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Study on the increment of the production of gastric mucus in rats treated with *Opuntia ficus indica* (L.) Mill. cladodes

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Abstract

Opuntia ficus indica cladodes are used in traditional medicine of many countries for their cicatrisant activity. The major components of cladodes are carbohydrate-containing polymers, which consist of a mixture of mucilage and pectin. In this paper we studied the cytoprotective effects of cladodes on experimental ethanol-induced ulcer in rat. The *O. ficus indica* cladodes administration gives rise to cytoprotection phenomena by breaking up the epithelial cells and stimulating an increase in mucus production. When *O. ficus indica* cladodes are administered as a preventive therapy, keep the gastric mucosa under normal condition by preventing mucus dissolution caused by ethanol and favouring mucus production. An increase of mucus production is also observed during the course of the curative treatment. The treatment with *O. ficus indica* cladodes provokes an increase in the number of secretory cells. Probably, the gastric fibroblasts are involved in the antiulcer activity.

Keywords: Gastric ulcer; Mucus; *Opuntia ficus indica*; Light microscopy

1. Introduction

Opuntia ficus indica cladodes are used in traditional medicine of many countries for their cicatrisant activity (Meyer and Mc Lauglin, 1981; Barbera and Inglese, 1993; Mondello et al., 2000). The major components of cladodes are carbohydrate-containing polymers, which consists of a mixture of mucilage and pectin (Trachtenberg and Mayer, 1981a,b, 1982).

In a recent study (Galati et al., 2001) we investigated the preventive and curative effects of *O. ficus indica* Mill. cladode preparations on rats affected by ethanol-induced ulcers, and we assessed the ultra-structural alterations to the mucous membrane by transmission electron microscopy. The isthological evaluation of the mucus amount is a method widely used by many authors to study the ulcer healing process (McManus, 1946; Terasaki et al., 1996). In the present work we evaluate by light microscopy whether treatment with *O. ficus indica* cladodes produces cytoprotection by increasing mucus secretion in the gastric mucosa of rats affected by ethanol-induced ulcers.

2. Materials and methods

2.1. Plant material

For the experiments we utilized *O. ficus indica* Mill. lopping cladodes, collected in May 1999 in a cultivation located in San Cono (CT, Sicily).

The identity of the plant was confirmed by bibliographic data (Tutin et al., 1968; Pignatti, 1982).

O. ficus indica cladodes, deprived from epidermis and cut in pieces, were homogenized in a high speed mixer, Ultra-Turrax, for 5 min. The homogenized obtained was

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lyophilized. Yield was 1%. The lyophilized cladodes, suspended in distilled water, in a volume of 1.5 ml/100 g b.w., were administered by gavage to rats, at the dose of 1 g/kg.

2.2. Animals

Male Wistar rats, weighing 180–200 g, kept in controlled stabulation (temperature 22±2 °C; humidity 60±4%, in a 12 h light/dark cycle), maintained on a standard diet (S. Morini Mill rat GLP) and water ad libitum, were used.

Before the experiment, all the animals fasted for 24 h, with free access to water.

2.3. Treatment

The rats were divided into four groups of six animals.

The first group of rats was treated with the lyophilized cladodes of *O. ficus indica* (1 g/kg).

The second group (control) was treated, by gavage, with ethanol 90% at the dose of 0.5 ml/rat (Del Soldato et al., 1984).

The third group of animals received by gavage ethanol 90% at the dose of 0.5 ml/rat; after 15 min from the administration of the ulcerogenic agent, the rats were treated orally with lyophilized cladodes of *O. ficus indica* (1 g/kg) (curative treatment).

The fourth group received by gavage the lyophilized cladodes (1 g/kg) and, 1 h after, received 0.5 ml of ethanol 90% (preventive treatment).

After 60 min all the animals were sacrificed under ether anaesthesia. Their stomachs were cut along the greater curvature and washed with saline solution not to remove the mucus layer from the mucosa surface.

2.4. Histology

The stomachs were extended on a cork surface to avoid deformities and small pieces of every stomach were cut and fixed in neutralized 4% paraformaldehyde in 0.2% phosphate buffer for 4 h at 4 °C. The samples were washed with the same buffer and dehydrated in graded ethanol (30–100 °C) and, finally, embedded in bioplast (Biooptica, Milano, Italia). Sections (5 μm), obtained by a rotative microtom, were stained with periodic acid—Schiff, (McManus, 1946) which reacts with mucopolysaccharides. The last one constitutes the gastric mucus and produces a characteristic carmine colour.

