An increase in the length of the alkyl chain (compound 4) resulted in a significant loss of inhibition. Bicyclic analogues of the two isomers of CAPE that differed in the placement of hydroxyl substituents showed a drastic change in the inhibitory potency of the two analogues; the isomer 5 was completely ineffective, whereas the isomer 6 completely abolished the binding, once again indicating that the placement of the hydroxyl groups played a critical role in inhibiting NF- κ B activation. In the saturated amide analogues, the analogue with three additional hydroxyls (compound 7) and the reverse amide analogue (compound 8), which lacked an additional hydroxyl group, were less active than CAPE. Thus it is possible to find structural analogues of CAPE that are more active than CAPE (compound 6), as active as CAPE (compound 1), and less active than CAPE (compounds 2-5, 7, and 8).

The analogues that were maximally active in inhibiting NF- κ B activation were different from those with maximum inhibitory activity for either HIV integrase or cell growth, suggesting a difference in the mechanism. For instance, compound 6, one of the conformationally constrained CAPE variants (5, 6 dihydroxy derivative), was more potent than native CAPE for NF- κ B activation but less potent than the parent compound for inhibition of HIV integrase and cell growth (Burke et al., 1995).

The antibacterial activity of cinnamic acid derivates has been reported (Ramanan & Rao, 1987), and some QSAR studies have been done, and they reported that the introduction of halogen onto the benzene ring of cinnamic acid enhance the antimicrobial activity against gram negative bacteria (Ramanan et al., 1987). The reactive $\alpha\beta$ -unsaturated carbonyl is a common factor in compounds showing antimutagenic activity, it is speculated that $\alpha\beta$ -

unsaturated carbonyl systems react with nucleophiles and exert their antimutagenic activity by trapping thiol groups of target proteins (Kakinuma, 1986). Structure-activity analysis suggests that 3', 4' catechol ring is important for the antioxidant potential, metal chelation and free radical captures (Kerry & Rice-Evans, 1998), although both groups hydroxyl decrease its lipophilicity and thus their ability to cross cell membranes. On the other hand, it has been reported that increasing the length of the carbon chain reduces the fungitoxic activity of CAPE analogues (Jun ZHU, 2000).

