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***Mikania glomerata* and *M. laevigata*: Clinical and Toxicological Advances**

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1. Introduction

Mikania laevigata and *M. glomerata*, commonly known as guaco, are important medicinal plant species used in South America for the treatment of respiratory diseases. In folk medicine, their leaves have ample use due to their balsamic, antiophidic, appetite stimulant, antispasmodic, expectorant, and antimalarial properties, among others (Coimbra, 1942; Lucas, 1942; Neves & Sá, 1991; Alice et al., 1995; Gasparetto et al., 2010; Napimoga & Yatsuda, 2010).

There is also pre-clinical evidence of the anti-inflammatory, anti-allergy, and bronchodilation activities of these species (Fierro et al., 1999; Moura et al., 2002; Suyenaga et al., 2002; Graca et al., 2007a). Due to their important effects, pharmaceutical preparations, including syrup and oral solutions, are freely distributed through various government phytotherapy programs and, thus, are widely used by the population (Gasparetto et al., 2010).

The pharmacological effects of guaco are attributed mainly to the presence of coumarin (1,2-benzopyrone); however, other metabolites have been shown to produce significant pharmacological effects. Studies that have evaluated isolated markers in the mouse model of allergic pneumonitis have demonstrated that coumarin and *o*-coumaric acid are part of the phytocomplex that is responsible for the therapeutic activities of guaco species (Santos et al., 2006). In addition, dihydrocoumarin and syringaldehyde have antioxidant, immunologic and anti-inflammatory properties (Farah & Samuelsson, 1992; Hoult & Paya, 1996; Bortolomeazzi et al., 2007; Stanikunaite et al., 2009; Gu & Xue, 2010). Finally, kaurenoic acid, isolated in high quantities from both species (Fierro et al., 1999; Veneziani et al., 1999; Yatsuda et al., 2005), has been shown to contribute to the effects of guaco through its antimicrobial, antinociceptive, anti-inflammatory and smooth muscle relaxant activities (Block et al., 1998; Costa-Lotufo et al., 2002; Wilkens et al., 2002; Cunha et al., 2003; Cotoras et al., 2004; Tirapelli et al., 2004; Cavalcanti et al., 2006).

The presence of these metabolites is directly related to the benefits of guaco, but studies have shown them to be toxic. Dihydrocoumarin administered to groups of rodents led to carcinogenic activity, ulcers, forestomach inflammation, parathyroid gland hyperplasia and increased nephropathy (National Toxicology Program, 1993a). Kaurenoic acid has been shown to kill sea urchin embryos and to cause hemolysis in mouse and human erythrocytes

(Costa-Lotufo et al., 2002); it also induces DNA breaks, cytogenetic abnormalities in human peripheral blood leukocytes, and positive genotoxic effects in the liver, kidney and spleen of mice (Cavalcanti et al., 2010). In addition, kaurenoic acid has been shown dose-dependent genotoxicity in Chinese hamster lung fibroblast cells (Cavalcanti et al., 2006).

Isolated coumarin has been shown to be carcinogenic, especially in the liver and lungs of rats and mice (Lake, 1999). With long exposure, this substance may change biochemical and hematological parameters and cause ulcers and necrosis, fibrosis, and cytologic alterations in the liver (National Toxicology Program, 1993b). In humans, the majority of tests for mutagenicity and genotoxicity suggest that coumarin is not toxic. This low toxicity is attributed to the mechanism of the detoxification of coumarin, which occurs via the 7-hydroxylation pathway in humans. In rats and mice, the main route is by 3,4-epoxidation, resulting in the formation of toxic metabolites (Lake, 1999).

Considering that the toxic and therapeutic effects of these metabolites are dose dependent, understanding their mechanisms and scientific advances is a key point to validate their therapeutic indications without putting human health at risk. This chapter describes the scientific aspects of guaco, especially the pre-clinical and clinical studies, with a particular emphasis on the pharmacological and toxicological effects of the extracts, preparations and isolated metabolites.

Keywords: *Mikania laevigata*, *Mikania glomerata*, guaco, toxicity, pharmacological effects, review, coumarin, *o*-coumaric acid and kaurenoic acid.

2. General overview

Mikania glomerata Sprengel and *M. laevigata* Schultz Bip. ex Baker, commonly known as guaco, are medicinal species used to treat several inflammatory and allergic conditions, particularly in the respiratory system due to their bronchodilator properties (Gasparetto et al., 2010).

Both species grow in the same regions and have similar morphological characteristics, which make them hard to distinguish. The leaves are similar, and both species have the characteristic odor of coumarin. The main difference between the species is the flowering period, which occurs in January for *Mikania glomerata* and September for *M. laevigata*. Therefore, humans use these plants without distinction (Lima, 2003; Ritter & Miotto, 2005).

An similar chemical profile has also been described for these plants (Oliveira, 1986; Lima & Biasi, 2002). Therefore, detailed studies of their morphological and anatomical features are necessary to allow botanical identification and quality control of these medicinal species in the absence of another way to make the distinction.

In folk medicine, these plants have a long history of use by rainforest inhabitants, especially by native peoples in South American, who have an ancient tradition of using guaco for the treatment of several diseases. Amazonian tribes have used the crushed leaf topically on skin eruptions and on snakebites. They also consume teas made from the leaves and/or stems against snake venom and to cure fevers, stomach disorders and rheumatism. South American tribes also believe that the aroma of the freshly crushed leaves left around sleeping areas keeps snakes away (Napimoga & Yatsuda, 2010).

In recent decades, guaco has been used as a home and commercial remedy. In popular medicine, the leaves have been widely used due to their tonic, antipyretic, balsamic, antiphidic, appetite stimulant, neuralgia, antispasmodic, expectorant, and antimalarial properties and for the treatment of rheumatism, eczema, influenza, asthma and sore throat.

Guaco can be used as infusion and decoction, but it is most commonly used in the commercialization of crude extracts for medicinal purposes (Coimbra, 1942; Lucas, 1942; Neves & Sá, 1991; Ruppelt et al., 1991; Galvani & Barreneche, 1994; Alice et al., 1995; Matos, 2000; Souza & Felfili, 2006; Botsaris, 2007).

Because of the therapeutic effects attributed to guaco species, syrups and oral solutions are widely used by the South American population and have been distributed in free government phytotherapy programs (Gasparetto et al., 2010). These preparations have been used as an effective natural bronchodilator, expectorant and cough suppressant in treatment of respiratory problems such as bronchitis, pleurisy, cold, flu, coughs and asthma, and sore throats, laryngitis and fever (Napimoga & Yatsuda, 2010).

3. Chemical constituents

Numerous studies have been conducted to evaluate the chemical composition of guaco species. Detailed screenings revealed the presence of alcohols, acids, esters, aldehydes, organic esters, terpenes, diterpenes, triterpenes and steroids, among other metabolites; some of them are associated with the therapeutic effects of guaco (Gasparetto et al., 2010).

A wide variation in metabolite content has been observed among different extracts and pharmaceutical preparations (Gasparetto et al., 2011). In fact, the geographic origins, agronomic aspects, extraction solvent and extraction techniques have been described as crucial factors to obtain a desirable substance. Thus, to maximize the yield of any metabolite and to standardize the extracts, these aspects must be considered (Gasparetto et al., 2010).