All samples were evaluated by light microscopy (BH_2 Olympus).

3. Results

The results of the present study indicate that following the *O. ficus indica* cladodes administration, a gelatinous layer composed of mucus forms and, probably, provides a favourable environment for rapid epithelial restitution (Fig. 2). In fact in the rats treated only with the lyophilized cladodes (I group), the gastric mucosa shows at the normal features and the mucus layer appears on the surface epithelium on cells near the glandular pit (Fig. 1A). In the control rats, treated only with ethanol (II group), intracellular granules are visible both in the glandular surface and in deeper glandular portion. The interglandular spaces are dilated (Fig. 1B). In the rats treated with ethanol (III group) and then with the lyophilized cladodes (curative treatment), intracellular mucus is localized inside the cells of the gastric gland. The glandular spaces are reduced (Fig. 2A). In the rats treated with the lyophilized cladodes and then with ethanol (preventive treatment) (IV group), the surface epithelium shows a regular layer of mucus, while, in the cells neck, the mucus is in a greater amount. The glandular spaces are reduced (Fig. 2B). In the 'lamina propria' of gastric mucosa of rats treated with the lyophilized cladodes, fibroblasts are visible near the glandular portion, especially in preventive treatment (IV group).

4. Conclusions and discussion

The major components of *O. ficus indica* cladodes are carbohydrate-containing polymers which consist of a mixture of mucilage and pectin (Karawya et al., 1980). Recent studies have demonstrated antiulcer activity of polysaccharide fraction from plants (Trachtenberg and Mayer, 1981a; Sun et al., 1991a,b, 1992a,b; Nagaoka et al., 1994). In this way the pectic polysaccharides from *O. ficus indica* cladodes probably may affect the gastrointestinal mucosa regeneration. Our own results demonstrate that the administration of *O. ficus indica* cladodes give rise to cytoprotection phenomena by breaking up the epithelial cells and stimulating an increase in mucus production. Ethanol tends to dissolve the components of the mucous membrane of the stomach and lowers the level of tissue proteins, bringing gastric blood flow to a standstill (Szabo et al., 1985) but preventive treatment with *O. ficus indica* cladodes can stop the ulcerogenic agent from inflicting damage. Results reported in a previous study (Galati et al., 2001) show that treatment with *O. ficus indica* cladodes stimulates a protective response from the gastric mucosa which prevents the development of ethanol-induced ulcers (preventive treatment) or promotes a faster recovery (curative treatment). Ulcer healing takes place either by a regeneration process which starts from the neck cells of the glands or

