Bioactivity Of Sesquiterpenes: Compounds That Protect From Alcohol-Induced Gastric Mucosal Lesions And Oxidative Damage

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Abstract: Sesquiterpene lactones of the guaianolide and eudermanolide types are considered of interest because they have an effect in the regulation and prevention of oxidative damage and inflammation-mediated biological damage. Dehydroleucodine, a natural sesquiterpene isolated from *Artemisia douglasiana* Besser, and ilicic aldehyde, a semi-synthetic sesquiterpene lactones, showed *in vivo* protection in ethanol-induced gastric mucosa damage. This action was determined by *in situ* gastric mucosa chemiluminescence and by tissue antioxidant content.

Keywords: Cytoprotection, dehydroleucodine, ethanol gastric damage, gastric ulcer, inflammation, sesquiterpene lactone.

INTRODUCTION

The therapeutic use of several plants, i.e. herbal medicine, can be traced back to China about 5000 years ago. Herbalists and indigenous healers have used botanical medicines traditionally worldwide for the prevention and treatment of different pathologies. Clinical research has confirmed the efficacy of several plants for the treatment of gastroduodenal diseases, while basic scientific research has uncovered the mechanisms by which some plants exert their biologic and therapeutic effect [1, 2].

The medicinal properties of folk plants are attributed to the presence of flavonoids, but they may be also influenced by other organic and inorganic compounds such as coumarins, phenolic acids, antioxidant micronutrients and sesquiterpene lactones [3].

Nowadays, plants of the family of Asteraceae (Compositae) are sources of sesquiterpene lactones, an interesting group of natural isoprenoids, with about 4000 compounds at the present time. Sesquiterpenes are defined as 15 carbon compounds derived of the assembly of 3 isoprenoid units. Simple sesquiterpenes are constituents of plant essential oils and sesquiterpene structures present acyclic, mono-, bi-, tri-, and tetracyclic systems [4]. The sesquiterpene lactones belong either to the eudermanolide, guaianolides or germacranolides classes, and the sesquiterpene lactones of the eudermanolide and guainolide types are considered of interest from a chemotaxonomical point of view because they have shown to have effects in the regulation and prevention of inflammatory and oxidative cellular damage [5].

Sesquiterpene lactones are isolated from several medicinal plants used for popular treatment of bronchitis, pneumonia, dysentery, gastric ulcers, cancer, as well as for its antipyretic, analgesic and anti-inflammatory effects. These compounds possess diverse biological activities and exhibit several effects such as immunomodulation, cytotoxicity, antitumor and antimicrobial effects, this latter including antibacterial and anti-fungal activities, an anorexic effect and the property of giving allergic contact dermatitis [6].

The species *Artemisia douglasiana* Besser and *Fluorencia oolepis* Blake have provided sesquiterpene-γ-lactones with the capacity of preventing the gastric mucosal damage produced by absolute ethanol [7, 8,9].

In this review, we attempt to summarize the current knowledge of biological and therapeutic properties of sesquiterpene lactones. First, we assess the experimental evidence for the bioactivity of sesquiterpene lactones obtained from both *in vitro* experiments [10] and *in vivo* animal models [11]. Sesquiterpene lactones exert their gastroprotective anti-ulcer capability by inhibition of inflammatory responses and oxidative damage. Second, we outline the possible molecular mechanisms involved in the bioactivity of sesquiterpene lactones, in particular, thiol reactions, antioxidant protection and cell signaling pathways [12]. Thirdly, we discuss the structure-activity relationship of sesquiterpene lactones, that are hydrophobic molecules with rings and with mild polar groups [13]. The functional structure, an α-methylene-γ-lactone, is the chemical functional group which is interpreted as the basis for the thiol-reactivity of sesquiterpene lactones and is responsible for their bioactivities [14]. It is apparent that some structural requirements are necessary in order to elicit the cytoprotective effect. The presence of an electrophilic center (α,β-unsaturated carbonyl group) together with a second polar group seem to be determinant, whereas the conformational requirements from the decaline system seems to play a secondary role [13].

The evaluation of the biological properties of these compounds permit to establish the chemical basis for new anti-ulcer agents and the treatment of gastric diseases with plant extracts.

