Antiulcer activity of *Opuntia ficus indica* (L.) Mill. (Cactaceae): ultrastructural study

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Abstract

In Sicily folk medicine, *Opuntia ficus indica* (L.) Mill. cladodes are used for the treatment of gastric ulcer. We studied the effect of administration of lyophilized cladodes on experimental ethanol-induced ulcer in rat. In this paper, we report the ultrastructural observations of gastric mucosa. The ultrastructural changes were observed by transmission electronic microscopy (TEM) confirming the protective effect exercised by administration of lyophilized cladodes. Pre-treatment test in rats revealed a protective action against ethanol-induced ulcer. Probably, the mucilage of *Opuntia ficus indica* is involved. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Ethanol-induced ulcer; Folk medicine; Gastric protection; *Opuntia ficus indica* (L.) Mill.; Transmission electronic microscopy

1. Introduction

*Opuntia ficus indica* (L.) Mill. is a plant indigenous to the dry region of the new world whose cultivation has been suggested for arid land because of its special adaptative mechanism and capacity to produce biomass. Moreover *O. ficus indica* fruits (prickly pears) are considered of great economic importance in Sicily.

Within the project, supported by Regione Siciliana, tending to exploit *O. ficus indica* raw material, we studied some biological activities (Trovato et al., 2000) of waste matter (unmarketable fruits and cladodes), which constitutes a problem for environmental pollution due to fermentation phenomena. In particular, we assayed diuretic, hypcholesterolemic, anti-inflammatory and cicatrizant activity (Galati et al., 2000; Galati et al., in press a, Galati et al., in press b, Mondello et al., 2000).

The cladodes of *O. ficus indica* are used in folk medicine in the treatment of gastric mucosal diseases (Meyer and Mc Laughlin, 1981; Barbera and Inglese, 1993). Therefore, the aim of the present work is to study the antiulcer activity of lyophilized cladodes of *O. ficus indica* on an experimental ethanol-induced ulcer in rat. In this paper, we also report the results of ultrastructural observations of the gastric mucosa of a rat treated with lyophilized cladodes.

2. Materials and methods

2.1. Plant material

*Opuntia ficus indica* lopping cladodes (modified stems in cacti) were obtained from a cultivation located in San Cono (CT, Sicily) in May 1999. The identity of the plant was confirmed by bibliographic data (Tutin et al., 1968; Pignatti, 1982). Voucher specimens are deposited at the Pharmaco-Biological Department at the University of Messina.

The fresh tissue, deprived from epidermis and glochides, was homogenized in a blender and lyophilized at once. The lyophilized fraction suspended in water (20 g/100 ml), was given to rats, by gavage, at a dose of 1 g/kg, in a volume of 1.5 ml/100 g.
Table 1
Ulcer scoring scale (Magistretti et al., 1988)

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>no lesion</td>
</tr>
<tr>
<td>1</td>
<td>1–3 small lesions (≥ 10 mm length)</td>
</tr>
<tr>
<td>2</td>
<td>1–3 large lesions (≥ 10 mm length)</td>
</tr>
<tr>
<td>3</td>
<td>1–3 thickened lesions</td>
</tr>
<tr>
<td>4</td>
<td>more than 3 small lesions</td>
</tr>
<tr>
<td>5</td>
<td>more than 3 large lesions</td>
</tr>
<tr>
<td>6</td>
<td>more than 3 thickened lesions</td>
</tr>
</tbody>
</table>

2.2. Animals

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

2.3. Ulcer induction

The rats were divided into four groups of six animals each and treated by gavage in the morning.

The first group of rats (control) received orally the ulcerogenic agent, EtOH 90% (Del Soldato et al., 1984), at a dose of 0.5 ml/rat.

The second group of rats received *O. ficus indica* lyophilized cladodes.

The third group received lyophilized cladodes. The ulcers were induced by administration of oral ethanol (0.5 ml) 1 h after lyophilized administration (preventive treatment).

The fourth group received the ulcerogenic agent orally (0.5 ml). After 15 min, the rats were administered *O. ficus indica* lyophilized cladodes (curative treatment).