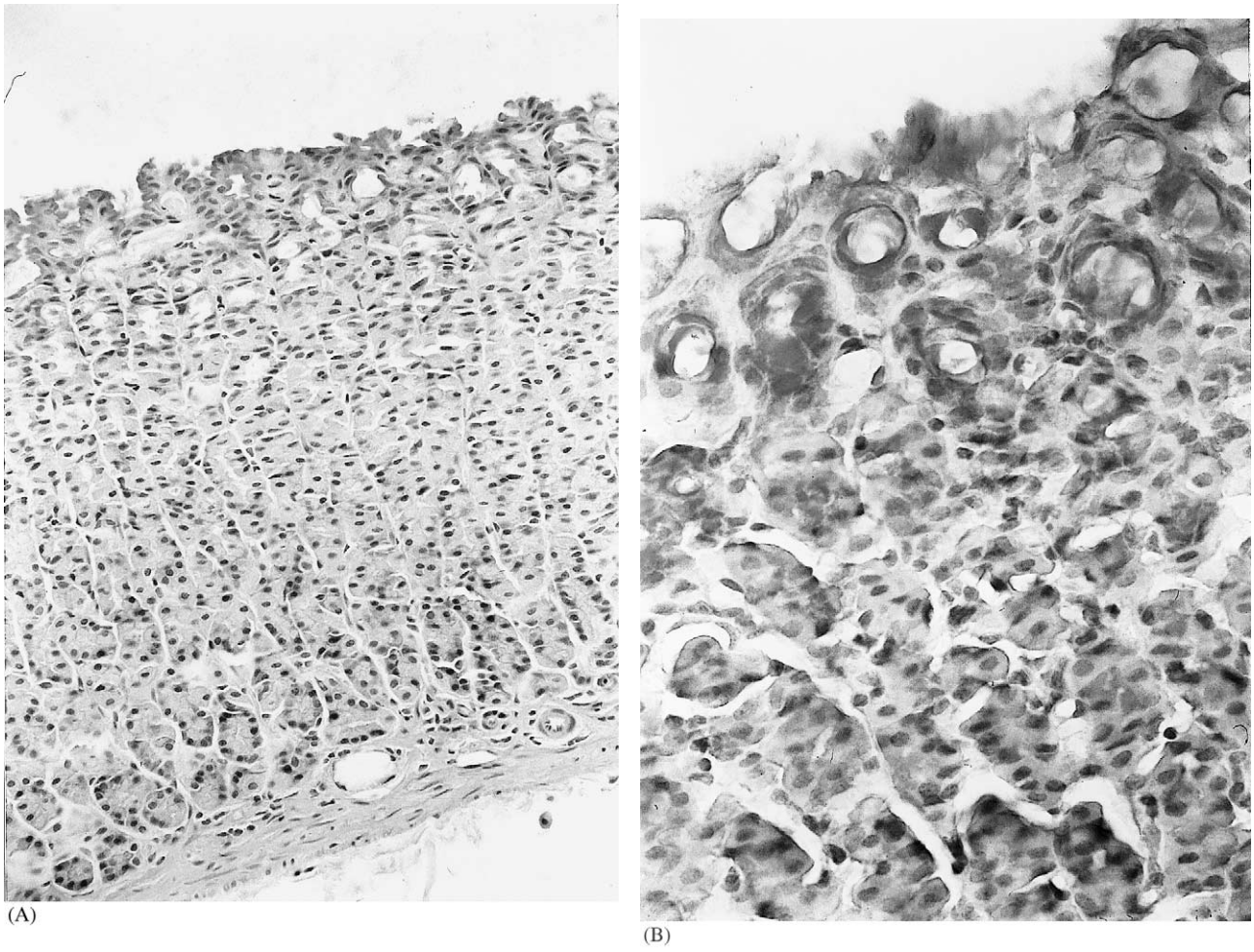
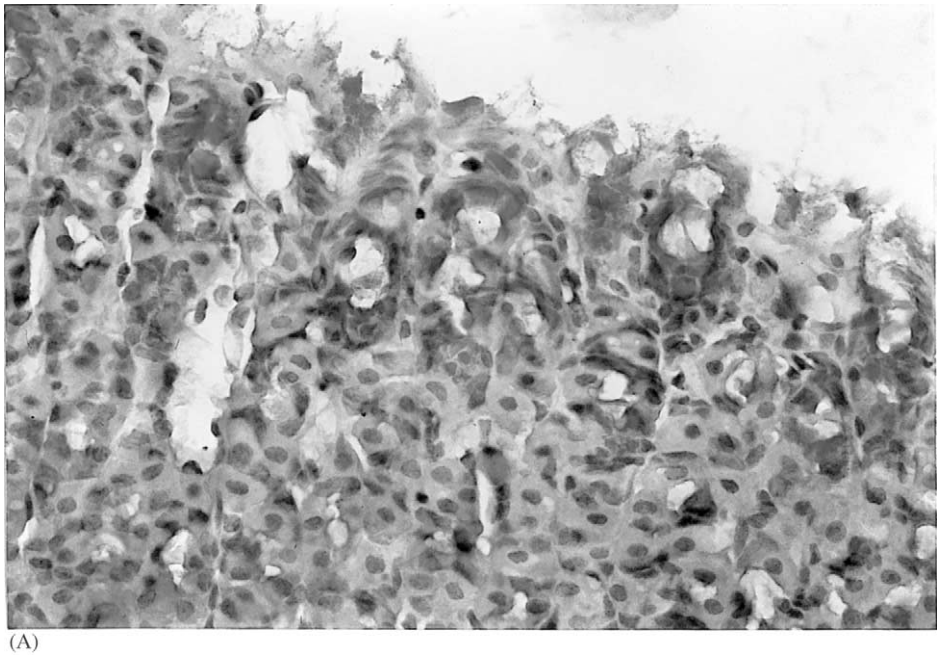


Fig. 1. Light microscopy observations of rat mucosa gastric section. (A) *O. ficus indica* liophilized cladodes-treated rats. Glandular pits and surface epithelium show normal secretion of mucus (20 ×). (B) Ethanol-treated rats. Both neck cells and deeper glandular layer cells produce mucus. The interglandular spaces are widened (40 ×).