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1389-5575/10 $55.00+.00

1. BIOLOGICAL PROPERTIES OF MEDICINAL PLANTS

The beneficial effects and the protection given by plants and plant extracts in several diseases and symptoms has been attributed to the various compounds, including flavonoids, contained in these plants and extracts [15]. Plants produce superoxide anion ($O_2^{\cdot-}$) and H_2O_2 as a result of their normal aerobic metabolism in chloroplasts and mitochondria during photosynthesis and respiration. Nonenzymatic scavengers of these reactive species include ascorbic acid, tocopherol, carotenes and reduced glutathione. Glutathione and ascorbic acid work in concert with enzymes such as ascorbate oxidase, dehydroascorbate reductase and glutathione reductase, to modulate the oxidation state of the cell and to prevent oxidative stress. Superoxide dismutase, catalase, and glutathione-S-transferase are enzymes that are present in plant cells and which form the enzymatic antioxidant defense [16]. Plant extracts have hundreds of constituents that are able to both increase or decrease the rate of free radical reactions. The first ones are the pro-oxidant effects of plant extracts and the second ones the anti-oxidant effects of plant extracts. These plant extracts contain redox active metal ions, such as $Fe^{2+/3+}$, Co^{2+}, Cu^{2+}, or Cd^{2+} that by themselves or as chelates stimulate free radical oxidative reactions. Plant extracts also contain enough organic compounds, e.g. citric acid, able to chelate redox active metals and catalyze free radical mediated reactions.

2. MEDICINAL PLANTS AND ACTIVE COMPOUNDS WITH THERAPEUTIC ACTION

Among the rich unexploited world flora a large number of species have folk medicinal uses. Recent years have witnessed a renewed interest in plants as pharmaceuticals. Many studies have focused this interest not only on the discovery of new biologically active molecules by the pharmaceutical industry, but also on the adoption of crude plant extract, for instance as infusions, for self-medication by the general public. In the field of the popular use of herbal medicine, plant extracts and infusions are widely used for the treatment and prevention of gastric mucosa and liver diseases.

Species and herbs are recognized as sources of natural compounds that provide chemo prevention of diseases resulting from oxidative damage and lipid oxidation [3]. The active compounds of plants, flavonoids and other polyphenols, tannins and triterpenes, belong to the recently popular phytochemicals with potentially beneficial effects on human health. These compounds my be regarded as possible active compounds against gastric lesions by acting as protective factors on increasing antioxidant activity [3].

2.1. Medicinal Plants Used for Popular Treatment of Gastric Ulcers

In Argentina the aerial part of *Artemisia douglasiana* and *Fluorencia oolepis* have been used in folk medicine as a cytoprotective agent against the development of gastric ulcers, external treatment of skin injury and dermal ulcers. These species contain great diversity in secondary metabolite composition: sesquiterpene lactones, essential oils (azulene and chamazulene), flavonoids (rutin), vitamin B_6, ascorbic, palmitic, caffeic, glutamic, stearic, gallic and ferulic acids, sanlonine, lignnan, betaine, coumarins and polyacetylenes [3, 10, 11].

Artemisia douglasiana and *Fluorencia oolepis* contain sesquiterpene-γ-lactones. *A. douglasiana* is a hexaploid species whose origin was attributed by Keck [17] as a hybrid between *Artemisia suksdorfii* Piper and *Artemisia ludoviciana* Nutt. It is found on the western slopes of the Rocky Mountains and in Northern California. The first report of its occurrence in Argentina appeared in 1967 when Ariza Espinar [18] reported the presence of *A. douglasiana* as an adventitious plant in the provinces of San Juan and Mendoza (Argentina). *Flourensia oolepis* is an endemic species that grow in the hills of the province of Córdoba (Argentina) [19].

A substance found in these plants has anti-inflammatory and anticancer effects. Plants containing sesquiterpenene lactones have been used in some cultures to treat gastric lesions by acting as protective factors on increasing antioxidant activity [3].

2.2. Sesquiterpene Lactones

Sesquiterpene lactones found in plants are remarkly diverse in terms of their structure, properties, and proposed functions. Among the Compositae, over 500 different members of the sesquiterpene lactone family have been described [13].

2.2.1. Dehydroleucodine

Dehydroleucodine (DhL) is the active cytoprotective principle isolated from the aerial parts of *Artemisia douglasiana*, a sesquiterpene lactone of the guaianolide type [structural formula of DhL is shown in Fig. (**1a**); DhL induces mucosal gastric protection and increases gastric glycoprotein synthesis determined by histological studies in gastric and duodenal mucosa [20], and prevents the lesions in the gastric mucosa produced by intra-gastric administration of absolute ethanol and other necrotizing agents [14]. Both, *A. douglasiana* extract and DhL, prevent the gastric injury induced by absolute ethanol [14]. The dose in rats that is effective in preventing gastric mucosa damage and ulcer by absolute ethanol is rather high: 400 mg/kg of rat for A. *douglasiana* and 40 mg/kg of rat for DhL, given 30 min before the intragastric administration of 3 g ethanol/kg of rat. The mechanism of the protective action of DhL is unknown although it seems that antioxidant effects are involved and is related to the biosynthesis of endogenous prostaglandins.