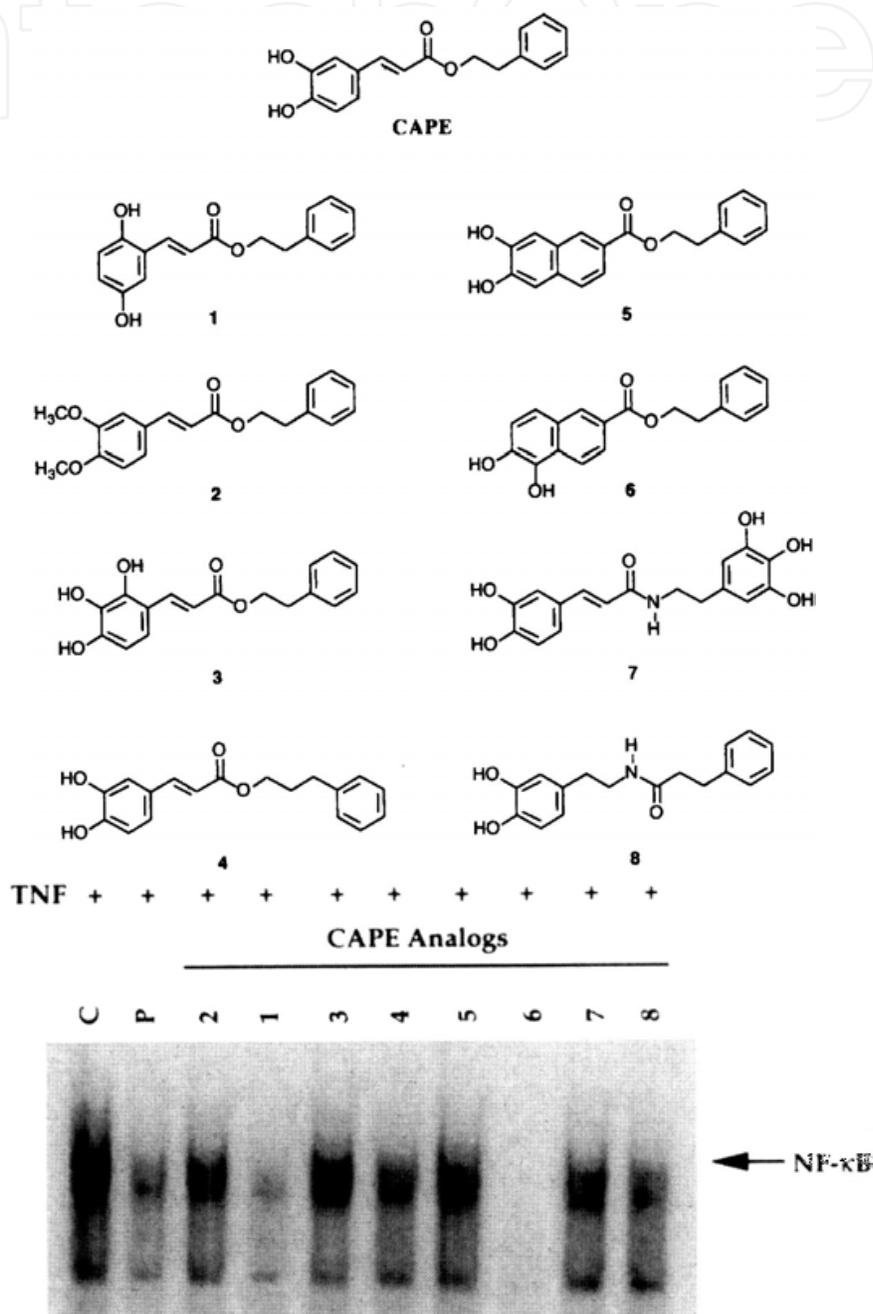


Fig. 3. Structure-activity relationship studies: several analogues of CAPE were synthesized including ring substituents (compounds 1 to 3), ester groups (compound 4), rotationally constrained Variants (compounds 5 and 6), and saturated amide analogues (compounds 7 and 8). Taken from Natarajan et al 1996.

Jun ZHU et al. tested the biological activity of several cinnamato and cinnamide derivatives in a fungitoxicity test in which *Pythium sp.* and *C. rolfisii*, were used as test plant pathogenic fungi. They were cultured in potato dextrose agar for 4-5 days at 27 °C, was added and the test compounds and fungitoxic activity was expressed as % inhibition of mycelial growth diameter.

They work too with a Phytotoxicity test in which the test compound in solution was poured into a filter paper, ten seeds of *Brassica rapa var. amplexicaulis* were sown on the filter paper in petri dishes. After three day incubation at 27 °C, inhibitory activity on the length of the plant's radicle and hypocotyl was measured and compared with the control. The cinnamic acid analogues remain in varying degrees phytotoxic and cytotoxic effect, they reported that cinnamic acid derivatives having methyl, propyl or isopropyl group as the substituent R₂ showed the highest fungitoxic activity. Derivatives of 4-Isopropylcinnamide showed high fungitoxic activity and derivatives of 4-Chlorocinnamide showed relatively high fungitoxic activity, the introduction of two chlorine atoms at 2 and 4 positions of cinnamic acid decreased activity against both pathogenic fungi and plant growth (Jun ZHU et al., 2000).

Compound	R	EC ₅₀	
		Cytotoxicity (μM)	NO Inhibition (μM)
1	H	3406.000 ± 714	165.295 ± 16.05
2	CH ₃	367.500 ± 133	3.199 ± 0.27
3	C ₂ H ₅	121.700 ± 17.16	3.183 ± 0.27
4	C ₃ H ₇	26.420 ± 4.38	0.440 ± 0.08
5	C ₄ H ₉	7.419 ± 0.93	0.240 ± 0.06
6	C ₆ H ₁₃	2.677 ± 0.96	0.340 ± 0.05
7	C ₇ H ₁₅	4.594 ± 1.12	0.236 ± 0.02
8	C ₈ H ₁₇	1.588 ± 0.22	0.060 ± 0.01
9	C ₉ H ₁₉	1.658 ± 0.02	0.052 ± 0.02
10	C ₁₀ H ₂₁	1.542 ± 0.18	0.045 ± 0.09
11	C ₁₁ H ₂₃	1.188 ± 0.06	0.018 ± 0.02
12	C ₁₂ H ₂₅	1.000 ± 0.08	0.556 ± 0.05
13	C ₁₄ H ₂₉	1.256 ± 0.07	0.292 ± 0.03
14	C ₁₆ H ₃₃	3.200 ± 0.36	0.713 ± 0.13
15	C ₁₈ H ₃₇	2.671 ± 0.29	0.573 ± 0.04
16	(CH ₃) ₂ CH	42.200 ± 5.97	0.302 ± 0.03
17	C ₂ H ₃ (CH ₃) ₂	13.000 ± 1.81	0.303 ± 0.08
18	cyclo-Hexyl	28.700 ± 3.88	1.655 ± 0.36
19	Benzyl	38.800 ± 3.57	0.347 ± 0.04
20	Prenyl	30.000 ± 1.61	0.578 ± 0.04
21	Geranyl	3.054 ± 0.16	0.223 ± 0.01
22	Farnesyl	2.658 ± 0.16	0.258 ± 0.03
	Phenethyl	4.518 ± 0.04	0.193 ± 0.04

