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Chemical and Pharmacological Properties of Dehydroleucodine, A Lactone isolated from *Artemisia douglasiana* Besser

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ABSTRACT

Plant medicines have commonly been used in basic health care in many countries throughout the centuries. One of the fairly well documented preparations in Argentinean traditional medicine is an infusion of the leaves of *Artemisia douglasiana* Besser, popularly known as 'matico'. Dehydroleucodine, a sesquiterpene lactone isolated from *Artemisia douglasiana* Besser, prevents gastrointestinal damage in response to necrosis-inducing agents in a dose-dependent manner. The mechanisms of action of dehydroleucodine, confirmed by biochemical and morphological studies, are mainly related to the ability of the drug to stimulate mucus production and to inhibit pro-inflammatory mediator release from mast cells. The purpose of this review, based on studies from our laboratory as well as from others, is to summarize salient features of the ethnobotany of *Artemisia douglasiana* Besser, as well as the chemistry, pharmacology and mechanisms involved in the cytoprotective action of dehydroleucodine. It may be expected that the lactone will be a promising drug in the treatment of disease processes involving the gastrointestinal tract, such as peptic ulcer and food hypersensitivity.

Key words: traditional medicine; *Artemisia douglasiana*; matico; dehydroleucodine; gastrointestinal cytoprotection; inflammation.

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List of abbreviations: DhL: dehydroleucodine; HPLC: high performance liquid chromatography; OPA: *o*-phthalaldehyde.

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INTRODUCTION

Natural products have served as a major source of drugs for centuries, and about half of the pharmaceuticals in use today are derived from natural products (Clark, 1996; Mahidol *et al.*, 2002). Since ancient times peoples have been exploring nature, more particularly the plants, in the search for new therapies. This has resulted in the use of a large number of medicinal plants and bioactive molecules to treat various diseases (Clark, 1996). Some of the molecules are pure compounds from the traditional medicinal plants and others are modifications of these substances (Clark, 1996).

Peptic ulcer disease is a chronic, inflammatory condition of the stomach or duodenum that affects as many as 10 % of the population at some time in their lives. The disease has relatively low mortality, but it results in substantial human suffering and high economic costs. Phytogenic agents have traditionally been used by herbalists and indigenous healers for the prevention and treatment of peptic ulcer. Botanical compounds with anti-ulcer activity include flavonoids (i.e. quercetin, naringin, silymarin, anthocyanosides, sophoradin derivatives), saponins (i.e. from *Panax japonicus* and *Kochia scoparia*), tannins (i.e. from *Linderae umbellatae*), gums and mucilages (i.e. gum guar and myrrh). Among herbal drugs, liquorice, aloe gel and capsicum (chilli) have been used extensively (Borrelli and Izzo, 2000). One of the fairly well documented preparations in Argentinean traditional medicine for the treatment of peptic ulcer disease is the infusion of the leaves of *Artemisia douglasiana* Besser, popularly known as 'matico'. Dehydroleucodine, a sesquiterpene lactone isolated from *Artemisia douglasiana* Besser, prevents gastrointestinal damage in response to necrosis-inducing agents.

The purpose of this review, based on studies from our laboratory as well as from others, is to summarize salient features of the ethnobotany of *Artemisia douglasiana* Besser, as well as the chemistry, pharmacology and mechanisms involved in the cytoprotective action of dehydroleucodine.

ETHNOBOTANY OF *ARTEMISIA DOUGLASIANA* BESSER AND PRELIMINARY PHARMACOLOGICAL EXPERIMENTS

Artemisia douglasiana Besser (family: Asteraceae) is a hexaploid species whose origin was attributed by Keck as a hybrid between *Artemisia suksdorfii* Piper and *Artemisia ludoviciana* Nutt (Keck, 1946). It is a perennial herb found on the western slopes of the Rockies, in the northern Baja in California and in Argentina. Probably, *Artemisia douglasiana* Besser would have been introduced into Argentina from Chile (Giordano *et al.*, 1990, 1997). The first report of its occurrence in Argentina appeared in 1967. In that year Ariza Espinar reported the presence of *Artemisia douglasiana* Besser as an adventitious plant in San Juan and Mendoza provinces (Ariza Espinar, 1967; Ariza Espinar and Bonzan, 1992). A herbarium sample representative of the plant is deposited in the herbarium of Universidad Nacional de San Luis, Argentina.