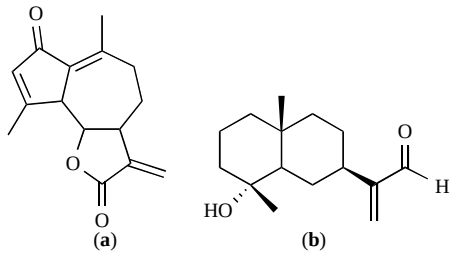
Chemical structure of DhL contain β-unsubstituted cyclopentanone ring (useful for antitumor, antimicrobial and antifeedant properties) and the α-methylene-γ-lactone functionality (a requirement for the cytoprotective activity on ethanol-induced ulcer damage) [6, 8, 13], Fig. (**1a**).

2.2.2. Ilicic Aldehyde

Ilicic aldehyde (IA) is a semi-synthetic sesquiterpene lactone of the eudermanolide type. The structural formula of IA is shown in Fig. (**1b**), derived from the natural compounds, ilicic alcohol and ilicic acid, isolated from air-dried aerial parts of *Fluorensia oolepis* and purified by chromatography [21]. IA is obtained by oxidation of ilicic alcohol with

the Jones´ reagent for alilyc alcohols. IA shows a pharmacological cytoprotective effect and is particularly effective in preventing the formation of gastric lesions induced by several necrotizing agents [21]. The effective dose in preventing gastric mucosal damage by absolute ethanol is: 40 mg/kg of rat given 30 min before the intragastric administration of 3 g ethanol/kg of rat.

Chemical structure of IA contain α-β-unsaturated aldehyde, with a polar group at C-4 [21], Fig. (**1b**).



(**a**) (**b**)

Fig. (1). Chemical structure of sesquiterpene lactones: (**a**) Dehydroleucodine, the natural compound isolated from Artemisia douglasiana; and (**b**) ilicic aldehyde, the semi-synthetic sesquiterpene lactone isolated from Fluorencia oolepis.

3. BIOLOGIC ACTIVITY OF NATURAL COMPOUNDS AND THEIR DERIVATES. *IN VITRO* AND *IN VIVO* STUDIES.

3.1. *In Vitro* Evidences of Antioxidant Activity of Natural Compounds and Sesquiterpene Lactones

It was reported that *A. douglasiana* extract (10% w/v) and DhL (25-300μg/mL) showed antioxidant capacity *in vitro*, as determined by total reactive antioxidant potential (TRAP) and total antioxidant reactivity (TAR) using the luminol chemiluminescence method developed by Wayner [22]. TRAP is an index of the total antioxidant charge present in the sample and TAR reflects the capacity of the added substance to be a trap for luminol-derived radicals, and allows the estimation of the efficiency of an antioxidant substance. This antioxidant activity is more marked in *A. douglasiana* (TRAP = 95 μM Trolox and TAR = 30 μM Trolox in an extract dilution 1/100) than in DhL (IC_{50} = 15 and 117 μg/ mL for *A. douglasiana* and DhL, respectively). This difference can be accounted by the presence of flavonoids with antioxidant activity in the plant extract. Considering that the substitution of the molecule nucleus with hydroxyl groups is essential to acquire significant antioxidant activity, it is clear why DhL is not an efficient antioxidant [10].

Concerning the molecular mechanism of the therapeutic action of *A. douglasiana* and DhL, this seems to be due to their capacity of scavenging oxygen free radicals, as suggested by the TRAP and TAR determinations, which are involved in ulcer formation and inflammatory diseases.

3.2. *In Vivo* Etanol--Induced Gastric Erosions: Experimental Model

3.2.1. Histological Damage

Intragastric administration of absolute ethanol (3 g/kg animal weight) to rats produces necrosis of the mucosal cells with erosion of the adherent mucus gel layer which is the normal protection against stomach mechanical and chemical injury [23,24]. Ethanol produces arteriole dilatation and constrictions of collecting venules with mucosal congestion and tissue injury [25], producing inflammation and cell death. The ethanol-induced lesions depend on the dose of ethanol and can be prevented by prostaglandines (PGE2), which inhibits gastric motility and increases the mucus secretion [8]. If gastric mucus is decreased, the gastric mucosa is susceptible to oxidative damage [11] Histological damage in gastic mucosa by ethanol is summarized in Fig. (**2**).

3.2.2. Oxidative Damage

Mucosal damage can be easily produced by the generation of exogenous and endogenous active oxygen and nitrogen species which produces an unbalanced oxidant/antioxidant cellular process (Fig. (**2**)).

Necrosis of the gastric mucosal cells is associated with tissue oxidative damage, as determined in mucosal homogenates by decreased reduced glutathione (GSH) content, increased phospholipids oxidation (TBARS) and xanthine oxidase activities, mitochondrial depolarization, DNA oxidation and damage [24]. Oxidative process induced by ethanol toxicy in gastric mucosa is summarized in Fig. (**2**).

Since antioxidant substances are able to reduce the severity of the mucosal injury, lipid peroxidation was suggested to contribute to the ethanol-induced gastric injury [26]. The involvement of oxygen radicals in ethanol-induced gastric injury was confirmed by *in vitro* experiments made in cultured mucosal cells [27].