In the essential oil of guaco, a variety of compounds have been found, including α -acorenol, α -cadinol, α -copaene, α -humulene, α -muurolol, α -pinene, α -terpinol, β -pinene, β -farnesene, β -bourbonene, β -cubebene, β -elemene, β -caryophyllene, γ -elemene, (E)- β -ocimene, (E)-nerolidol, *p*-cymene, α , β , γ and Δ cardinene, α and TAU-caudynol, epi- α -bisabolol, epi- α -muurolol, aromadendrene, bicyclogermacrene, caryophyllene oxid, citronellyl acetate, coumarin, cubebene, elemol, germacrene-B, germacrene-D, globulol, limonene, linalol, myrcene, nerolidol E, nonanal, sabinene, silvestrene, spathulenol, terpin 4-ol, *trans*-ocymene, *trans*-cariophyllene and 1,4-dimethoxybenzene (Radunz, 2004; Duarte et al., 2005; Rehder et al., 2006).

In hexanic and dichloromethane extracts, the presence of coumarin, *o*-coumaric acid, campesterol, kaurenoic acid, grandifloric acid, stigmasterol, lupeol, lupeol acetate, germacrene, sesquiterpenes, 11-methylbutanoic acid, *ent*-15 β -benzoyloxykaur-16(17)-en-19-oic acid, 17-hydroxy-*ent*-kaur-15(16)-en-19-oic acid, β -sitosterol and peroxides has been described (Oliveira et al., 1984; Vilegas et al., 1997a; Vilegas et al., 1997b; Santos et al., 1999; Veneziani et al., 1999; Cabral et al., 2001; Schenkel et al., 2002; Contini et al., 2006).

Hydroalcoholic extracts are the most common preparations that have been commercialized for therapeutic purposes, and the majority of phytochemical assays that have been conducted have been to evaluate their chemical compositions. Thus, using different analytical procedures, the presence of a large number of compounds has been described, including stigmasterol, phytol, 1-ethoxy-1-phenylethanol, 4-hydroxy-3,5-dimethoxybenzaldehyde, hexanoic acid, ethyl hexadecanoate, ethyl linoleate, kaurenol, an isomer of kaurenoic acid, spathulenol, hexadecanoic acid, 9,12,15-octadecatrienoic acid, cupressenic acid, isopropiloxigrandifloric acid, 2-5-ciclohexadiene-1,4-dione, 2,6-bis, 1-octadecene, octadecanoic acid, ester diterpenic, caryophyllene oxide, 10,13-octadecadienoic acid, isobutylloxigrandifloric acid, *trans*-cariofileno, 8,11-octadecadienoic acid, lupeol, lupeol

acetate, benzoylgrandifloric and cinnamoylgrandifloric acids (Oliveira et al., 1993; Moura et al., 2002; Biavatti et al., 2004; Santos, 2005; Yatsuda et al., 2005; Bertolucci et al., 2008; Alves et al., 2009; Bolina et al., 2009; Muceneeki et al., 2009).

In quantitative terms, the most prevalent metabolites of hydroalcoholic extracts are coumarin (1,2-benzopyrone) (Biavatti et al., 2004; Bueno & Bastos, 2009), *o*-coumaric acid (Santos, 2005), dihydrocoumarin (Alves et al., 2009), syringaldehyde (Muceneeki *et al.*, 2009) and kaurenoic acid (Vilegas et al., 1997a; Vilegas et al., 1997b; Yatsuda et al., 2005; Bertolucci et al., 2008). These substances have been associated with the therapeutic effects of guaco because they have anti-inflammatory and bronchodilator properties. The chemical structures of each compound are shown in Figure 1:

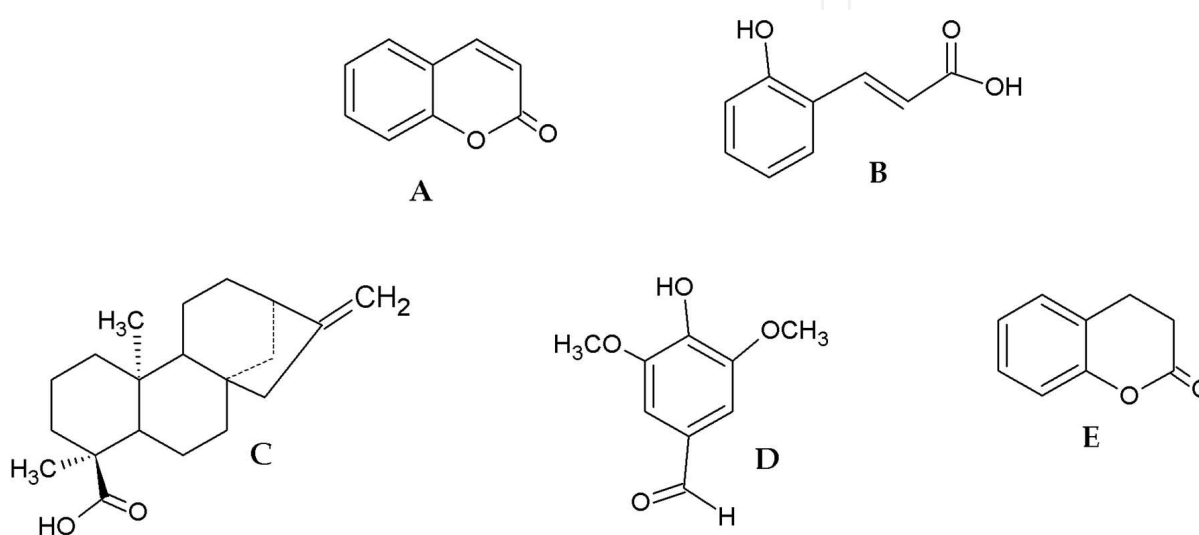


Fig. 1. Chemical structures of the main substances associated with the therapeutic effects of guaco. Data: (A) coumarin, (B) *o*-coumaric acid, (C) kaurenoic acid, (D) syringaldehyde and (E) dihydrocoumarin.

4. Pre-clinical and clinical trials

In addition to the use of guaco in popular medicine, pre-clinical studies have justified the main therapeutic uses of guaco species. Aqueous extracts prepared from several plant parts efficiently inhibit the different toxic, pharmacological, and enzymatic effects induced by the venom of *Bothrops* and *Crotalus* snakes. For example, guaco root extracts reduced the hemorrhage zone stimulated by the intradermal injection of *Bothrops* venom by 80% in rats. This result suggests that there is an interaction between the components of guaco and metalloproteases involving the catalytic sites of these enzymes or essential metal ions, thereby inhibiting their hemorrhagic activities (Maiorano et al., 2005).

Guaco extracts have also been considered to be powerful inhibitors of clotting activity, probably due to the interaction with thrombin-like enzymes. Guaco leaves and stems significantly diminished the coagulant activity induced by *Crotalus* and *Bothrops* venoms, especially the root extract, which led to clotting times of more than 45 min. Root extracts (1:50 *w/w*) also neutralized the edema caused by *Crotalus durissus terrificus* venom by 40%, with additional phospholipase A2 activity inhibition (95%). Nevertheless, no significant inhibition was observed against *Bothrops jararacussu* venom by incubating different ratios of guaco extracts and snake venom (1:50, 1:100 and 1:200 *w/w*) (Maiorano et al., 2005).

The tea of guaco leaves, administered orally in mice, had analgesic and anti-inflammatory activities following the intra-peritoneal administration of 0.1 N acetic acid or the intravenous administration of 0.2 mL Evans blue dye solution. The number of contortions was measured, and after 30 min of acid administration, a reduction of 63% was reached following oral administration of 10 mg/kg of the extract. The inhibition of dye diffusion to the peritoneal cavity was 49%, indicating an anti-inflammatory activity, but this result was not consistent with the analgesic effects (Ruppelt et al., 1991).