One hour later, all the rats were anesthetized using ether anesthesia, and the stomachs were removed, opened along the great curvature and delicately washed with saline solution so as not to remove the mucus layer from the mucosa surface.

2.4. Macroscopic observations

For the macroscopic observations, the number, lengths and severity of ulcers were noted and scored on an arbitrary 0–6 point scale (Magistretti et al., 1988) (Table 1).

The ulcer index (U.I.) of each stomach was the sum of its scores.

The ulcer index was reported as arithmetic means ± S.E. The significance of differences between means was evaluated by Student’s *t*-test for unpaired data. *P* < 0.05, versus control, was taken as significant.

2.5. Ultrastructural observations

After macroscopic examination of the stomach, fragments were taken along the small curve, not in the lesion site, in order to study histological modifications.

The fragments were fixed in 2.5% glutaraldehyde and sodium 0.1 M cacodylate 0.1 M buffer for 2 h at 4°C. After washing in the same buffer and dehydration by gradual ethanol (30–100°), the samples were included in EPON 812.

The ultrathin sections, obtained by the ultramicrotom Ultratom V of the LKB, were stained with uranyl acetate and lead citrate, according to Reynolds (1963) and observed using a TEM Philips CM10.
Fig. 4. Ultrastructural observations of rat gastric mucosa sections observed by transmission electronic microscopy (TEM). *O. ficus indica* lyophilized cladodes-treated rats (a, b); ethanol-treated rats (c, d).

3. Results

3.1. Macroscopic observations

In the control rats that received only ethanol, intense and widespread gastric hyperemia and a thickened lesion were evident (Fig. 1), and the ulcer index (U.I. 8.24 ± 1.5) was calculated.

The pretreatment test, with lyophilized cladodes of *O. ficus indica*, revealed a protective action against ethanol-induced ulcer. The stomach showed an aspect close to normality. In fact, a significant reduction in gastric hyperemia in both number and severity of the lesion was observed (Fig. 2). The ulcer index significantly decreased to 1.48 ± 1.3 (*P* < 0.05) with respect to the control (U.I. 8.24 ± 1.5).

*O. ficus indica* cladodes produced a reduction in the ulcer index (U.I. 3.25 ± 2.0) (*P* < 0.05), also when it was administered as curative, after ethanol administration, but there was no time sufficient to heal the gastric mucosa (Fig. 3).

3.2. Ultrastructural observations

The ultrastructural changes of gastric mucosa were
observed by transmission electronic microscopy (TEM) to confirm the antiulcer effect exercised by administration of lyophilized cladodes.

The results revealed some ultrastructural differences between the groups of rats that were treated respectively with:

1. only *O. ficus indica* lyophilized cladodes;
2. only ethanol (EtOH 90%);
3. *O. ficus indica* lyophilized cladodes + EtOH (preventive treatment);
4. EtOH + *O. ficus indica* lyophilized cladodes (curative treatment).
Fig. 5. Ultrastructural observations of rat gastric mucosa sections observed by transmission electronic microscopy (TEM). Preventive *O. ficus indica* treatment (a, b, c); curative *O. ficus indica* treatment (d, e, f).

A. The mucosa of the rats treated only with *O. ficus indica* cladodes shows a normal morphology of the gastric mucosa. Ultrastructurally, it shows characteristic functional aspects of the normal mucosa: the parietal cells highlight a turnover of the canaliculi with an initial and final stage of secretion; the chief cells appear to be rich in zymogen granules, with typical fusion of membrane and endoplasmic reticulum normally represented (Fig. 4a and b).

B. In the rats treated only with the ulcerogenic agent, we observe numerous alterations in the different cytotypes, with an injury that can be defined as type III, according to the Lacy and Ito (1982) classification. The ultrastructural observation shows the degeneration of the superficial epithelium (Fig. 4c). Few cells seem in fact very lengthened and full of secretion granules, others are completely empty, and in a few cases, they dissolve in the organ lumen. Moreover, it is possible to observe a wide intra-cellular space that is characteristic in cases of exfoliation. The most evident morphologic data are from the parietal cells with dilated secretion canaliculi and microvilli of various lengths (Fig. 4d); even the junction complexes are reduced, and on the cytoplasmic membrane, many introflections are present.
In the chief cells, the distribution of secretion granules seems normal, and the reticulum is well developed, but we do not observe the typical omega figures present in the excretion phase.