The open stomach of rats provides a preparation in which the cells of the gastric mucosa are the layer of cells that yield the light emission measured as organ chemiluminescence [28]. Rats given intragastrically absolute ethanol show a marked increase, about 2.4-times, *in vivo* chemiluminescence [11]. Light emission from *in situ* organs is related to the *in vivo* steady state concentration of excited carbonyls and singlet oxygen [28]. An increased *in situ* organ chemiluminescence reflects an increased rate of the free radical reactions of the lipid peroxidation, the occurrence of oxidative stress, and an increased intracellular steady state concentration of the excited states of carbonyl compounds and singlet oxygen [29]. In addition, intragastric administration of absolute ethanol to rats produces a significant morphological damage in the gastric mucosa with ulcer formation and histological cell necrosis. Chemiluminescence levels increase almost immediately after the production of erosions and lesions by ethanol in the stomach. From the available data, it can be concluded that ethanol administration produces a marked oxidative damage and necrosis in gastric mucosal cells. The situation of less morphological damage with oxidative stress and damage, is associated with a higher rate of the lipid peroxidation process and of the generation of carbonyl compounds and singlet oxygen. This increase in excited species is simultaneous with an increase in reactive oxygen species, oxidative stress, and lipid peroxidation, a series of processes that certainly lead to the damage of essential molecules , Fig. (**2**).

Ethanol exposure appears to induce the increase over physiological values in the intracellular concentrations of

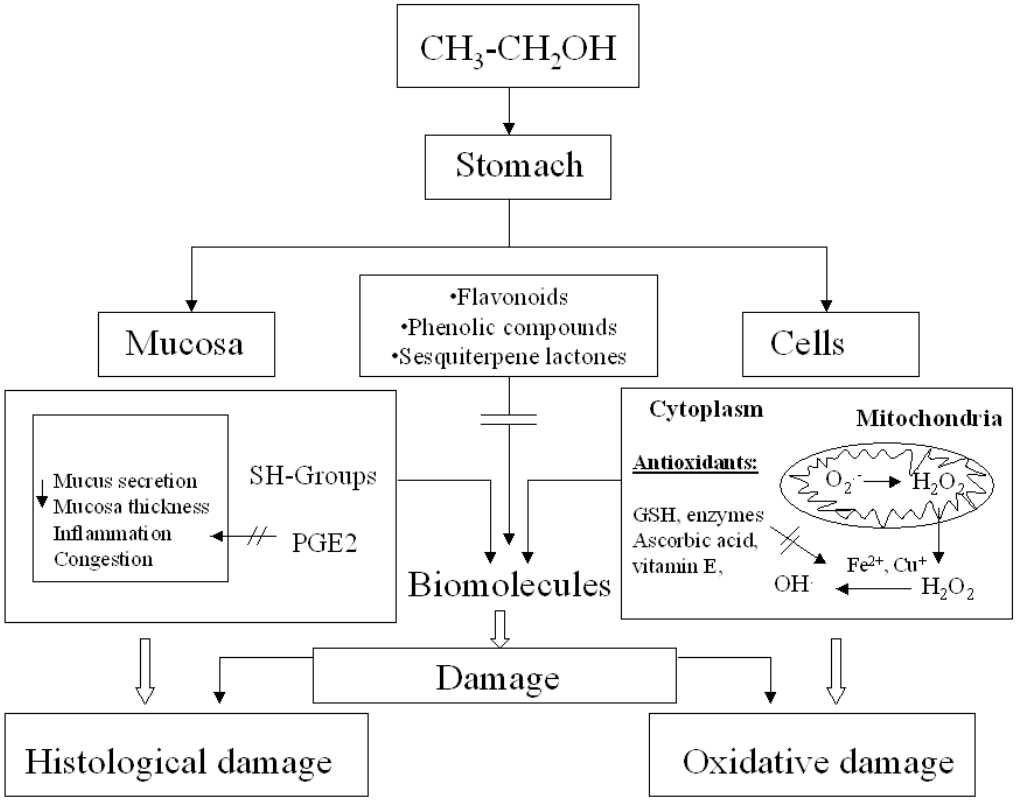


Fig. (2). Biochemical mechanisms of histological and oxidative damage in ethanol-induced gastric erosions. Protective effect of antioxidant compounds and sesquiterpene lactones. (//: inhibitory effects).

active oxygen species: superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (HO·), peroxyl radical (ROO·) and singlet oxygen ($^1O_2$). The disturbance in the pro-oxidant/antioxidant balance in favor of the pro-oxidant state is defined as oxidative stress, and the irreversible oxidation of biological molecules produces oxidative damage [30]. This condition is supported for the ethanol-induced gastric mucosa damage by the observed increase in *tert*-butyl hydroperoxide initiated chemiluminescence, a measurement that is inversely related to the tissue vitamin E content [31] and by the significant decrease in catalase activities [11]. The process of lipid peroxidation is initiated by the interaction of HO· radicals with the unsaturated phospholipids of the cell membranes, subsequently producing lipid-derived free radicals, such as conjugated dienes and lipid hydroperoxides [32]. Acute ethanol administration is highly effective in decreasing GSH levels, what lead to a state of diminished antioxidant levels.