Table 1. Effect of Caffeic acid derivatives on NO production in RAW264.7 macrophage. Obtained from Uwai et al., 2008.

As shown in table 1, a structure activity relationship analysis showed that caffeic acid esters in different degrees preserved their activity to inhibit nitric oxide (NO) production induced by lipopolysaccharide in murine RAW264.7 macrophages, which is reflected in the median effective concentration (EC50) of each compound. The inhibitory effect of these derivatives on NO production in RAW264.7 macrophage was dependent on the length and size of the alkyl moiety, and undecyl caffeate was the most potent inhibitor of NO production (Uwai et al., 2008).

Additionally, these authors showed that the connection between caffeic acid and the alkyl chain is critical for activity. Amide and ketone derivatives showed that not only the ester functional group but also the amide and ketone functional groups exhibit an inhibitory effect on NO production (Uwai et al., 2008).

These examples suggest that compounds with similar structure to CAPE could keep on varying degrees the chemoprotective effect that CAPE has shown on *in vivo* models like is the resistant hepatocyte modified model.

2.3 *In vivo* assays on the resistant hepatocyte modified model

It has been possible to study the chemical carcinogenesis and the chemoprotective effect of some chemical compounds using experimental animal's models. In the animal models, the chemical carcinogenesis is reproducible, and has advantages over cell culture and clinical biopsies as the possibility of study the carcinogenesis from the initiation through the different stages until the tumor establishment, also is possible to obtain information of the participation of cell not belonging to the tumor, or test the secondary effect of these compounds, while human precancerous lesions such as dysplastic nodules are rather difficult to obtain because of their small size, difficult detection, and coexistence with other liver pathologies. The models of chemical carcinogenesis require chemical compounds able to alter the DNA, so they are mutagenics, many of them need a metabolic bioactivation and they showed a direct relation in its ability to form adducts, produce mutations and lead to cancer. The modified Semple-Roberts model in rats, allow the study of hepatocarcinogenesis (Semple-Roberts et al., 1987; Solt & Farber, 1976), providing valuable information about the changes that occur throughout the process.

On the initiation, produced by the carcinogen diethylnitrosamine (DEN) in the resistant hepatocyte modified model, Fischer 344 male adult rats weighing between 180 and 200 g are administrated with the carcinogen DEN at dose of 200 mg/kg *i.p.* at day 0 of treatment, as promotion stimulus is administered a daily dose of 20 mg/kg *o.p.* of 2-acetyl-aminofluorene (2-AAF) at days 7, 8 and 9, and is performed a partial hepatectomy at 10th day. In addition, using histoenzimatic staining for gamma-glutamyl-tranpeptidasa (GGT) and Glutation-S-transferasa (GST-p) as markers of preneoplastic lesions (Carrasco-Legleu et al., 2004; Carrasco-Legleu et al., 2006) was determined that the highest number of preneoplastic lesions in rat liver is reached between 25 and 30 days after start the treatment, and lead to tumor after one year of the treatment.

Mutations in DNA and hepatocyte proliferation are common to models of hepatocarcinogenesis, DEN is a genotoxic and mutagenic alkylating agent, its metabolic bioactivation by cytochromes P-450 produce oxidative stress and reactive chemical species such as ethyl carbonium ion, producing ethylated and oxidized adducts with

macromolecules as DNA and proteins, both of them have an important role in the carcinogenesis (Sánchez-Pérez et al., 2005; Takabe et al., 2001). As example of the adducts formed by DEN are the N-7-ethylguanine and the O6-ethylguanine because the nitrogenated base more likely to be ethylated by DEN is guanine in the N7 and O6 positions, due to the electrophilic character of the ethyl carbonium ion, it react covalently with nucleophilic sites of the cell components to produce ethylated adducts (Verna et al., 1996). As result of cell damage DEN is necrogenic and cellular death is a stimulus to restore the lost tissue, inducing a cell division that fixes mutations in cells with unrepaired adducts on its genetic material. Hepatocyte regeneration have been implicated in the development of HCC, 2-AAF has a mitoinhibitory effect on the uninitiated hepatocytes, while allow the selective proliferation of initiated hepatocytes as consequence of the proliferative stimulus of partial hepatectomy (Ohlson et al., 2004), it means, only proliferate the hepatocytes resistan to the mitoinhibitory effect of 2-AAF.