The hydroalcoholic extract also affected the inflammatory and oxidative stress caused by a single coal dust intratracheal instillation in rat. Histopathological analyses revealed that animals pretreated subcutaneously with the hydroalcoholic extract (100 mg/kg) had a reduction in lung inflammation, with an additional decrease in protein thiol levels, suggesting that guaco has an important protective effect on the oxidation of thiol groups (Freitas et al., 2008).

With regard to the antiedema activity of guaco, *in vivo* studies conducted in rats treated orally with an extract made from leaves (400 mg/kg) showed a complete reduction in the paw edema induced by carrageenan. A 28.26% decrease in leukocyte migration at the lesion site was also observed (Suyenaga et al., 2002). In mice, the subcutaneous administration of the same extract (3 mg/kg) significantly reduced the vascular permeability and leukocyte adhesion to inflamed tissues with carrageenan-induced peritonitis. The antiedema activity of guaco species has been associated with the inhibition of the pro-inflammatory cytokine production at the inflammatory site (Alves et al., 2009).

The ability of guaco to decrease ulcerative lesions was also tested by treating rats orally with 1000 mg/kg crude hydroalcoholic extract. A 50% decrease in the ulcerative lesions produced by reserpine was achieved, with higher levels of reduction in lesions caused by hypothermic restraint stress (82%), indomethacin (85%) and ethanol (93%). The antisecretory mechanism was confirmed by measuring acid hypersecretion induced by histamine, pentagastrin and bethanechol. Duodenal administration of the hydroalcoholic extract inhibited only the gastric acid secretion induced by bethanechol, a selective agonist of the muscarinic receptors of the parasympathetic nervous system (Bighetti et al., 2005).

The dichloromethane fraction obtained from the ethanolic extract was evaluated in rats for its anti-allergic and anti-inflammatory properties on ovalbumin-induced allergic pleurisy and in models of local inflammation induced by biogenic amines, carrageenan and Platelet-Activating Factor (PAF). The subcutaneous injection of 100 mg/kg of the dichloromethane fraction significantly reduced the plasma exudation, leukocyte infiltration and PAF. Because the pre-treatment of the animals did not alter the pleurisy induced by histamine, serotonin or carrageenan, the fraction was considered effective only for inhibiting immunologic inflammation and not the acute inflammatory response caused by other agents (Fierro et al., 1999).

Guaco also has antidiarrheal effects by decreasing the propulsive movements of the intestinal contents in mice. The percentage distances of the small intestine (from the pylorus to the cecum) traveled by the charcoal plug were determined. Oral administration of aqueous guaco extract (1000 mg/mL) produced a significant reduction in the distance of the charcoal marker in the animal feces ($66.99 \pm 10.60\%$). This extract was considered to give an excellent outcome because the reduction was as effective as that produced by loperamide ($62.34 \pm 11.21\%$), a reference antidiarrheal drug (Salgado et al., 2005).

The antiparasitic effects of lyophilized hydroalcoholic extracts on the growth of *Leishmania amazonensis* and *Trypanosoma cruzi* were also established. By inoculating the parasites in

medium containing 100 µg/mL of the extract, approximately 50% growth inhibition was observed for the *Trypanosoma* epimastigote and *Leishmania* promastigote forms. Additionally, under the tested concentration, a nearly complete reduction was achieved for the *Leishmania* amastigote form ($97.5 \pm 2.6\%$) (Luize et al., 2005).

Different guaco extracts were also tested for their antimicrobial properties. Using the minimal inhibitory concentration (MIC) assay, the essential oil obtained from guaco leaves had only limited action (MIC values from 300 to >1000 µg/mL) against *Candida albicans* and different serotypes of *Escherichia coli* (Duarte et al., 2005, 2007). Lyophilized hydroalcoholic extracts showed some degree of antibacterial activity, with MIC values of 500 µg/mL for *Staphylococcus aureus*, 250 µg/mL for *Bacillus subtilis*, 500 µg/mL for *Escherichia coli*, >1000 µg/mL for *Pseudomonas aeruginosa*, 500 µg/mL for *Candida krusei* and *C. tropicalis*, and >1000 µg/mL for *C. albicans* and *C. parapsilosis* (Holetz et al., 2002).

The antimicrobial activities of the hexane, ethanolic and ethyl acetate fractions from the ethanolic extract of both guaco species were also evaluated by the MIC and minimum bactericidal concentration (MBC) assays. Negligible activity was observed using ethyl acetate fractions against strains of *Streptococcus mutans*, *S. cricetus* and *S. sobrinus*. The ethanolic extract fraction had moderate activity (MIC and MBC values from 25 to > 800 µg/mL) against different strains of *S. cricetus*, *S. sobrinus* and *S. mutans* but no bactericidal activity (MIC and MBC values > 800 µg/mL) against *S. mutans* D1 and P6 strains. Only the hexane fraction showed remarkable antibacterial activity, having the lowest MIC (12.5–100 mg/mL) and MBC (12.5–400 mg/mL) values (Yatsuda et al., 2005).

Regarding the use of guaco for the treatment of respiratory diseases, *in vitro* studies revealed that the hydroalcoholic extract produced dose-dependent relaxation in denuded and intact rat epithelium tracheal precontracted with acetylcholine, with a median effective concentration (EC₅₀) of 1400 µg/mL and a maximum effect (E_{max}) of 95%. The mechanism of relaxation has also been established, leading to the conclusion that the antispasmodic activity of guaco does not depend on epithelium-derived substances but instead involves changes in the cellular mobilization of calcium (Graça et al., 2007a). A dose-dependent relaxation was also observed in human bronchi precontracted with potassium, with a median inhibitory concentration (IC₅₀) of 0.34 mg/mL, supporting the indication that guaco is effective for the treatment of respiratory diseases in which bronchoconstriction is present (Moura et al., 2002).

In addition to guaco extracts, isolated compounds, especially the main metabolites, also have substantial pharmacological effects. Studies conducted in a mouse model of allergy pneumonitis recognized that both coumarin and *o*-coumaric acid are part of the phytocomplex responsible for the therapeutic activities of guaco species because a reduction in the influx of total leukocytes and eosinophils in lung tissue was observed upon treatment with these substances. Anti-inflammatory and antioxidant properties have been described for dihydrocoumarin, reported to be one of the major compounds in hydroalcoholic extracts. Syringaldehyde has been shown to have a moderate antioxidant activity (Bortolomeazzi et al., 2007) and a dose-dependent inhibition of cyclooxygenase-2 (COX-2) activity (IC₅₀ = 3.5 µg/mL), thereby contributing to the anti-inflammatory properties of guaco extracts (Farah & Samuelsson, 1992; Stanikunaite et al., 2009).

Kaurenoic acid (*ent*-kaur-16-en-19-oic acid) has lately been of considerable interest relating to the pharmacological activities of guaco species. At a concentration of 0.69 mg/mL, it has *in vitro* activity against trypomastigote forms of *T. cruzi*. It also has a moderate antimicrobial activity against strains of *S. aureus*, *S. epidermidis*, *Mycobacterium smegmatis* and *B. cereus*.

However, no antimicrobial action has been reported against Gram negative bacteria such as *E. coli* and *P. aeruginosa* (Silva et al., 2002; Zgoda-Pols et al., 2002).

