

Opuntia ficus indica (L.) Mill. mucilages show Cytoprotective Effect on Gastric Mucosa in Rat

E. M. Galati^{1*}, M. T. Monforte¹, N. Miceli¹, M. R. Mondello¹, M. F. Taviano¹, M. Galluzzo¹
and M. M. Tripodo²

¹Pharmaco-Biological Department, School of Pharmacy, University of Messina, Vill. SS. Annunziata, Messina, Italy

²Department of Organic and Biological Chemistry, University of Messina, Vill. S. Agata, Messina, Italy

***Opuntia ficus indica* cladodes possess a protective action against ethanol-induced ulcer in the rat. The major components of cladodes are carbohydrate polymers, mainly mucilages and pectin. To clarify the cytoprotective effects of cladodes on experimental ethanol-induced ulcer in rat, mucilages and pectin were extracted and were administered instead of cladodes. The above mentioned effects induced by cladodes may be attributed to mucilages, and not significantly to pectin. Copyright © 2007 John Wiley & Sons, Ltd.**

Keywords: mucilages; *Opuntia ficus indica* (L.) Mill.; pectin; gastric ulcer.

INTRODUCTION

In folk medicine *Opuntia ficus indica* cladodes are employed for their antiinflammatory, cicatrizant and antiulcer activity (Meyer and Mc Laughlin, 1981). Furthermore, the *nopalitos*, small cladodes, 10–15 cm wide, are utilized in South America also for food use. A first series of tests employed fresh cladodes collected in East Sicily (S. Cono, Catania) and homogenized. Their administration to hypercholesterolemic rats decreased cholesterol, LDL and triglyceride plasma levels (Galati *et al.*, 2003b). These effects might be related to an interference with lipid absorption, or a stimulation of the cholesterol catabolism, or an increase apolipoprotein B/E receptor expression. Moreover, *Opuntia ficus indica* cladodes significantly inhibited carragenan-induced oedema probably by affecting prostaglandins (Galati *et al.*, 2000) and showed a protective action against ethanol-induced ulcer and an increase in mucus production. The ultrastructural changes of gastric mucosa, observed by transmission electronic microscopy, confirmed the protective effect (Galati *et al.*, 2002).

The cicatrizant activity on injury produced on the back of the rats was studied. They were treated with a cream containing homogenized cladodes (15%) and macroscopical and microscopical controls effected. After treatment, the skin of wounded animals showed a healing process more marked than in the control rats (data unpublished). The cladodes may stimulate the remodelling and regeneration of keratinocytes (Galati *et al.*, 2003a).

Opuntia ficus indica cladodes contain many active principles including vitamins, flavonoids, β -sitosterol,

but they are particularly rich in fibrous compounds, that are mucilages and pectin (Burret *et al.*, 1982; Salt *et al.*, 1987; Lee *et al.*, 2002). Mucilages are complexes polysaccharides which consist of arabinose, galactose, rhamnose and galacturonic acid and in solution form large molecular aggregates (Cardenas *et al.*, 1997). Pectin is constituted particularly by galacturonic acid, present in different degrees of methylation.

The observed biological activity of cladodes may be related to the above mentioned compounds, and in fact, the fibres contained in vegetables play an important role in the prevention of many pathological conditions including constipation, haemorrhoids, colon-rectal cancer as well as several metabolic diseases such as obesity, diabetes and dyslipidaemias.

The aim of the present work was to clarify whether the cytoprotective effects of *O. ficus indica* cladodes exhibited in rats with ulcers (Galati *et al.*, 2001), may be attributed to mucilages or pectins or both. With this aim, a system was set up to extract them from cladodes. Mucilages and pectins were extracted and administered to rats instead of cladodes.

MATERIALS AND METHODS

Plant material. *Opuntia ficus indica* cladodes were harvested from San Cono cultivation (CT, Sicily).

The identity of the plant was confirmed by bibliographic data (Tutin *et al.*, 1968). Voucher specimens are deposited at the Pharmaco-Biological Department at the University of Messina (Number of voucher specimen UME DFB/13/04). Mucilages and pectin were extracted by means of the method described by Karawya *et al.* (1980) and modified to reduce the amount of reagents and the liquid effluents produced.

The cladodes, deprived of epidermis and glochides, were cut into wafers and mixed with water acidified

* Correspondence to: Professor E. M. Galati Pharmaco-Biological Department, School of Pharmacy, University of Messina, Vill. SS. Annunziata, Messina, Italy.
E-mail: emgalati@unime.it

with HCl (pH 3.5) at room temperature; the wafers were pressed against a stainless steel strut and plunged again into acidified water. This treatment, which was repeated twice, was employed to facilitate the passage of acidified water which had dissolved the mucilages through the vegetable tissues of cladodes. The final liquid obtained was concentrated, reducing its volume to 33%, and treated with 4 volumes of ethanol (95%), to precipitate mucilages, which were recovered by filtration and dried.

The solid wet marc obtained after the third treatment, was utilized to extract pectin. At this aim it was treated twice with HCl 0.05 N at 90 °C for 2 h. The liquids obtained by filtration were blended and the pectin precipitated by the addition of 1.5 volumes of 95% ethanol acidified at pH 2 and then separated by filtration under vacuum with paper filters. Finally, they were dried in a vacuum desiccator at 40 °C. The yields of both mucilages and pectin were about 9–10% of the dry weight of the cladodes.