In Argentina, *Artemisia douglasiana* Besser is used in folk medicine and is known by the common name of 'matico' (Fig. 1). The popular use of the infusion of the boiled leaves of matico as a cytoprotective agent against peptic ulcer and in the external treatment of sores, prompted preliminary pharmacological experiments with the aqueous extract of the aerial parts of "matico". These studies were designed to evaluate whether *Artemisia douglasiana* Besser showed the aforementioned property. In fact, in preliminary evaluations the aqueous extract showed reproducible cytoprotective activity against ulcerogenic agents such as absolute ethanol in rats (Giordano *et al.*, 1990). The chloroform extract of the aerial-dried parts of *Artemisia douglasiana* also showed significant cytoprotective activity (Giordano *et al*., 1990). The results of this test led to a phytochemical study of the plant.

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Figure 1. *Artemisia douglasiana* Besser, popularly known as 'matico'.

CHEMISTRY OF DEHYDROLEUCODINE

The phytochemical study of *Artemisia douglasiana* Besser led to the isolation of dehydroleucodine, a sesquiterpene lactone of the guaianolide type (Giordano *et al*., 1990, 1997). Fig. 2 shows the chemical structure of dehydroleucodine.

Dehydroleucodine was obtained from the aerial parts of *Artemisia douglasiana* Besser. Extraction was performed according to method of Giordano *et al.* (1990). Stems and dry leaves of plants collected in the San Luis province (Argentina) were soaked in chloroform at room temperature. The extracts were evaporated *in vacuo* and dissolved in 95% ethanol. Then, 1.5% lead tetracetate aqueous solution was added. The mixture was extracted three times with chloroform and the solution was again concentrated under vacuum. The residue was chromatographed in a medium pressure chromatography system. Different mixtures of ethanol and hexane were used as eluents. The chromatographic purification of the chloroform extract yielded almost exclusively dehydroleucodine (Giordano *et al*., 1990). Dehydroleucodine (8 g/kg) was first identified by ^{1}H- and ^{13}C-nuclear magnetic resonance, infrared, ultraviolet, mass spectrometry, and melting point analysis. At present, the lactone can be detected and quantified in animal tissues, plants extracts and biological fluids by high performance liquid chromatography (HPLC) using a separation column packed with strong cation exchange resin, postcolumn *o*-phthalaldehyde (OPA) automatic derivatization and fluorescence detection (Penissi *et al*., 2003 c).

This method is sensitive enough for detecting dehydroleucodine with a detection limit of 1.7 ng/mL and quantitation limit of 17 ng/mL in less than 8 min with a high degree of accuracy.

PHARMACOLOGY OF DEHYDROLEUCODINE

Pharmacological tests showed that dehydroleucodine is the compound responsible for the antiulcerogenic activity exhibited by the crude extract of *Artemisia douglasiana* Besser (Giordano *et al*., 1990).

Dehydroleucodine prevents gastrointestinal damage in response to necrosis-inducing agents such as absolute ethanol in a dose-dependent manner. Dehydroleucodine was orally given in increasing doses of 3, 10, 20 and 40 mg/kg, 60 min before the oral administration of absolute ethanol. The stomachs and duodena were removed, opened and washed gently with ice-cold saline solution. The degree of erosion in these organs was assessed from a scoring system designed by Marazzi-Uberti and Turba (1961).

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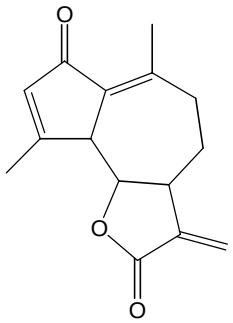

Figure 2. Chemical structure of dehydroleucodine.