Using the microculture tetrazolium assay (MTT), it was shown that 78 μM kaurenoic acid led to a 95% growth inhibition of CEM leukemic cells and a 45% growth inhibition of MCF-7 breast and HCT-8 colon cancer cells (Costa-Lotufo et al., 2002). In experiments conducted by the trypan blue dye-exclusion method, 70 μM kaurenoic acid reduced the viability of MCF7 and SKBR3 cells by 40% and 25%, respectively. However, resistance to treatment was observed in the HB4A cell line, demonstrating a selective activity in cancerous cells (Peria et al., 2010).

Kaurenoic acid also contributes to the anti-inflammatory activity of guaco. To determine this effect, lipopolysaccharide (LPS)-induced RAW264.7 macrophages were treated with different concentrations of kaurenoic acid, and a dose-dependent inhibition of nitric oxide production ($\text{IC}_{50} = 51.73 \mu\text{M}$) and prostaglandin E_2 release ($\text{IC}_{50} = 106.09 \mu\text{M}$) was observed. A reduction in the protein levels of COX-2 and the expression of inducible nitric oxide synthase was also seen. Additionally, kaurenoic acid dose-dependently inhibited the LPS-induced activation of the NF- κB mediator as assayed by electrophoretic mobility shift assay (EMSA), and it almost abolished the binding affinity of NF- κB for at 100.0 μM (Choi et al., 2011).

The anti-inflammatory effect of kaurenoic acid on acetic acid-induced colitis in rats has also been proven. Colitis was induced by intracolonic instillation of 2 ml of a 4% (*v/v*) acetic acid solution; 24 h later, the colonic mucosal damage was analyzed microscopically for the severity of mucosal damage, myeloperoxidase (MPO) activity and malondialdehyde (MDA) levels in the colon segments. A significant reduction in the gross damage score (52% and 42%) and wet weight of damaged colon tissue (39% and 32%) were observed in rats that received 100 mg/kg kaurenoic acid by rectal and oral routes, respectively. This effect was confirmed biochemically by a two- to three-fold reduction of the colitis-associated increase in MPO activity, a marker of neutrophilic infiltration, and by a marked decrease in the level of MDA, an indicator of lipoperoxidation in colon tissue. Furthermore, light microscopy revealed a marked decrease of inflammatory cell infiltration and submucosal edema formation in the colon segments of rats treated with kaurenoic acid (Paiva et al., 2002).

The *in vivo* anti-inflammatory effect of 50 mg/kg kaurenoic acid was examined in carrageenan-induced paw edema in mice. Kaurenoic acid dose-dependently reduced paw swelling up to 34.4% 5 h post-induction, demonstrating inhibition in an acute inflammation model. Taken together, the action of kaurenoic acid on COX-2 and inducible nitric oxide synthase expression is one of the mechanisms responsible for its anti-inflammatory properties (Choi et al., 2011).

At 160 μM , kaurenoic acid significantly decreased the contraction of rat uterine muscle precontracted with oxytocin ($E_{\text{max}} = 83\%$) and acetylcholine ($E_{\text{max}} = 91\%$) (Cunha et al., 2003). At 10 μM and above, kaurenoic acid also had concentration-dependent activity on vascular smooth muscle (endothelium-intact or denuded rat aortic rings) precontracted with phenylephrine and potassium chloride (Tirapelli et al., 2002, 2004). The mechanism of the vasorelaxant action involves the block of extracellular Ca^{2+} influx, but it is partly mediated by the activation of the nitric oxide cyclic GMP pathway and the opening of K^+ channels sensitive to charybdotoxin and 4-aminopyridine. Activation of the endothelial and neuronal nitric oxide synthase isoforms is also required for the relaxant effect induced by kaurenoic acid.

Although several guaco metabolites have been described as having therapeutic relevance, the simple coumarin (1,2 benzopyrone) has been considered to be the main component, and it has been used for the treatment of various clinical conditions. For example, in Brazil, the daily uptake (0.5–5 mg) of this substance has been assured by regulatory agencies (Brasil, 2008), but the recommended doses for the treatment of several diseases can vary largely according to the therapy (Lacy & O’Kennedy, 2004).

Coumarin is an anticoagulant and antithrombotic agent. It has been widely used in combination with troxerrutine to improve peripheral venous and lymphatic circulation and is also used to reduce the swelling caused by lymphatic and venous vessel problems. Pre-clinical studies also revealed that coumarin administered to the rat duodenum (100 mg/kg) produces antiulcerogenic activity by inhibiting the acid secretion mediated by the parasympathetic system (Bighetti et al., 2005).

In clinical trials, coumarin had *in vivo* macrophage-derived actions and has been used as an adjuvant in melanoma therapy and for recurrence prevention (Thornes et al., 1994). In carcinoma, coumarin (100 mg/day) in combination with cimetidine (1200 mg/day) led to metastatic reduction without toxic side effects (Thornes et al., 1982). Patients with metastatic prostate cancer were treated with 3 g of coumarin daily, and stable levels of prostate specific antigen (PSA) were maintained for over 7 years (Lacy & O’Kennedy, 2004).

Coumarin also activates macrophages and cells of the immune system (Hoult & Paya, 1996; Lacy & O’Kennedy, 2004). It has also been reported to reduce acute and chronic protein edema. In rodents, coumarin decreases the swelling caused by thermal damage; in humans, a significant reduction of lymphoedema was confirmed through a double-blind trial involving patients with elephantiasis and postmastectomy (Hoult & Paya, 1996).

Coumarin induces a concentration-dependent relaxation in guinea pig trachea pre-contracted with histamine ($EC_{50} = 35.0 \mu\text{g/mL}$) or carbachol ($EC_{50} = 33.4 \mu\text{g/mL}$) (Ramanitrahasimbola et al., 2005). However, this effect was not associated with the antispasmodic activity on rat jejunum and ileum cells isolated from guinea pig (Aboy et al., 2002). Coumarin was also less effective in guinea pig trachea ($EC_{50} = 130.8 \mu\text{g/mL}$) and endothelium-denuded trachea ($EC_{50} = 153.4 \mu\text{g/mL}$) pre-contracted with potassium chloride. When coumarin was combined with theophylline, a significant additive relaxing effect on pre-contracted trachea was observed, and this effect was not blocked by propranolol. These results indicate that the bronchodilator effect of coumarin is partly due to endothelium-dependent tracheal relaxation and also mediated through a non-specific tracheal relaxation (Ramanitrahasimbola et al., 2005).

5. Absorption, distribution, metabolism and excretion of coumarin, the main substance of guaco

Coumarin (1,2-benzopyrone) is a naturally occurring compound, which is present in a wide variety of plants, micro-organisms and in some animal species (Lake, 1999). In the 1990s, coumarin was widely used as a trial drug in cancer treatment and is still used to improve peripheral venous and lymphatic circulation, to stimulate the proteolytic effect of macrophages, and to treat edema. As a consequence, the metabolism of coumarin, including the excretion of some of its metabolites, has been widely studied in humans and other animal species.

Following oral administration, coumarin is rapidly absorbed from the gastrointestinal tract and distributed throughout the body (Egan et al., 1990; O'Kennedy & Thornes, 1997). The quick absorption is related to its non-polar characteristics and high partition coefficient (21.5%), which are considered favorable for rapid absorption, suggesting that coumarin should easily cross the lipid bilayer by passive diffusion (Lacy & O'Kennedy, 2004).