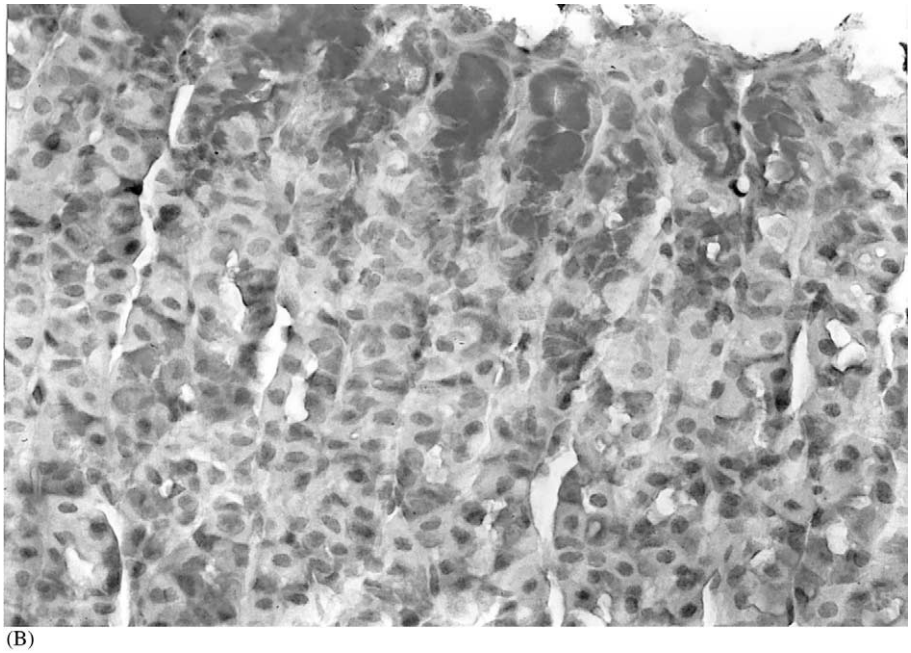
by a rapid process of 'restitutio ad integrum' which involves the migration of cells towards the luminal surface and their deposition on the area stripped by the ulcerogenic agent. The mechanisms which protect the gastric mucosa against acute attack by necrotic agents of different types involve, however, a variety of events (Wallace et al., 1982; Bolton et al., 1978). Among these, a crucial role is played by mucus production (Wallace and Whittle, 1986). This depends on a delicate balance of factors which control its synthesis and the protein, glycoprotein and lipid composition necessary to give it the right viscosity and its characteristic hydrophobicity (Wood and Dubois, 1983; Hills et al., 1983). The cytoprotective effect of *O. ficus indica* cladodes has been attributed to the physico-chemical properties of mucilage (Galati et al., 2001). However, the observations by light microscopy reported in the present study show in addition that surface cells are still present and that there is a recovery of the organisation typical of the gastric mucosa, with normal mucus production. When administered as a preventive therapy, *O. ficus indica* cladode mucilage keeps the gastric mucosa under normal conditions. In fact this treatment prevents mucus dissolution induced by ethanol, reduces the folding of the mucosa so causes it to extend (Dubois et al., 1977) and favours mucus production from neck cells. An increase in mucus production by the cells of the gastric glands, mainly localized in the apical portion, is also observed during the course of curative treatment.

The significant increased mucus production may also be due in part to the action of fibroblasts that are more ordered and more numerous in the 'tonaca propria' of the rats treated with *O. ficus indica* and ethanol compared to those treated with ethanol alone.

The importance of the gastric fibroblasts in the ulcer repair process is known (Kasugai et al., 1997; Nakamura et al., 1998). In fact fibroblasts take part to the functional normalization of the 'tonaca propria' and



(A)



(B)

Fig. 2. Light microscopy observations of rat gastric mucosa section. *O. ficus indica* liophilized cladodes-treated rats. (A) Curative treatment: both neck cells and deeper glandular cells produce mucus. The interglandular spaces are reduced. In the 'tonaca propria' the fibroblasts are present (40 ×). (B) Preventive treatment: mostly neck cells produce mucus. The interglandular spaces are reduced. In the 'tonaca propria' the fibroblasts are present (40 ×).

they play a key role in ulcer healing by favouring the regeneration of the superficial epithelium and the apical pole of the gastric glands that produce glandular mucus (Wallace, 2001).

The results of this study show that treatment with *O. ficus indica* cladodes provokes cell proliferation and differentiation and causes a restoration of the normal mucosal architecture with an increase in the number of secretory cells. Besides it is possible that the components of cladodes enhanced the mucus production by influencing the prostaglandins. Further studies, concerning the isolation of active fraction from *O. ficus indica* cladodes and the mechanism of action, are currently in progress in our laboratory.

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