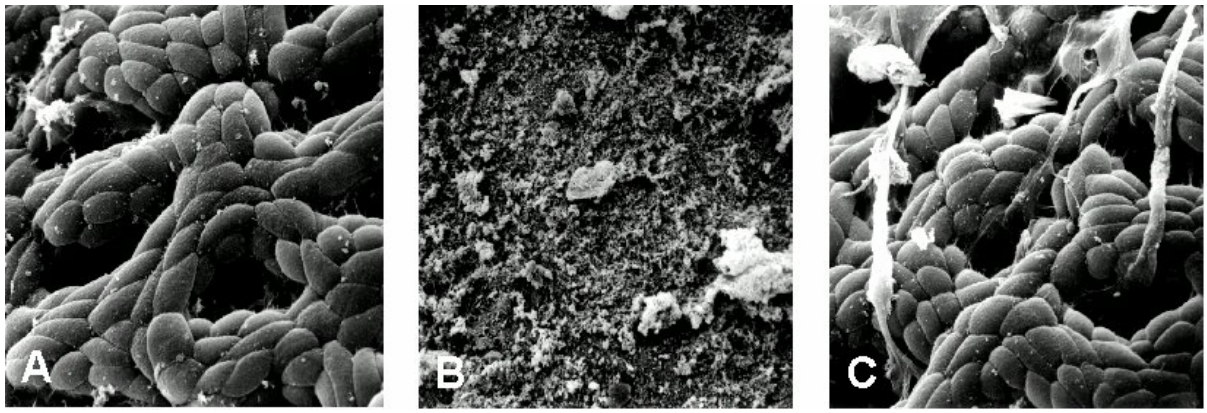

Figure 3. Scanning electron micrographs showing gastric mucosa of mice (X 600). (**A**) Control. Epithelial cells have a cobblestone appearance. Scarce filaments of mucus are present. (**B**) Ethanol. Necrotic tissue is covered by masses of flocculated material. (**C**) Dehydroleucodine + Ethanol. The epithelium is similar to that of control.

The results were expressed as an ulcer factor. Dehydroleucodine also prevents gastrointestinal damage produced by 0.6 N HCl, 0.2 N NaOH and 25% NaCl (Giordano *et al*., 1990). Morphological studies by light and electron microscopy have confirmed that dehydroleucodine pretreatment prevents the significative damage induced by ethanol in the rat stomach and small intestine (Penissi *et al*., 1998). Fig. 3 shows the gastric protective effect examined with the scanning electron microscope. Recently, it has also been demonstrated that dehydroleucodine exhibits a strong antiinflammatory action in acute (carrageenan-induced rat paw oedema model) and chronic (cotton pellet method) models of experimental inflammation (Guardia *et al*., 2003).

Pharmacological and structure-activity relationship studies with related guaianolides have shown that: (1) the presence of an exocyclic methylene group conjugated to a gamma lactone is structural requirement needed for the observed antiulcerogenic activity (Giordano *et al*., 1990, 1997; Enriz *et al*., 1994); and (2) the presence of the β-substituted or -unsubstituted cyclopentenone ring is not a structural requirement for cytoprotective activity contrary to its requirement for antitumor, antimicrobial and antifeedant properties (Giordano *et al*., 1990).

In this way, the first guaianolide checked was desacetoxymatricarin, a guaianolide isolated from *Artemisia mendozana* var. *paramilloensis* (Giordano *et al*., 1990, 1997). Different assays


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carried out with desacetoxymatricarin have shown no cytoprotective activity against chemical induced ulcer in rats (Giordano *et al*., 1990, 1997). On the other hand, ludartin, a guaianolide isolated from *Stevia yaconensis*, showed itself as an active cytoprotective product (Giordano *et al*., 1990,1997). Dehydroleucodine, desacetoxymatricarin and ludartin provide model compounds with: β-substituted cyclopentenone and α-methylene-γ-lactone, β-substituted cyclopentenone and α-methyl-γ-lactone, and α-methylene-γ-lactone moieties, respectively (Giordano *et al*., 1990, 1997).

Similar pharmacological and structure-activity relationship studies were carried out with related pseudoguaianolides (Giordano *et al*., 1990, 1997). The first pseudoguaianolide checked was hymenin, isolated from *Parthenium hysterophorus*. The prevention, by hymenin, of gastric lesions produced by ethanol showed a dose-dependent variation, similar to that exhibited by dehydroleucodine. Mexicanin (a pseudoguaianolide isolated from *Gaillardia megapotamica* var. *scabiosoides*), helenalin (another pseudoguaianolide also isolated from *Gaillardia megapotamica var. scabiosoides*) and 9-*O*-desacetylspathulin-2-*O*-angelate (a pseudoguaianolide obtained from *Helenium alternifolium*) were also checked. All the previously examined pseudoguaianolides exhibited antiulcerogenic activity and have the α-methyl-γ-lactone functionality. Hymenin, mexicanin and helenalin have the β-unsubstituted cyclopentenone ring. The *O*-desacetylspathulin-2-*O*-angelate provides a model compound with the α-methyl-γ-lactone moiety exclusively.