In systemic circulation, only 2 to 6% of coumarin molecules remain intact (Ritschel et al., 1979). In the liver, coumarin is converted to 7-hydroxycoumarin by a specific cytochrome P-450-linked mono-oxygenase enzyme (CYP2A6). Then, 7-hydroxycoumarin undergoes a phase II reaction, a glucuronide conjugation, that results in the formation of 7-hydroxycoumarin glucuronide, which is subsequently eliminated in the urine (O'Kennedy & Thornes, 1997; Wang et al., 2005).

In addition to 7-hydroxycoumarin, the formation of other metabolites is possible, and the metabolic pathways are species-specific. Thus, coumarin may be hydroxylated at one of the other five possible positions, carbons 3, 4, 5, 6, and 8, to yield 3-, 4-, 5-, 6- and 8-hydroxycoumarin, respectively. In addition, the lactone ring can also open and lead to the formation of a variety of metabolites, including *o*-hydroxyphenylacetaldehyde, *o*-hydroxyphenylethanol, *o*-hydroxyphenylacetic acid and *o*-hydroxyphenylacetic acid. The formation of 6,7-dihydroxycoumarin, *o*-coumaric acid, *o*-hydroxyphenylpropionic acid and dihydrocoumarin has also been described (Lake, 1999).

In humans, the half-life of intravenously administered coumarin can vary slightly according to the dosage (Ritschel et al., 1976), but its metabolism is usually fast. The low availability along with the short half-life (1.02 hrs peroneal vs. 0.8 hrs intravenous) lead coumarin to be considered as a pro-drug and 7-hydroxycoumarin as the substance with more therapeutic relevance (Lacy & O'Kennedy, 2004). In other species, the half-life of coumarin can vary from 1 to 4 hours and is quickly eliminated from systemic circulation.

The mechanism of the excretion of coumarin and its metabolites also depends of the species. For example, a large amount of biliary excretion has been described followed by a considerable elimination via feces in rats. In the Syrian hamster, rabbit and baboon, elimination is via urine. In humans, the rapid and total excretion via urine suggests that there is little or no biliary excretion (Shilling et al., 1969).

Regarding dermal application, coumarin is amply absorbed, distributed and excreted in the urine and feces of humans and rats. Following the applied dose of 0.02 mg/cm², the total absorption was 60% in humans and 72% in rats with a 6-h exposure. The mean plasma half-life of coumarin and its metabolites was approximately 1.7 h for humans and 5 h for rats. As in oral administration, the dermal application of coumarin resulted in the formation of 7-hydroxycoumarin and excretion in the urine as 7-hydroxycoumarin glucuronide. In rats, at least twenty metabolites were found, but only *o*-hydroxyphenylacetic acid was identified (Ford et al., 2001).

In summary, the 7-hydroxylation pathway is characteristic for human and a minor route for rat and mouse, which primarily use the 3,4-epoxidation pathway (Lacy & O'Kennedy, 2004). Another possible route in rat, Syrian hamster, gerbil and human is the 3-hydroxylation pathway leading to the formation of 3-hydroxycoumarin (Lake et al., 1992). The 3-hydroxycoumarin is a minor *in vivo* metabolite in rat and human and a major urinary metabolite in rabbit. The possible metabolic pathways of coumarin are shown in Figure 2.

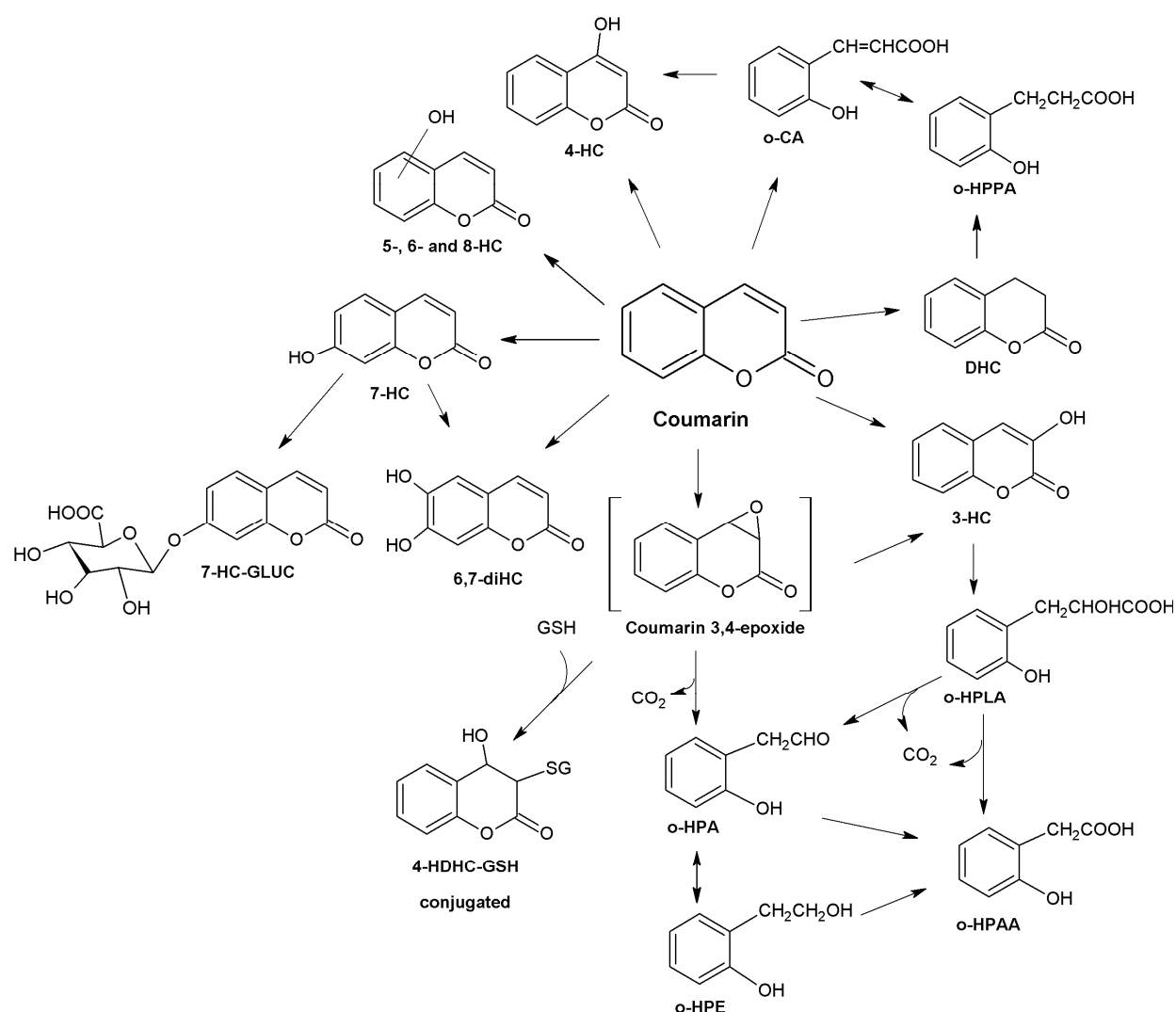


Fig. 2. Pathways of coumarin metabolism. Dihydrocoumarin (DHC); *o*-hydroxyphenylpropionic acid (*o*-HPPA); *o*-coumaric acid (*o*-CA); 3, 4, 5, 6, 7 and 8-hydroxycoumarin (HC); 7-hydroxycoumarin glucuronide (7-HC-GLUC); 6,7-dihydroxycoumarin (6,7-diHC); *o*-hydroxyphenylacetic acid (*o*-HPLA); *o*-hydroxyphenylacetic acid (*o*-HPAA); *o*-hydroxyphenylacetaldehyde (*o*-HPA); *o*-hydroxyphenylethanol (*o*-HPE); 4-hydroxydihydrocoumarin-glutathione-conjugated (4-HDHC-GSH).