The resistant hepatocyte model modified allows the study of the chemopreventive properties observed in various compounds such as the anti-inflammatory drug (NSAID) celecoxib (Arellanes-Robledo et al., 2006; Arellanes-Robledo et al., 2010) and antioxidants quercetin (Vasquez-Garzon et al., 2009) and CAPE (Beltrán-Ramírez et al., 2008), these compounds decreased the percentage of GGT+ area in rat liver, used as a marker of preneoplastic lesions, compared to the livers of rats receiving carcinogen treatment only. Each chemopreventive compound can be studied under different conditions in the model, such as a different stage (Three main steps: initiation, promotion, and progression), dose or route of administration, given that many chemicals possess multiple modes of action. CAPE has shown anticarcinogenic properties on initiation and in progression stages in the modified resistant hepatocyte model.

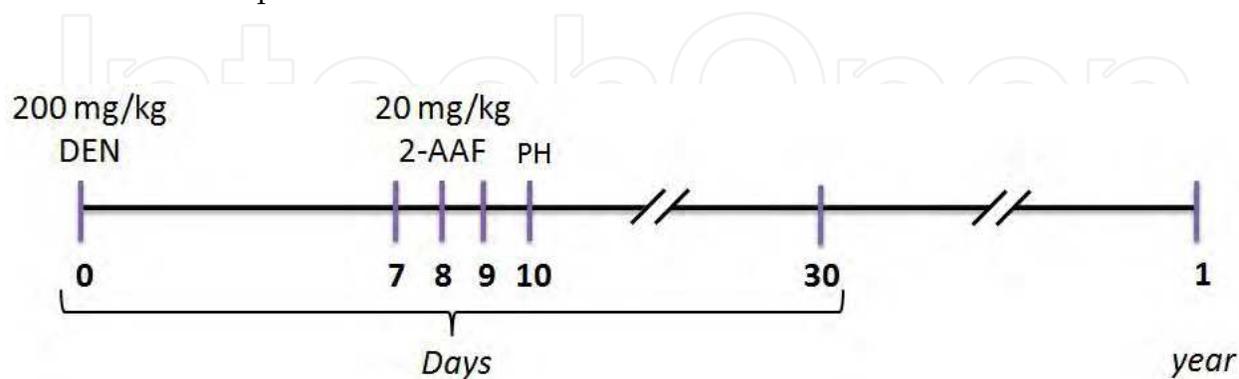
When given during promotion, CAPE decreased the expression of number and area of altered hepatic foci (GGT+ AHF) by 91% and 97%, respectively at 25 d of carcinogenic treatment. Glutathione S-transferase placental (GST-P), another protein marker for preneoplastic lesions was decreased 82%. Additionally, were evaluated the effect of CAPE on the expression of nuclear factor NF- κ B and found an 85% decrease in nuclear localization of NF- κ B (Carrasco-Legleu et al., 2004).

When is given 12 h before initiation, CAPE prevents preneoplastic lesions, as were shown by GGT histoenzymatic staining of liver sections, showing that CAPE decrease 84% the number and 91% the area GGT+ AHF in the liver rats at 25 days with respect to the group that received only the carcinogenic treatment as see in Figure 4, and in case of the GSTp the protein level was reduced by 90% (Carrasco-Legleu et al., 2006).