C. The gastric mucosa of rats treated with lyophilized cladodes followed by the ulcerogenic agent (EtOH 90%) shows, in general, an aspect close to normality. The ultrastructural observation shows a tendency to normalization of different cytotypes, even if the intercellular relationship remains damaged. In the parietal cells, the nucleus at the center is surrounded by mitochondria with well-defined crests, and the secretion canaliculi are
Fig. 5. (Continued)

full of microvilli of varying lengths (Fig. 5a and b). The chief cells show granules of zymogen partially fused among themselves and with the membrane, where they form the omega figure that is characteristic of the normal cell physiology (Fig. 5c).

D. In the rats that underwent treatment with ulcerogenic agent (EtOH 90%) followed by lyophilized cladodes, ultrastructural observation of the gastric mucosa reveals degenerating cells in the superficial epithelium (Fig. 5d). In the gland, the chief cells are occupied by numerous secretion granules in the apex, surrounded by tubules and cisterns of the rough endoplasmic reticulum. These formations are even more evident in the basal portion, where the spherical nucleus is present (Fig. 5e).

The parietal cells have a central nucleus surrounded by numerous tightly packed mitochondria with numerous crests, and the canaliculi have numerous microvilli (Fig. 5f).

The intraglandular tunica is dilated because of the presence of fibroblasts and collagene fibres. Even the intercellular space is wide.
4. Discussion

From the results of this work, it is evident that acute administration of *O. ficus indica* lyophilized cladodes generally maintains the cytoarchitecture of the gastric mucosa in the normal arrangement of its components, although it is possible to observe an induction to exfoliation of the superficial epithelium near the glandular apex, which is closed normally.

Ethanol treatment induces a direct damage of gastric mucosal cells, probably by the development of free radicals and hyperoxidation of lipid (Puurunen et al., 1980; Pihan et al., 1987; Ito et al., 1993). Moreover, in the stomach, ethanol causes solubilization of mucus constituents and depresses tissue levels of proteins, leading to flow stasis in gastric blood (Szabo et al., 1986). The preventive treatment with *O. ficus indica* lyophilized cladodes blocks the ulcerative effect of ethanol.

It is possible to suppose an involvement of *O. ficus indica* mucilages in the observed effects. The mucilage may prevent penetration of the necrotizing agent into the gastric mucosa. Perhaps, it forms a protective layer and averts the deep necrotic lesions and the extensive exfoliation of surface epithelium induced by ethanol. Probably, the mucilage, a high MW acid polysaccharide (Trachtenberg and Mayer, 1981) mainly formed by arabinogalactan and galacturonic acid (Saag et al., 1975), can act synergetically with defense factors of gastric mucosa. The polymer present in *O. ficus indica* is probably a glycoprotein (Paulsen and Lund, 1979). This arabinogalactan protein has the potential to interact with macromolecules or small ligand of gastric mucosa (Clarke et al., 1979). *O. ficus indica* mucilage is a negatively charged polyelectrolyte, strongly viscous because of the negative charges causing strong intermolecular repulsion, resulting in expansion of the molecules. The viscosity is influenced by pH and Ca++ concentration (Trachtenberg and Mayer, 1982), and in the gastric lumen, the gelation properties change because of the changes in the conformation of the molecule. This effect on molecular shape and conformation could be related to protective activity on the gastric mucosa.

The results are different in preventive and curative treatment. In the curative treatment, epithelial cells of gastric mucosa look injured since, after administration of the ulcerogenous agent, acute treatment with lyophilized cladodes probably had no time to restore mucosal defensive factors.

In conclusion, the *O. ficus indica* lyophilized cladodes show a significant antiulcer activity in our experimental model. Our previous study indicates that lyophilized cladodes have a significant anti-inflammatory activity, too (Galati et al., 2000). New experiments are under way to correlate the two effects.

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References