6. Toxicological studies

Guaco species have been widely used by the South America population; thus, several studies, although insufficient, have been done to evaluate the toxicity of the extracts, phytomedicines, and isolated compounds.

The aqueous extract of *M. laevigata* was screened for anti-mutagenic activity using the Salmonella/microsome assay. The infusions was negative for mutagenic activity, showing high percentages of inhibition of mutagenesis induced by mutagens 2-aminofluorene (2AF), in the presence of exogenous metabolism (S9 fraction), for frameshift (TA98) and base pair substitution (TA100) lesions. In addition, these inhibitions were observed against mutagen

sodium azide in assays with the TA100 strain, without exogenous metabolism (S9 fraction). A synergistic effect was also observed in frameshift mutagenic events, with direct action in the presence of 4-oxide-1-nitroquinoline and a tendency to a low percentage of action enhancement in the presence of the 2AF mutagen (Fernandes & Vargas, 2003).

In contrast to the outcomes from the Salmonella/microsome trials, studies conducted by the comet assay revealed that guaco extracts have deleterious effects. DNA damage was observed in rat hepatoma cells treated with hydroalcoholic maceration (10 and 20 $\mu\text{L/mL}$) and infusion (20 and 40 $\mu\text{L/mL}$) of the leaves. The genotoxic potential of the infusion was also observed by the micronucleous test at a very high concentration (40 $\mu\text{L/mL}$), suggesting a limitation in the phytotherapeutic use of guaco species (Costa et al., 2008).

Caution is recommended for patients who use lyophilized extracts or medicines containing isolated compounds, such as coumarin and *o*-coumaric acid. Hemorrhaging lung tissue was observed in mice treated with these substances and with the extract. However, this effect was not observed in animals treated with the whole hydroalcoholic extract, leading to the conclusion that some protective effect of the whole extract can be lost during the lyophilization process (Santos et al., 2006).

Because guaco showed an effect against *L. amazonensis* and *T. cruzi*, it was important to assess its toxic effects on mammalian host cells to determine the ratio of selectivity to biological activity. For this purpose, a test of cytotoxicity in sheep erythrocytes was performed using hydroalcoholic extracts of leaves at different concentrations and times of incubation. At 100, 500 and 1000 $\mu\text{g/mL}$, guaco extracts caused, respectively, 25, 50 and 75% hemolysis in erythrocytes incubated at 120 min. However, the hydroalcoholic extract was not considered cytotoxic to sheep erythrocytes because no significant hemolytic effect was observed at 100 and 500 $\mu\text{g/mL}$ after 60 minutes of incubation (Luize et al., 2005).

The hydroalcoholic extract did not impair the fertility of rats following 52 days of oral treatment with a chronic dose of 3.3 g/kg of animal. In females, no changes in mating, gestation, preimplantation loss, the number of implanted embryos or offspring, weaning and the implantation and resorption indexes were observed using this kind of extract (SÁ et al., 2006). In males, the treatment did not alter body and organ weights and did not interfere in gamete production, serum testosterone levels or food intake (SÁ et al., 2003). Following 90 days of treatment, no significant change was observed in body and organ weights, gamete concentration on the epididymis cauda, serum testosterone level or food consumption, suggesting the absence of toxicity or antifertility activity of the hydroalcoholic extract (SÁ et al., 2010).

The absence of any effect on body weight gain or behavioral patterns in mice subjected to a repeated-dose over 14-, 28- or 60-day treatments (3 mg/kg) indicated that the *M. laevigata* ethanolic extract does not induce significant toxicity. The lack of alterations in hematological parameters, liver cell injury and serum aminotransferases (AST and ALT) was indicative of normal hepatic and biliary function. In addition, there was no change in urea levels, indicating the absence of alterations in the kidney. Additionally, the LD_{50} was found to be almost 75-fold higher than the pharmacological dose tested (Alves et al., 2009).

The potential genotoxicity of the dichloromethane fraction of the hydroalcoholic extract was evaluated on plasmid DNA using an alkaline lysis procedure, in which plasmid DNA was treated with SnCl_2 and the *M. glomerata* extract fraction. The role of reactive oxygen species in DNA damage was also evaluated by incubating the extract fraction with sodium benzoate, a hydroxyl radical scavenger. The results showed that the dichloromethane

fraction was not genotoxic because this fraction did not damage DNA directly or by producing the hydroxyl radical reactive oxygen species (Moura et al., 2002)

The pharmaceutical preparation of guaco syrup did not produce any disturbances in the hematological or biochemical parameters in rodents following 90 days of treatment with subchronic and chronic doses (75, 150 and 300 mg/kg). Additionally, no evidence of toxicity in the hepatic, renal or pancreatic systems was reported. At reproductive endpoints, no alterations in body and organ weights, sperm, spermatid number, testosterone levels, or sperm morphology were observed after exposure to guaco syrup (Graca et al., 2007a, 2007b). In humans, only two phase I clinical studies have been conducted to evaluate the clinical safety of guaco syrup. The volunteers (n= 24 – 26) received an oral dose of 15 mL phytomedicine four times a day over 21 to 28 days; after the treatment, any clinically significant changes in coagulation parameters were observed. In some cases, low variations in biochemical, hematological and serological analysis were observed, but none of the volunteers had values out of the established normality limits. Among them, only two volunteers reported mild drowsiness during the treatment, and one reported diarrhea and nausea. However, it is unclear if these effects were caused by guaco ingestion and in addition, clinical, electrocardiographic and laboratory tests did not show any evidence of toxicity. Nevertheless, more conclusive studies should be made because only phytomedicines containing low amounts of guaco extract associated with other plants were evaluated (Soares et al., 2006; Tavares et al., 2006).

The toxicity of the main isolated compounds has also been assessed. For example, kaurenoic acid has been shown to kill sea urchin embryos by inhibiting the first cleavage of the fertilized eggs ($IC_{50} = 84.2 \mu M$). Additionally, this compound progressively induced the destruction of embryos in other development stages ($IC_{50} = 44.7 \mu M$ for blastulae stages and $< 10 \mu M$ for larvae stages) (Costa-Lotufo et al., 2002).

Kaurenoic acid has been shown to have a weak to negligible capacity for killing human sperm. The estimated ED_{50} for sperm immobilization was $374.1 \mu g/mL$, using 15×10^6 sperm/500 μL (VALENCIA et al., 1986). This compound has also been shown to induce dose-dependent hemolysis of mouse and human erythrocytes with an EC_{50} of 74.0 and 56.4 μM , respectively (Costa-Lotufo et al., 2002).

By the microculture tetrazolium test (MTT) assay, 78 μM kaurenoic acid causes cytotoxicity in CEM leukemic cells, leading to a 95% growth inhibition. This effect was also observed in MCF-7 breast and HCT-8 colon cancer cells, with a growth inhibition of 45% (Costa-lotufo et al., 2002). Moderate antiproliferative effects were also observed in K562, HL60, MDA-MB435 and SF295 human cancer cell lines ($IC_{50} = 9.1 - 14.3 \mu g/mL$). Fluorescence microscopy using acridine orange/ethidium bromide staining indicated that kaurenoic acid induced apoptosis and necrosis in HL-60 cell cultures, consistent with the findings described in the MTT assay. However, the antiproliferative effects were not selective to cancer cells because inhibition of lymphocyte proliferation also occurred ($IC_{50} = 12.6 \mu g/mL$) (Cavalcanti et al., 2009).