Certainly, *in situ* organ chemiluminescence provides a determination that, after comparing with a proper control, really establishes the condition of oxidative stress and oxidative damage in a tissue under physiological conditions.

3.3. *In Vivo* Evidences of Antioxidant Activity of *Artemisia Douglasiana* Extracts and Sesquiterpene Lactones in the Prevention of Ethanol-Induced Gastric Damage

Artemisia douglasiana extracts (400 mg/kg animal weight), DhL (40 mg/kg animal weight) and IA (40 mg/kg animal weight) exhibited gastroprotective and antioxidant effects in the ethanol-induced gastric ulcers in rats. The extract and the substances prevented the formation of necrotizing lesions and the production of the oxidation products [11, 21, 33,3].

The gastric mucosa of the stomach of rats treated with absolute ethanol showed a marked increase in *in vivo* chemiluminescence (from 12 ± 1 to 28 ± 2 cps/cm^2). When animals were pretreated with *A. douglasiana* extracts one hour before ethanol administration, the light emission decreased considerably to 17 ± 1 t cps/cm^2 ($p<,0.05$, n=8 rats per group)

At variance, DhL was not able to decrease the increased *in situ* gastric mucosa chemiluminescence after absolute ethanol administration [11]. Consistently, the *A. douglasiana* aqueous extract was also able to reduce the emission of *tert*-butyl hydroperoxide-initiated chemiluminescence (an estimation of the antioxidant content in the tissue).

3.4. Gastroprotective and Anti-Ulcer Effects of *Artemisia Douglasiana* Extracts and of Sesquiterpene Lactones

The extract of the air-dried aerial parts of *A. douglasiana* has significative cytoprotective activity, at a dose of 400 mg/kg of animal weight. Aqueous extract of *A. douglasiana* and its active principle DhL (40 mg/kg animal weight) exhibit anti-ulcer properties against absolute ethanol- and acid-induced gastric mucosal damage. These effects have been studied associated with the pharmacological effect on the prostaglandin synthesis in gastric epithelium. The *A. douglasiana* extract and DhL are able to increase gastric mucosal

prostaglandin concentration and protect the mucosa from damage induced by necrotizing agents [33].

Giordano *et al* [13] studied the cytoprotective action of *A. douglasiana* according to the gastric lesion produced by the acute administration of ethanol. The erosion degree, in semi-arbitrary units but related to the diameter of erosion and ulcers, was 4.5 (big erosions and ulcerative perforations) in ethanol-treated rats and only 0.5 in rats treated with either *A. douglasiana* extracts or DhL, previous to ethanol administration [13].

The compound DhL prevented the gastric damage produced by caustic agents (0.6 N HCl; 0.2 N NaOH and 25 % NaCl). Light and transmission electron microscopic studies showed that *A. douglasiana* and DhL pretreatment prevent the hemorrhagic lesions and the mucosal erosions produced by ethanol [33]. Piezzi *et al* [33] observed that the stomachs of rats pretreated with DhL presented a reduction of lesions. No hemorrhages and erosions were observed. The mucosal epithelium had a cobblestone appearance, similar to control rats and was covered by a fine layer of mucus on the surface. The increased vascular permeability precedes the development of grossly visible mucosal hemorrhagic erosion. The authors suggested that the vascular damage play an important role in the development of the hemorrhagic erosions [33].

Gastric mucus is an important protective factor for the gastric mucosa and consists of a viscous, elastic, adherent and transparent gel formed by 95% water and 5% glucoproteins that covers the entire gastrointestinal mucosa. However, mucus capable of acting as an antioxidant compound, and thus can reduce mucosal damge mediated by oxygen free radicals. The protective properties of the mucus barrier depend not only on the gel structure but also on the amount of thickness of the layer covering the mucosal surface [34].

In the stomachs of animals pretreated with DhL and treated with the absolute ethanol, mucus filaments and gross deposits occur as a mucosal response that dilute ethanol and provide a favorable environment for rapid epithelial restitution. This increase in mucus secretion is one of the gastric defense mechanisms involved in the protection of superficial gastric mucosal cells.

The semisynthetic sesquiterpene Ilicic aldehyde gave a high level of gastric protection (96 %) in the ethanol-induced damage. The cytoprotective effect of IA was comparable to that previously reported for DhL (97%) [21].

The cytoprotective effect of DhL and IA compounds have been studied by our laboratory group [8-14, 17, 18, 30, 31). Further investigation are needed to define the limit of concentration concerning the possible toxic effects of sesquiterpenes.