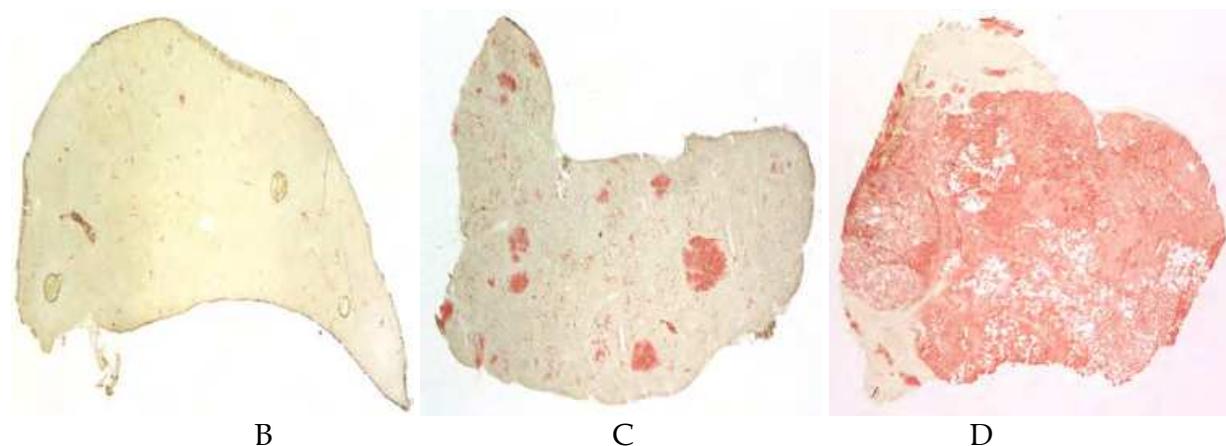
On initiation stage the mechanism of action were further investigated testing the effect of CAPE during the early stages of liver carcinogenesis. When CAPE is administered at dose of 20 mg/kg to the rats 12 h before initiation, the hematoxylin-eosin histological staining of liver sections showed that CAPE prevents necrosis at 24 h after DEN administration, in relation to the group of rats that received only DEN; indicating that CAPE administration reduces the toxicity of DEN.

DEN requires metabolic activation by CYP to lead to the formation of diazoalkanes or carbocations and ultimately to the alkylation of nucleophiles, reactive species are known to

induce cancer in mammals. With the research in this model were reported that CAPE modifies the enzymatic activity of CYP isoforms involved in the activation of DEN, such CYP1A1, CYP1A2, CYP2B1/2, and CYP2E1 (Beltrán-Ramírez et al., 2008). Suggesting that CAPE may modify the enzyme activity of CYP isoforms involved in DEN activation, and the modification of DEN metabolism could lead to a detoxification without the formation of reactive chemical species.



A



B

C

D

Fig. 4. A) Resistant hepatocyte modified model. DEN: Diethylnitrosamine, 2AAF: 2-acetylaminofluorene, HP: Partial hepatectomy. B) Untreated liver. C) preneoplastic lesions on liver 1 month after carcinogenic treatment. D) Liver tumor 1 year after carcinogenic treatment.

Increased concentrations of active oxygen, organic peroxides and free radicals can promote initiated cells to neoplastic growth, inducing alterations in DNA structure or producing epigenetic mechanisms. It has been reported antioxidant activity for catechol rings, like in CAPE related compounds (Bors et al., 2004), and the effect of CAPE on lipid peroxidation (LPX) was measured by the thiobarbituric acid reactive species assay (TBARS), 12 h after DEN administration was detected a 68% increase of (TBARS). When CAPE was administered before DEN, it completely protected from liver TBARS induction (Carrasco-Legleu et al., 2006).

This model has been analyzed by DNA microarray methodologies at different stages, for the gene expression profile of preneoplastic nodules and hepatocellular carcinomas (HCC) to define the genes implicated in cancer progression (Pérez-Carreón et al., 2006), as a result we

have a big database of genes that will allow to investigate the mechanism by which the cancer evolve, with the option of inquiring how this genes participate in carcinogenesis and how could be modulated. Among the main possibilities it also allows to search for possible early markers, that is important to prevention or to design a treatment. Gene expression profiles induced by DEN have been compared with those obtained from rats previously administered with a single dose of CAPE, as example of the results obtained by microarrays, it has been found that CAPE alone did not alter the expression profile, DEN treatment modified the expression of 665 genes, and CAPE plus DEN changes 1371 genes in the expression profile. Some of the genes found decreased in its expression on CAPE plus DEN treatment were Glutation reductasa, *GST-k*, *GST-0*, *p53* and *CYP2b1*, the last one involved in DEN bioactivation. The database obtained will help to elucidate the mechanism by which CAPE exert its chemoprotective activity (Beltrán-Ramírez et al., 2010).