The cytotoxic effects of kaurenoic acid have been partly associated with its partial inhibitory effect on human topo-isomerase (topo) I activity. In contrast, 14-hydroxy-kaurane, xylopic acid, and semi-synthetic derivatives of kaurenoic acid [16*a*-methoxy-(–)-kauran-19-oic acid, 16*a*-methoxy-(–)-kauran-19-oic methyl ester and 16*a*-hydroxy-(–)-kauran-19-oic acid] lack genotoxic and mutagenic effects. This result suggests that the exocyclic double bond (C16) moiety may be the active pharmacophore for the genetic toxicity of kaurenoic acid (Cavalcanti et al., 2009).

At 30 and 60 µg/mL, kaurenoic acid also induces DNA breaks and cytogenetic abnormalities in human peripheral blood leukocytes (PBLs), as evaluated by comet, cytokinesis-block micronucleus and chromosomal aberration assays. Using a yeast cell model, cytotoxic and mutagenic effects of kaurenoic acid were also observed in the XV185-14c strain: there was an increase in the frequencies of point, frameshift, and forward mutations in the stationary phase at high concentrations (0.5–2 µg/mL). However, these effects were more pronounced when cells were treated in the exponential phase than in growth or non-growth conditions (Cavalcanti et al., 2010).

Positive genotoxic effects have also been described testing kaurenoic acid *in vivo* in multiple organs, such as the liver, kidney and spleen of mice (alkaline comet assay). DNA migration in liver cells was considerable at all tested doses (25, 50 and 100 mg/kg, i.p.) and at higher doses (50 and 100 mg/kg) in kidney cells. No DNA breaks were observed after the treatment in spleen cells (Cavalcanti et al., 2010). Finally, genotoxicity in Chinese hamster lung fibroblast cells was also observed using the comet and the micronucleus assays. However, lower concentrations (2.5, 5, and 10 µg/mL) failed to induce significant effects, whereas higher concentrations (30 and 60 µg/mL) lead to an increase in cell damage index and frequency. These data indicated that kaurenoic acid induces dose-dependent genotoxicity (Cavalcanti et al., 2006).

Dihydrocoumarin is one of the most studied guaco metabolites in regards to its toxic effects. In the human TK6 lymphoblastoid cell line, dihydrocoumarin caused an increase in p53 acetylation and cytotoxicity. Flow cytometric analysis to detect annexin V binding to phosphatidylserine demonstrated that dihydrocoumarin also increased apoptosis more than 3-fold over controls. In addition, dihydrocoumarin disrupted epigenetic processes in the yeast *Saccharomyces cerevisiae* and also inhibited several human sirtuin deacetylases (SIRT1 and SIRT2), a class of proteins that control some epigenetic processes and has, interestingly, been implicated in extending the longevity of several organisms (Olaharski et al., 2005).

Toxicity and carcinogenicity studies were also conducted by administering 99% pure dihydrocoumarin to groups of rats and mice in short (16 days), 13-week, and long (2 years) exposures. The short exposure lead to the death all male and female rats treated with 3000 mg/kg of dihydrocoumarin. At 1500 mg/kg, half of the animals died and a gain of body weight was observed; however, there were no clinical findings of organ-specific toxicity or evidence of impaired blood coagulation. A similar finding was also observed in mice groups, however with total mortality observed at a lower body/weight concentration than the rat groups (2250 mg/kg).

Following 13 weeks of administration, groups of 10 male and 10 female rats were studied, and a difference of exposure sensitivity was observed between the groups. In this case, two male and five female rats died after the administration of 1200 mg/kg dihydrocoumarin. The platelet counts were diminished in males receiving 600 mg/kg and in the female groups receiving 300 mg/kg dihydrocoumarin. Hemoglobin and hematocrit values were significantly lower in males that received 300 mg/kg dihydrocoumarin; this dose caused hepatocellular hypertrophy in both sexes. Additionally, the absolute and relative liver and kidney weights were significantly greater than those of the controls following a treatment of 600 mg/kg dihydrocoumarin. In mice groups, mortality was 80% in male and 50% in female receiving 1600 mg/kg dihydrocoumarin. With this exposure, the absolute and relative liver weight in both sexes and the relative kidney weight in males were significantly greater than those of the controls. However, no variation in body weight or changes in hematologic parameters were observed in either sex.

Under long dihydrocoumarin exposure (2 years), carcinogenic activity in male rats was evident based on the increased incidence of renal tubule adenoma and focal hyperplasia. The transitional cell carcinomas in two males were chemical related. No evidence of carcinogenic activity was observed in female rats receiving 150, 300, or 600 mg/kg dihydrocoumarin. In mice, no evidence of carcinogenic activity was observed in male groups receiving 200, 400 or 800 mg/kg dihydrocoumarin; however, these doses led to an increase in the incidence of hepatocellular adenoma and carcinoma (combined) in females. In addition, ulcers, forestomach inflammation, parathyroid gland hyperplasia, and increased nephropathy were observed in these groups of rodents (National Toxicology Program, 1993a).

Coumarin, a main compound of guaco extracts, is a substance known to cause hepatotoxicity in liver rats. Prior to the existence of any available carcinogenicity and mutagenicity data, it was classified as a toxic substance by the Food and Drug Administration. Thus, it was banned in the USA in 1954 and in the UK in 1965 (Lake, 1999).

Various tests have been conducted to evaluate the toxicity and health effects of coumarin in laboratory animals. For example, doses of 25 mg/kg or higher were reported to produce liver damage in dogs (Felter et al., 2006). In primates (baboons) that received dietary coumarin for 2 years (0 to 67.5 mg/kg/day), no evidence of toxicity from biochemical and histochemical analyses was observed. However, an increase in the relative liver weight occurred at a high dosage, with additional dilatation of the endoplasmic reticulum observed after 10 months of treatment (Felter et al., 2006). The Syrian hamster has also been found to be resistant to coumarin-induced toxicity (Lake, 1999).

In groups of rats and mice, 97% pure coumarin administered orally has toxic effects with a short exposure (16 days), after 13 weeks, and a long (2 years) exposure. All groups of male and female rats died following 16 days of treatment with 400 mg/kg of coumarin. Increases in mean body weight also occurred, but no clinical signs of organ-specific toxicity were observed. Additionally, coagulation parameters were not impaired. In mice, groups of 5 male and 5 female rats were studied, and all 10 mice receiving 600 mg/kg, two male mice receiving 300 mg/kg, and one male mouse receiving 75 mg/kg died. With a short exposure, coagulation parameters were not impaired; however, an increase in the mean body weight and excessive lacrimation, piloerection, bradypnea and ataxia were observed for the 300 mg/kg dose in the first hours of administration (National Toxicology Program, 1993b).