MECHANISMS INVOLVED IN THE PHARMACOLOGICAL ACTION OF DEHYDROLEUCODINE

The mechanisms involved in the antiulcer action of dehydroleucodine, confirmed by biochemical and morphological studies, are mainly related to the ability of the drug to stimulate mucus production (Penissi *et al*., 1998; Penissi and Piezzi, 1999) and to inhibit pro-inflammatory mediator release from mast cells (Penissi *et al*., 2003 a,b). Both mucus production and mast cells are important components of the gastrointestinal mucosal defence system.

a. Gastrointestinal Mucosal Defence.

The term 'gastrointestinal mucosal defence' refers to the combination of factors that allow the gastrointestinal mucosa to withstand exposure to substances with a wide range of pH, osmolarity, and temperature, solutions with detergent properties (*e.g*., bile), and bacterial products capable of eliciting local and systemic inflammatory reactions (Wallace and Granger, 1996; Wallace and Ma, 2001). The mucosa is not impervious to damage by the various substances we eat and the endogenous secretions. Indeed, it is likely that mucosa injury occurs regularly. However, the mucosa can repair such injury quickly, thereby limiting it to the most superficial layer of cells and preventing entry into the systemic circulation of substances detrimental to the organism (Wallace and Granger, 1996; Wallace and Ma, 2001). The resistance of the mucosa can also be enhanced when irritants are present in the gastrointestinal tract. Thus, the ability of the mucosa to resist significant injury is attributable to a dynamic process rather than a static barrier (Wallace and Granger, 1996; Wallace and Ma, 2001).

The various components of mucosal defence can be viewed as being organized as a hierarchy, corresponding to the anatomical organization of the mucosa (Wallace and Granger, 1996; Wallace and Ma, 2001).

The first level of defence consists of the factors secreted into the lumen including hydrochloric acid, bicarbonate, mucus, immunoglobulins, some antibacterial substances (*e.g.*, lactoferrin), and surface-active phospholipids (Wallace and Granger, 1996; Wallace and Ma, 2001).

The second level of defence is the epithelium, which is remarkably resistant to acid-induced injury and forms a relatively tight barrier to passive diffusion (Wallace and Granger, 1996; Wallace and Ma, 2001). The epithelium is capable of undergoing extremely rapid repair if its continuity is disrupted.

The third level of defense is the mucosal microcirculation, in concert with sensory afferent nerves within the mucosa and submucosa (Wallace and Granger, 1996; Wallace and Ma, 2001). Back-difussion of acid or toxins into the mucosa results in a neurally mediated elevation of mucosal blood flow that is critical for limiting damage and facilitating repair.

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The fourth level of defense is the mucosal immune system, consisting of various 'alarm cells', such as the mast cell and macrophage, which sense entry of foreign material into the mucosa and orchestrate an appropriate inflammatory response (Wallace and Granger, 1996; Wallace and Ma, 2001). These cells sense the entry of foreign matter or antigen into the lamina propia, and respond by releasing soluble mediators and cytokines that initiate a defensive inflammatory response to prevent the foreign matter from gaining access to the systemic circulation (Wallace, 1996; Boros *et al*., 1999b). Many of the inflammatory mediators and cytokines that are released exert chemotactic effects on leukocytes, resulting in their recruitment into the region where the immunocytes have been activated (Wallace, 1996).

The fifth level of mucosal defense is called into play when an ulcer has formed; an ulcer being defined as a break in mucosa that extends through the muscularis mucosae. In these circumstances, the ulcer is repaired through growth and re-development of gastric glands, tissue remodeling, growth of new blood vessels (angiogenesis), and re-innervation of the mucosa by the extrinsic and intrinsic nerves (Wallace, 2001). Enhanced numbers of inflammatory cells, mainly mast cells and eosinophils, are well known to occur during angiogenesis and tissue remodeling (Armetti *et al*., 1999).

b. Role of Mucus in the Gastrointestinal Cytoprotective Action Induced by Dehydroleucodine.

Mucus has a central role in the protection of the gastrointestinal mucosa from the potentially hostile environment created by acid and peptic secretions, the mechanical and chemical irritants in ingested materials, and the reflux of bile secretions (Khanvilkar *et al*., 2001).