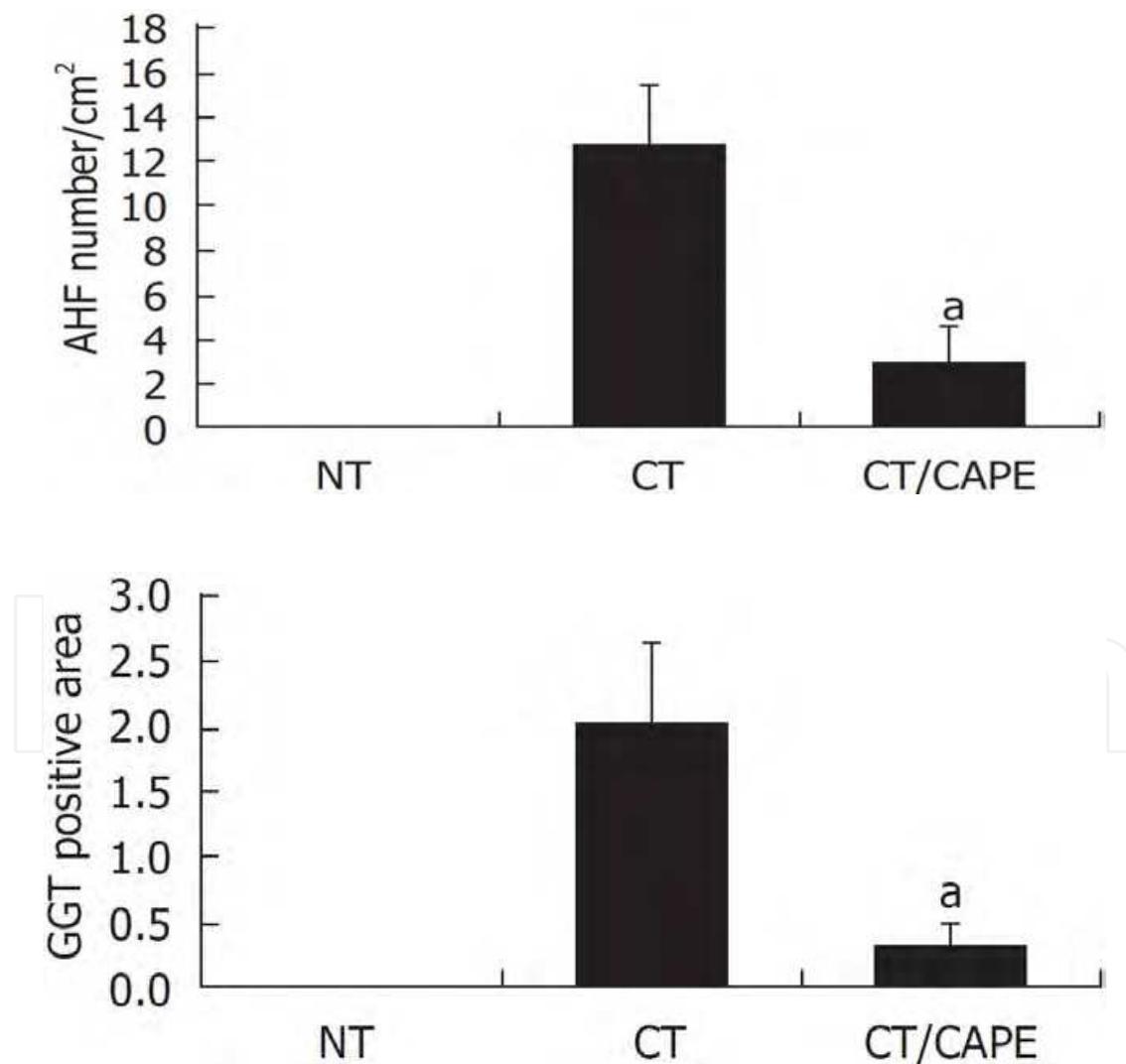


Fig. 5. Effect CAPE pretreatment on number/cm² of AHF and percentage of GGT+ area/tissue. Taken from Carrasco-Legleu et al 2006.