The therapeutic activity of sesquiterpene lactones are summarized in Table **1**.

4. MECHANISM OF BIOLOGICAL ACTION FOR SESQUITERPENE LACTONES

Sesquiterpene lactones have effects in several inflammatory processes, such as the exocytosis of cathepsin G and of acid phosphatase from the azurophilic granules of rat polymorphonuclear leukocytes, the releases of histamine from mast cells and of serotonine from blood platelets, and the exocytosis of elastase from human neutrophils [35]. The sesquiterpene lactones inhibit the 5-lipoxygenase and leukotriene C-4 synthase in human blood cells and possess proapoptotic effects, which can be desiderable in eliminating nonfunctional cells in inflammatory tissues. Sesquiterpene lactones inhibit the transcription factor NF-kB, a central mediator of the immune response, but do not interfere with the generation of reactive oxygen species following the stimulation of polymorphonuclear cells, and prevent the induced degradation of the inhibition factor IkB in the regulation of the inflammatory responses [36,37].

The gastric cytoprotection seems mediated by two main different mechanisms. The first one concerns endogenous prostaglandins [33] associated with the ability of the drug to stimulate mucus synthesis [4]. The mechanism of cytoprotection is mediated, at least in part, by the reaction between sulphydryl-containing compounds of the mucosa and an electrophilic acceptor, in a process that protect the mucosa from the damage induced by necrotizing agents. The fact that the increase in the mucus thickness after DhL treatment is similar in stomach (83 %) and duodenum (82 %), indicates that both tissues present a similar secretory response to the drug [34]. Prostaglandins have been shown to increase the amounts of luminal mucus and to increase the resistance of the gastrointestinal tract to injury in several experimental models. Prostaglandins are believed to exert their cytoprotective actions through the stimulation of mucus and bicarbonate secretion, maintenance of mucosal blood flow and by enhancing the resistance of epithelial cells to injury induced by cytotoxins [34]. María *et al* [14] observed an increase in gastric prostaglandin E_2 levels, measured by RIA, in subchronically DhL-treated rats. It is also possible that DhL alter the activity of certain receptors in the gastroduodenal mucosa, such as serotonin and/or muscarinic receptors. The muscarinic receptors mediate the secretion of hydrochloric acid, pepsinogen and mucus, coupled to stimulation of the phosphoinositide second messenger system. It is also possible that the massive mucus secretion after treatment with DhL is mediated by the activation of certain histamine receptors, such as the receptor H_3, responsible in increasing the amount of mucus in deep crypt cells of the fundic mucosa [34].

Giordano *et al.* [8, 13], and Donadel et *al.* [21], showed that the presence of an exocyclic methylene group conjugated to a γ-lactone appears to be a structural requirement for the biologic activity exhibited for DhL and IA respectively. The presence of the α-methylen-γ-lactone moiety is a requirement for the cytoprotective activity observed and the presence of the β-substituted or unsubstituted cyclopentanone ring is not a structural requirement for cytoprotective activity, contrary to their antitumor, antimicoby and antifeedant properties.

The relationship between prostaglandins and leukotrienes, the arachidonic acid products of prostaglandin H synthase (PGHS) and 5-lipoxygenase, respectively, seems to be an important factor in gastric ulcers.

Table 1. Chemical Structure Requirements of Sesquiterpene Lactones for the Therapeutic Action and the Related Biochemical and Histological Events

Therapeutic action	Biochemical and histological events	Chemical structure requeriments
Cytoprotective (gastric mucosa)	Increase mucus secretion Increase synthesis of glycoproteins Increase mucosa thickness Increase resistance of epithelial cells, Stimulation of bicarbonate secretion Local release of sensory neuropeptides	Electrophilic center α-β-unsaturated carbonyl group Reaction with SH-groups of gastric mucosa Polar function in C-4
Antinflammatory	Increase synthesis of PGE2 Exocytosis of acid phosphatase (PMN leukocytes) Exocytosis of cathepsin G (PMN) Release of histamine (mast cells) Release of serotonine (platelets) Release of elastase (neutrophils) Activate histamine receptors (H_3) Inhibit 5-lipoxygenase Inhibit leukotriene C-4 synthase Inhibit NF-kB by preventing degradation of IkB	α-methylen-γ-lactone
Antioxidant	Increase levels of Nitric oxide Increase cycloxygenase activity (COX-2) Inhibit lipid peroxidation Decrease oxidative damage of gastric mucosa Increase availability of mucosal sulphydryl groups	o-dihydroxy (cathecol) of B ring 2,3-double bond in conjugation with a 4-oxo function 7 and 5 HO-groups additionally 3-HO groups
Cytotoxic	Inhibition of inflammatory responses Inhibition of NF-kB Inhibition of mitogen-activated protein kinases	Functional groups in α-position β-substituted and unsubstituted cyclopentanone ring