The main constituents of mucus, irrespective of its origin, are glycoproteins and lipids (0.5-5%), water (*ca.* 95%), sloughed epithelial cells, electrolytes (0.5-1%), free proteins (1%), and bacteria (Khanvilkar *et al*., 2001).

Mucin is the glycoprotein of mucus and is found in two forms: soluble secretory mucin and membrane bound mucin (Khanvilkar *et al*., 2001). Secretory mucins form viscous gels due to their high molecular weights and their ability to form intermolecular disulfide bridges (Khanvilkar *et al*., 2001). Membrane-bound mucins differ from secretory mucins in that they contain a hydrophobic domain anchoring the molecules in the plasma membrane, and they lack intermolecular associations through disulfide bridges. Both types of mucins are found on epithelial surfaces where they contribute to the formation of a mucous layer to protect the surface (Khanvilkar *et al*., 2001).

Adherent mucus gel is proposed to form a continuous blanket over the mucosal surface, which traps bicarbonate secreted by the epithelium and therefore acts as a layer in which luminal acid that diffuses toward the epithelium is neutralized (Williams and Turnberg, 1981; Allen *et al*., 1993; Lichtenberger, 1995; Wallace and Granger, 1996; Grübel *et al*., 1997). Mucus also provides a diffusion barrier for certain low molecular weight solutes and a physical barrier for microorganisms and their toxins (Lamont, 1992). Moreover, mucus is capable of acting as an antioxidant, and so can reduce mucosal damage mediated by oxygen free radicals (Wallace and Granger, 1996; Seno *et al*., 1995). The protective properties of the mucus barrier depend not only on the gel structure but also on the amount or thickness of the layer covering the mucosal surface (Kerss *et al*., 1982; Engel *et al.*, 1995). Furthermore, the thickness of the adherent mucus gel represents a dynamic balance between the rate of mucus secretion by the mucosal cells and the rate of mucus erosion by enzymatic degradation and mechanical shear forces (Szentkuti *et al*., 1990).

Qualitative examinations from conventional histological samples and quantitative studies in unfixed tissues, have shown that dehydroleucodine by itself causes an increase in the thickness of the adherent mucus gel layer in the gastrointestinal tract (Penissi *et al*., 1998; Penissi and Piezzi, 1999). These observations have confirmed that one of the main mechanisms involved in the cytoprotective action of dehydroleucodine is increased mucus secretion.

Several different mechanisms have been proposed to explain the massive and accelerated mucus release induced by dehydroleucodine. It is probable that prostaglandins, particularly prostaglandins E and F, mediate the effect


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produced by dehydroleucodine. This hypothesis is reinforced by the findings of Maria *et al*. (1998), who found an increase in gastric PGE_2 levels in subchronically dehydroleucodine-treated rats. It is also possible that dehydroleucodine may alter the activity of certain receptors in the gastrointestinal mucosa, such as serotonin and /or muscarinic receptors. For example, it has been shown that some 5-HT_{1A} agonists exert a gastroprotective effect, not only through the reduction of aggressive elements in the gastrointestinal tract (acid and pepsin secretion), but also through the enhancement of defensive gastrointestinal factors, such as adherent mucus (Farrae *et al*., 1995). It has been also shown that muscarinic receptors mediate the secretion of gastric acid, pepsinogen, and mucus in gastric mucosa, through coupling to stimulation of the phosphoinositide second messenger system. Early experiments suggested that vagal or splanchnic nerve stimulation resulted in copious mucus release. Subsequently, cholinergic agonists such as carbachol have been shown to cause gastric mucus release in various species (Lamont, 1992).

c. Role of Mast Cells in the Gastrointestinal Cytoprotective Action Induced by Dehydroleucodine.

Mast cells are important components of the normal architecture of the gastrointestinal tract. Changes in the number of mast cells at various anatomic sites, or evidence of activation of the cells for mediator release, have been observed in a wide spectrum of adaptive or pathologic immune responses, and in a large number of disease processes, many involving the gastrointestinal tract. Evidence such as this supports the notion that mast cells can substantially influence immunologic and pathologic processes in the gastrointestinal tract and may even affect certain normal functions of the stomach or intestine (Barczyk *et al*., 1995; Bischoff *et al*., 1996, 2000; Echtenacher *et al.*, 1996; Wallace and Granger, 1996; Furuta *et al*., 1997; Wershil, 2000; Andoh *et al*., 2001; Kolaczkowska *et al*., 2001).