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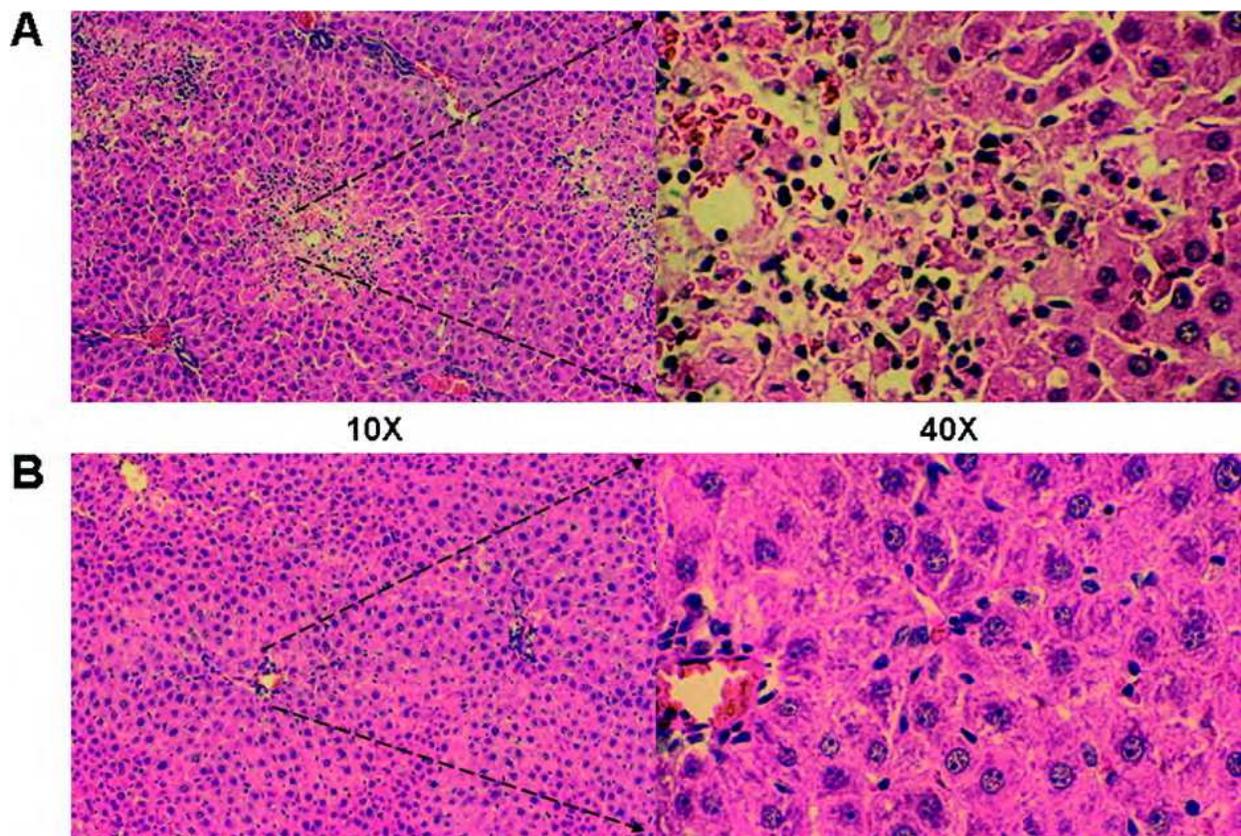


Fig. 6. Effect of CAPE on Necrosis induced by DEN. A) Necrosis produced by DEN 24h after administration. B) Diminution of necrosis produced by DEN 24h after administration by effect of CAPE. Taken from Beltrán-Ramírez et al., 2008