The biologic activity of sesquiterpene lactones seems the antioxidant and antinflammatory properties of flavonoids and other phenolic compounds. Polyphenos are plant compounds whose protective effects against a number of hepatotoxic agents in experimental animals have studied extensively by Fraga *et al.* [38]. Flavonoids such a s sylimain and quercetin, and polyphenols as cynarin, caffeic acid and chlorogenic acid have also proposed as hepatoprotective drugs. Many phenolic compounds have been shown to modulate the prostaglandins (PGH) synthesis and 5-lipooxygenase pathways of arachidonic acid [39]. Phenols have dual effect on prostaglandin biosynthesis, low concentrations stimulate and high concentrations inhibit PGHS, by inhibiting the cyclooxygenase activityPhenols stimulate prostaglandin synthesis by acting as reducting substrates for the oxidized intermediates of PGHS, thereby accelerating the peroxidase cycle and by functioning as electron-donating cosubstrates for the peroxidase component of PGHS. Phenols inhibit cyclooxygenase activity of PGHS by competing for the arachidonic acid binding site and by competitive reduction of PGHS. The modulation of hydroperoxide tone by phenols is probably the key element in explaining the suppression of arachidonic acid metabolism by PGHS. The stimulatory effect of phenols on PGE2 formation may be based on their action as cosubstrates for peroxidase reaction [39].

The gastroprotective effect of *A. douglasiana* extracts and DhL on the gastric mucosal injury could also be mediated by local release of sensory neuropeptides, nitric oxide and prostaglandins. Cyclooxygenase (COX-2), which catalyzes the rate limiting step in prostaglandins biosynthesis, is rapidly induced in a proinflammatory condition. Inhibition of lipid peroxidation and of COX-2 activity are considered as important targets for chemoprevention of gastrointestinal ulcers.

The active compounds of plants, flavonoids, triterpenes and tannins are active compounds against gastric lesions by acting as protective factors or increasing antioxidant activity. Three distinct mechanisms of protection may be involved: alteration of GSH metabolism, quenching of reactive oxygen species and the inhibition of Ca^{2+} influx that signals the last step in the cell death cascade induced by glutamate [39]. Plant extracts used for treatments of gastric ulcers produce a dose-dependent anti-ulcerogenic activity associated with a reduced acid output and increased mucin secretion, an in-

crease of PGE2 release, a decrease in leukotrienes [39], and prevent aspirin-induced damage to the rat gastric mucosa [4041].

Further investigations are necessary to elucidate if *A. douglasiana*,DhL and IA actively regulate stress responses of gastric mucosa through mechanisms that include heat shock proteins, prostanoids and nitric oxide besides their antioxidant properties.

The other molecular mechanism involved in the cytoprotective effect of sesquiterpene lactones is the reduction of intracellular oxidative stress and damage in gastric mucosal cells, resulting in an increased availability of mucosal sulphydryl groups.

5. STRUCTURE-ACTIVITY RELATIONSHIPS OF SESQUITERPENE LACTONES

Regarding the biological action of sesquiterpene lactones, some structural requirements appear as necessary in order to elicit the anti-inflammatory and cytoprotective activities. In this order, the presence of an electrophilic center (i.e. α,β-unsaturated carbonyl group) together with a second polar group seem to be determinant, whereas the conformational requirements from the decaline system seems to play a secondary role.

Concerning the anti-inflammatory activity, the α- methylene-γ-lactone moiety is the most decisive feature. Sesquiterpene lactones inhibit the transcription factor NF-kB. The NF-kB promotes the expression of over 150 target genes in response to inflammatory stimulators, such as interleukin-1, -2, and –6, or TNF-α, as well as genes encoding immunoreceptors, cell adhesion molecules, and enzymes such as cycloxygenase-2 and NO synthase. NF-kB is composed most frequently of p50 and p65 subunit and is retained in the cytoplasm bond to its inhibitory subunit Ik-B in its inactive form. Inducers of NF-kB such as inflammatory cytokines release active NF-kB by activating the Ik-B-kinase complex. The NF-kB inhibitory activity of sesquiterpene lactones is not only correlated to the number of unsaturated carbonyl structures in the molecule, but mostly to structure-coding parameters and correlates with the number of alkylating centers, such as the methylene lactone and conjugated keto or aldehyde functions [35]. Experiments of Henner *et al.* (1998) showed that the isoprenoid that lacked either the lactone or the exomethylene group in the α-position to the lactone function displayed no inhibitory effect on the pathway leading to the activation of NF-kB [42].

The antinflammatory activity of sesquiterpene lactones is related to the prevention of Ik-B complex degradation, without directly inhibiting Ik-B complex. Sesquiterpene lactones directly alkylates the NF-kB p65 subunit at cys 38, nevertheless, the p50 subunit is not modified. The inhibitory of Ik-B degadation is secondary to the alkylation of p65 subunit [42].