Gastrointestinal mast cells clearly play a role in many pathologic effects associated with food hypersensitivity (Stenton *et al*., 1998). Histamine and serotonin are valuable markers of mast cell activation (Theoharides *et al*., 1985; Glavin and Hall, 1991; Buckley and Coleman, 1992; Coelho *et a*l., 1998; Bueno and Fioramonti, 1999; Jiang *et al*., 2000), and have been regarded as critical pathogenetic factors in the development of peptic ulcers (Cho, 1994; Myers *et al*., 1998).

It has been suggested that stabilization of mast cells may be a key mechanism to protect the gastrointestinal tract from injury (Karmeli *et al*., 1991; Eliakim *et al*., 1992; Whittle, 1993; Hogaboam *et al*., 1993; Low *et al*., 1995; Kalia *et al*., 2000; Kirali *et al*., 2000).

Few molecules are known to possess both mast cell stabilizing and gastrointestinal cytoprotective activity. These include zinc compounds, sodium cromoglycate, FPL 52694, ketotifen (Cho and Ogle, 1992), aloe vera (Ro *et al*., 2000), certain flavonoids such as quercetin (Middleton, 2000), some sulfated proteoglycans such as chondroitin sulfate (Theoharides *et al*., 1999; Hori *et al*., 2001) and dehydroleucodine (Penissi *et al*., 2003 a,b).

The effects of dehydroleucodine on the intestinal mast cell population have been studied by Penissi *et al*. (2003 a,b), with the goal of testing the hypothesis that dehydroleucodine induces changes in these cells that are probably related to its mechanism of gastrointestinal cytoprotection.

It is well known that compound 48/80 is a connective tissue mast cell degranulator that releases histamine and serotonin from intracytoplasmic granules (Mc Neil and Austen, 1995). It has been established that this mast cell secretagogue causes mucosal injury in the gastrointestinal tract and mast cell mediator release (such as histamine and serotonin), and that released histamine and serotonin play a role in mediating the mucosal injury (Ohta *et al*., 1997; Boros *et al*., 1999 a). Incubation of jejuna with a compound 48/80 solution increases histamine and serotonin release, and this effect is inhibited by dehydroleucodine.

Light and electron microscopy have shown that the significantly increased histamine and serotonin release after compound 48/80 treatment is closely associated with a reduction in the number of granulated submucosal mast cells and with obvious mast cell ultrastructural changes (Penissi *et al*., 2003 b). The morphological findings also showed that dehydroleucodine inhibits the reduction in the number of granulated

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metachromatically stained submucosal mast cells, suggesting an interaction of the lactone with the mast cell population and an inhibition of the degranulation induced by compound 48/80.

Furthermore, dehydroleucodine induces some ultrastructural mast cell changes, but this last action is less dramatic than that elicited by compound 48/80 itself.

Dehydroleucodine also inhibits compound 48/80-induced serotonin release from rat purified peritoneal mast cells (Penissi *et al*., 2002).

The fact that dehydroleucodine inhibits compound 48/80-induced histamine and serotonin release from mast cells in the isolated mouse jejunum and in rat purified peritoneal mast cells, raises the possibility that the lactone acts as a mast cell stabilizer in the intact animal. Thus, dehydroleucodine could prevent histamine and serotonin release and, consequently, intestinal damage elicited by necrosis-inducing agents such as compound 48/80. This mechanism is quite analogous to that of ketotifen, a mast cell stabilizer that significantly protects the gastrointestinal mucosa against lesions induced by necrotizing agents, histamine or compound 48/80 (Karmeli *et al*., 1991; Eliakim *et al*., 1992; Kirali *et al*., 2000; Ruh *et al*., 2000).

CONCLUSIONS AND FUTURE IMPLICATIONS

Despite progress in conventional chemistry and pharmacology in producing effective drugs, the eradication of peptic ulcer disease remains an elusive goal. Dehydroleucodine, a sesquiterpene lactone isolated from *Artemisia douglasiana* Besser, have potential beneficial therapeutic actions and may represent an attractive pharmacological option in the management of inflammation and gastrointestinal ulcers. Further studies about the clinical efficacy of dehydroleucodine will lead to a more complete understanding of the interaction between the lactone and biological systems. It may be expected that dehydroleucodine will be a promising drug in the treatment of pathological processes involving the gastrointestinal tract, such as peptic ulcer and food hypersensitivity.

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