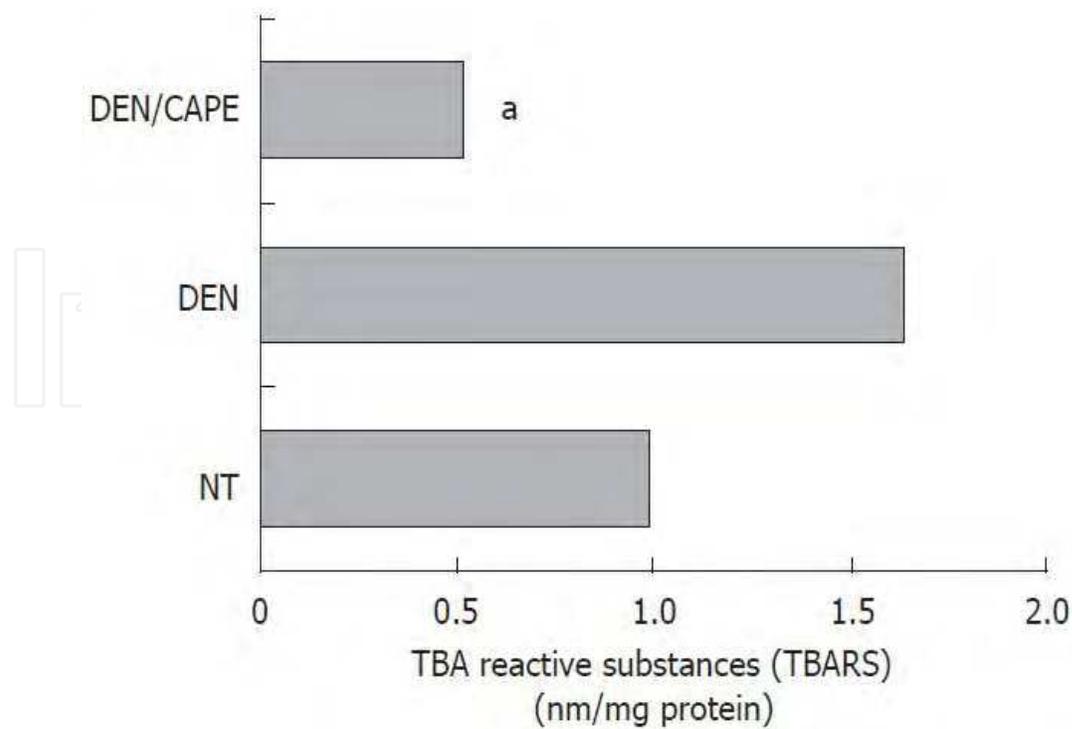


Fig. 7. Effect of CAPE on LPX levels induced by DEN. Tiobarbituric acid reactive substances in group NT: Not treated; DEN: 12h After DEN administration; DEN/CAPE: CAPE pretreatment 12h before DEN. Taken from Carrasco-Legleu 2006.

3. Conclusions

The standardization of natural products is necessary as well as the study of its components, given that substances with biological activity often have been found while studying substances purified from natural products to which folk medicine has attributed therapeutic properties. Many natural products are chemopreventive, several of them as dietary constituents, and experiments with cultured cells and animal models are revealing their potential mechanisms of action, and mainly these mechanism are related with the capability of prevent or greatly reduce initiation of carcinogenesis, or cell proliferation.

Honey and propolis are rich in phenolic compounds, and are becoming increasingly popular because of their potential role in contributing to human health. Data from laboratory studies indicate that CAPE has important effects on cancer chemoprevention and many mechanisms of action have been identified for CAPE and related compounds. First were reported that the antitumor activity of polyphenolic compounds like CAPE includes direct cytotoxic effects on tumor cells on in vitro experiments, this effect have been tested with positive results in several kinds of tumors in vivo, the effect on initiation was tested in an rat hepatocarcinogenesis model, were CAPE show a protective effect when a single dose was given before initiation, decreasing the induction of area and number of GGT+ AHF. As chemoprotection mechanism of CAPE was proposed it is due to its anti-oxidative and free-radical scavenging activities, and now have been added to chemoprotection mechanism on initiation stage that CAPE modifies the CYP-dependent DEN bioactivation and decreases reactive chemical species, inhibiting the initiation stage of carcinogenesis.

Although, the biological activities of caffeic acid esters and other analogues have been studied by analyzing their structure, the detailed mechanisms of their activities remain unclear. The research of mechanism-based compound can contribute to a greater understanding of cancer and a faster development of successful therapies, as suggested by the experiments analyzed here, compounds with similar structure to CAPE could keep or enhance the chemoprotective effect that CAPE has shown. It is of special importance that many protective effects of CAPE and related compounds could have common mechanisms for chemoprotection, by this reason it is needed to test on *in vivo* models the effect of modifications on the structure of CAPE that let us know the participation of properties like lipophilicity, anti-oxidative and free radical scavenging among others on the chemoprotection. If the CAPE analogues compounds share one or more of the mechanism of action and his preparation is easy, quick and inexpensive, these compounds may be promising anticancer agents as well as CAPE.

HCC is an aggressive tumor with a high fatality rate, early detection likely will be based on characterization of the molecular pathogenesis of this disease, and a successful treatment should be developed with this knowledge together with the understanding of the mechanism of action of drugs.

4. References

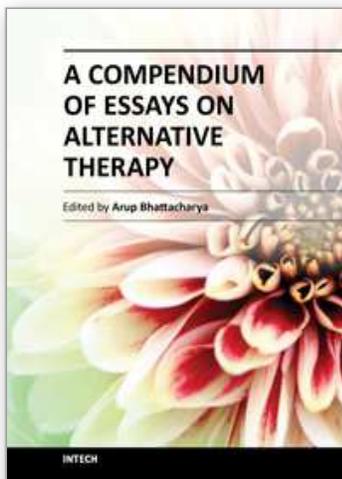
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A Compendium of Essays on Alternative Therapy is aimed at both conventional and alternate therapy practitioners, besides serving as an educational tool for students and lay persons on the progress made in the field. While this resource is not all-inclusive, it does reflect the current theories from different international experts in the field. This will hopefully stimulate more research initiatives, funding, and critical insight in the already increasing demand for alternate therapies that has been evidenced worldwide.

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