Following 13 weeks of exposure to 300 mg/kg of coumarin, 30% of rats in the male and female groups died. Both groups had increased erythrocyte counts and decreased hemoglobin and erythrocyte mean volumes. Serum levels of total bilirubin and one or more cytoplasmic enzymes were higher than those of control groups. The absolute and relative liver weights also increased significantly following the administration of 150 mg/kg coumarin, and centrilobular hepatocellular degeneration and necrosis, chronic active inflammation, and bile duct hyperplasia were also observed in the liver. In the mice groups, 20% of male and female groups receiving 300 mg/kg coumarin died; similar to rats, coumarin decreased the erythrocyte volume and hemoglobin. Centrilobular hepatocellular hypertrophy was observed in both sexes at 300 mg/kg, and the absolute and relative liver weights increased following treatment with 150 mg/kg coumarin.

During the long (2 years) exposure, groups of 60 male and 60 female rats were treated with coumarin at different dosages, and after 15 months, 10 animals from each group were evaluated. Treatment with 50 mg/kg led to a significant reduction in the activated partial thromboplastin times and the erythrocyte volume and hemoglobin values, and an increase

of platelet counts was also observed. With this dose, the values of alanine aminotransferase, sorbitol dehydrogenase, and g-glutamyltransferase significantly increased in males, whereas these effects were observed only from 100 mg/kg in females. Additionally, lesions associated with the administration of coumarin were also observed during the long exposure, which include an increase of the severity of nephropathy, increase of incidences of bile duct and parathyroid gland hyperplasias, increase of the incidences of ulcers, and necrosis, fibrosis, and cytologic alterations in the liver (National Toxicology Program, 1993b). A carcinogenic potential has also been described for coumarin, especially in the liver and lungs of rats and mice. However, the dose-response relationships are nonlinear with tumor formation and hepatic and pulmonary toxicity are associated only with high doses (Lake, 1999).

Regarding the mutagenic and genotoxic potential of coumarin, it showed weak clastogenic activity in Chinese Hamster ovary cells *in vitro*. However, this response was observed only at a very high concentration (10.95 μ M). Negative responses were reported in the *Salmonella typhimurium* assay in the TA98, TA1535, TA1537 and TA1538 strains, either with or without metabolic activation. However, gene mutations have been described in the TA100 strain in the presence of a metabolic activating system (S9) (Lake, 1999).

Using the Ames genotoxic assay, coumarin has not been shown to be a mutagenic agent in the TA100 strain assessed without metabolic activation (liver S9 fraction). With metabolic activation, coumarin produces only a weak positive effect at a high concentration. However, this effect has been widely discussed because a greater response was achieved in the presence of liver S9 fraction from untreated Syrian hamsters than from rats treated with Aroclor 1254, a substance used to stimulate coumarin metabolism by the 3,4-epoxidation pathway in rat hepatic microsomes. This result does not correlate with the extent of coumarin metabolism and coumarin-induced liver injury in these species. Because of the differences in their metabolic pathways, a chronic dose of coumarin induces liver lesions and tumor in the rat and not in the Syrian hamster, which appears to be refractory for coumarin-induced hepatotoxicity (Lake, 1999).

In addition, *in vivo* studies have shown that coumarin is unable to induce sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster*. Furthermore, no evidence for coumarin-induced genotoxicity has been observed in the *in vivo* micronucleus test in mouse peripheral blood cells. The conclusion is that coumarin is not DNA-reactive and that the induction of tumors at high doses in rodents is attributed to cytotoxicity and regenerative hyperplasia (Felter et al., 2006).

In humans, the majority of tests for mutagenicity and genotoxicity also suggest that coumarin is not a toxic agent (Lake, 1999). The lack of toxicity has been associated with the detoxification mechanism of coumarin, which in humans involves the 7-hydroxylation pathway, a minor route in rats and mice; these rodents use the 3,4-epoxidation pathway instead, which results in toxic metabolite formation (Gasparetto et al., 2011). Thus, the species-specific target organ toxicity has been attributed to the pharmacokinetics of coumarin metabolism, causing rats to be susceptible to liver effects and mice to have toxicity particularly in the lung. Therefore, it is possible to conclude that the use of rats and mice is not an adequate model to compare the metabolism and toxicity of coumarin with humans, due to their particular metabolism. Because *in vitro* genotoxicity studies demonstrated toxicity only at very high doses and no evidence for *in vivo* studies was observed, it is possible to conclude that there is no human health risk from coumarin exposure in natural dietary sources, such guaco species.

7. Conclusion

For centuries, medicinal plants have been used worldwide for the treatment of several diseases. In South American populations, plant products significantly contribute to primary health care and are sometimes the only therapeutic resources of some communities and ethnic groups.

Among the medicinal species used in South America populations, *M. glomerata* and *M. laevigata* are especially important due to their relevant therapeutic properties. In popular medicine, both species have a long history of use, and they are still employed especially for the treatment of respiratory diseases.

Pre-clinical trials have been conducted on guaco extract that have revealed scientific evidence for its anti-inflammatory, anti-allergy, and bronchodilation properties. However, there are currently no clinical studies for assessing the efficacy of guaco extracts and preparations in patients who present respiratory complaints.

Both guaco species have many bioactive compounds that probably contribute to the pharmacological effects. Thus, the properties of guaco should not be attributed only to coumarin because high contents of kaurene diterpenes and cinnamic acid derivatives were found in the extracts. Studies involving the quality control of different brands of guaco phytomedicines and extracts have been conducted and have shown a wide variation in the content of the main metabolites. A number of studies have reported that this discrepancy is due to the geographic origins, agronomic aspects, extractor solvent and extraction techniques of the guaco. Therefore, depending of the region and period of plant collection, the effects and/or toxicity of guaco may change or not be evident.

Regarding the safety of the extracts, phytomedicines and isolated compounds, guaco species did not present significant toxic and genotoxic effects in humans. However, the majority of studies were conducted in rat and mice, which have a unique metabolism, suggesting that new studies must be conducted. Additionally, relevant information on metabolism, bioavailability and toxicity has only been reported for coumarin, without substantial information concerning the other main metabolites.

In general, is possible to conclude that there is a need for clinical studies using standardized phytomedicines or extracts. This may be the most important step to ensure conclusive studies of guaco species. By conducting clinical studies, it will be possible to know the most effective extract for therapeutic purposes and to correlate the metabolite content with its relevance in a pharmacological and toxicological context. Meeting this requirement will guarantee the benefits and safe use of guaco.

8. References

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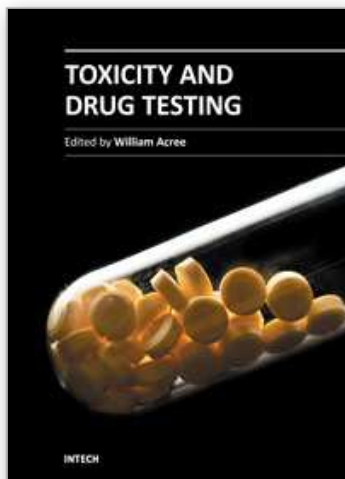
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Modern drug design and testing involves experimental in vivo and in vitro measurement of the drug candidate's ADMET (adsorption, distribution, metabolism, elimination and toxicity) properties in the early stages of drug discovery. Only a small percentage of the proposed drug candidates receive government approval and reach the market place. Unfavorable pharmacokinetic properties, poor bioavailability and efficacy, low solubility, adverse side effects and toxicity concerns account for many of the drug failures encountered in the pharmaceutical industry. Authors from several countries have contributed chapters detailing regulatory policies, pharmaceutical concerns and clinical practices in their respective countries with the expectation that the open exchange of scientific results and ideas presented in this book will lead to improved pharmaceutical products.

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