The cytoprotective activity seems related and depending upon the sesquiterpene lactones nucleophilic reaction with the SH-groups of the gastric mucosa. These reactions seem to protect the mucosa and the cells from the ulcerations induced by necrotizing agents. The presence and the adequate molecular accessibility of an α,β-unsaturated carbonyl in the structure and its inclusion in a ring system or at least in the proximity of a cyclic system were claimed as essential factors [36].

Another biological effect of the sesquiterpene lactones is their cytotoxic activity. Structure activity studies done for sesquiterpene lactone cytotoxicity showed that the mechanism of action involves the functional group in α-positions (α-methylene-γ-lactone) [13]. The presence of the α-methylene-γ-lactone moiety is a requirement for the cytoprotective activity observed, and the presence of the β-substituted or unsubstituted cyclopentanone ring is not a structural requirement for cytoprotective activity, contrary to its requirement for antitumor, antimicrobial, and antifeedant properties [13]. In the IA chemical structure, the polar function at C-4 plays a determinant role for the cytoprotective effect [21].

Furthermore, it has been determined that the lipid peroxidation process that occurs in the hydrophobic domains of the membranes accounts for a large part of the emission of chemiluminescence [31]. In contrast, the major antioxidant components of *A. douglasiana* are mostly hydrophilic, and this reduces their ability to reach the hydrophobic domains of its molecular structure and its redox properties, but also by its location in the membrane. The antioxidant activity against cytotoxicity depends on three structural groups: (a) the 0-dihydroxy (cathecol) structure of the B ring; (b) the 2,3-double bond in conjugation with a 4-oxo function; and (c) the presence of both 7- and 5- and additionally, 3-HO-groups. DhL presents only conjugated double bonds and oxo-functions. According with the results obtained by Maria *et al.* [10] and Repetto *et al.* [13], it is clear that in the aqueous extract of *A. douglasiana* other substances compound responding to these structural requirements, as, for instance, flavonoids, could be present [38].

Ilicic aldehyde, considered comparatively with DhL, seems to be a better basic structure to provide derivatives with anti-ulcer effects. The protective action of IA was similar to the protection given by *A. douglasiana* extracts [10, 13], with the advantage that IA is more effective, considering effects/concentration, than DhL and *A. douglasiana* extracts.

Regarding the biological action and the antioxidant effectiveness of the sesquiterpenes DhL and IA, it is interesting to describe a potential connection between their chemical structures. Previous studies performed with DhL [13] and with IA [21] show that the pharmacological cytoprotective effect is related to the presence of an electrophilic center, an α,β-unsaturated carbonyl groups, together with a second polar group. The presence of an α,β-unsaturated aldehyde on the C-7 side chain together with a hydroxyl group at C-4 in IA structure is the requirement for the observed antiulcerogenic activity and seems associated to the antioxidant activity.

However, DhL remains interesting as a basic chemical structure being able to increase the synthesis of gastric mucosal glycoprotein and to inhibit acid secretion in the pylorus-ligated rat [33].

6. CONCLUSIONS AND PERSPECTIVES

In summary, *A. douglasiana*, DhL and IA exert antioxidant actions *in vitro*and protect gastric mucosa from experi-

mental ulcerations *in vivo*These evidences indicate that *A. douglasiana* and DhL could protect gastric mucosa from acute injury and could promote the healing of chronic gastric ulcers by its antioxidant activity, by increasing synthesis of gastric mucosal glycoprotein [13] and of mucus [26], and by increasing gastric mucosal generation of prostaglandins (PGE2). Nitric oxide and prostaglandins contribute to the protective effect of DhL against ethanol-induced gastric mucosal damage [14].

A variety of plant extracts have been used from antiquity in herbal medicine against gastric diseases in experimental animals and humans. Sesquiterpenes and may have anti-inflammatory and anticancer effects [43-45]. Recent researches showed that polyphenols derived from apple extracts and olive oils have been recently demonstrated to exert anticarcinogenic effects on colon carcinogenesis [43, 44, 45]. However, there are no toxicology studies regarding these extracts, that may evolve useful for medical treatments in humans. Further investigations are necessary.

ACKNOWLEDGEMENTS

This study was supported by grants from the University of Buenos Aires, and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

The authors wish to express their gratitude to Professor Albertina Moglioni for technical assistance.

ABBREVIATIONS

DhL = Dehydroleucodine

IA = Ilicic aldehyde

I-kB = Inhibitory protein of nuclear transcription factor

NF-kB = nuclear transcription factor

GSH = reduced glutathione

TBARS = thiobarbituric acid reactive substances

TRAP = total reactive antioxidant potential

TAR = total antioxidant reactivity

PGE2 = prostaglandin E-2

PGHS = prostaglandin H-synthase

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