Animals. The biological activities of mucilages and pectin were tested on male Wistar rats weighing 180–200 g, kept in controlled conditions (temperature 22 ± 2 °C; humidity $60 \pm 4\%$, in a 12 h light/dark cycle), fed on a standard diet (S. Morini Mill rat GLP) and water *ad libitum*.

Ulcer induction. The animals were divided at random into four groups of six animals each and treated by gavage in the morning. Group I (control) received the vehicle, water 1.5 mL/100 g (b.w.); group II received the pectin at a dose of 150 mg/kg, suspended in water; group III received the mucilages, at a dose of 150 mg/kg, suspended in water; group IV received the standard reference drug, sucralfate (Bioprogress S.p.A., Roma) at a dose of 100 mg/kg.

Ulceration was induced 1 h after drug administration in all rats, by intragastric instillation of 0.5 mL of 90% EtOH (Del Soldato *et al.*, 1984).

One hour after ethanol administration, all the animals were killed under ether anaesthesia. The stomachs were removed, opened along the great curvature and washed with saline solution.

Macroscopic observations. The ulcers present in each stomach and the general conditions of the gastric mucosa were observed. 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).

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

The UI was reported as the arithmetic mean \pm SE. The significance of differences between means was evaluated by Student's *t*-test for unpaired data. A value of $p < 0.05$ versus control, was taken as significant.

Microscopic observations. After the macroscopic observations, the stomachs were extended on a cork surface to avoid deformities. 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 mm), obtained by a rotative microtome, were stained with periodic acid-Schiff (McManus, 1946) which reacts with mucopolysaccharides. These compounds constitute the gastric mucus and produce a characteristic carmine colour. All samples were evaluated by light microscopy (BH₂ Olympus).

RESULTS

Microscopic and macroscopic observations

The control rats, treated with vehicle and ethanol, showed an intense and widespread gastric hyperemia. Thickened and filiform lesions were evident and the ulcer index was 9.36 ± 1.3 (UI).

Group II treated with pectins (150 mg/kg) showed an insignificant reduction of ulcer index with respect to the control (UI 7.43 ± 1.2).

Both group III treated with mucilages (150 mg/kg) and group IV treated with sucralfate (100 mg/kg) revealed a protective action against ethanol-induced ulcer. A reduction of the number and the severity of the lesions was observed and the ulcer index significantly decreased respectively to 2.04 ± 1.5 ($p < 0.05$) and 1.23 ± 1.2 ($p < 0.05$) versus the control. The microscopy observation confirm the *restituito ad integrum* of the mucosa elements: glandular fundus was tubular, interglandular spaces were reduced and there were some fibroblasts (Figs 1, 2).

DISCUSSION AND CONCLUSION

The results showed that the treatment with pectin did not reduce significantly the damage induced by the ulcerogenic agent. Instead, the treatment with mucilages brought about a significant gastroprotective effect: actually the mucilages decreased the gastric hyperemia and the number and the severity of the lesions; the gastric mucosa appeared whole and the superficial

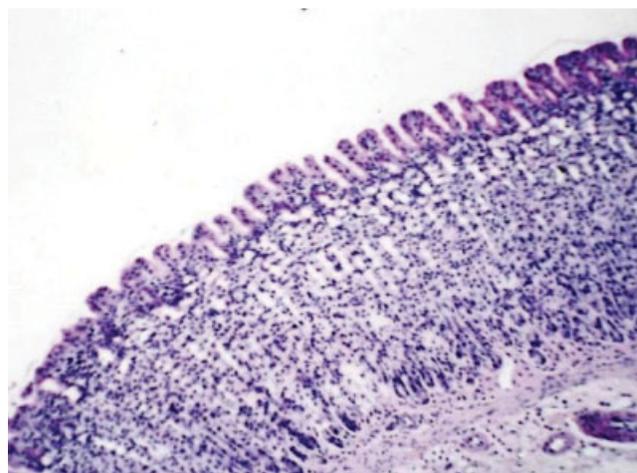


Figure 1. Light microscopy observations of rat mucosa gastric section. Control group: a thin layer of mucus in the surface epithelium is visible; the interglandular spaces are dilated (10 \times).

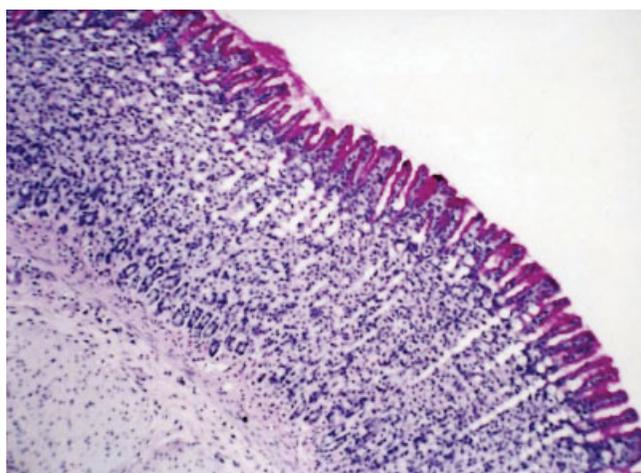


Figure 2. Light microscopy observations of rat gastric mucosa section. *O. ficus indica* mucilages-treated rats: glandular pits and surface epithelium show a increased secretion of mucus; the interglandular spaces are reduced; in the 'tunica propria' numerous fibroblasts are present (10 \times).

epithelium was continuous and covered by mucus, as in rats that received sucralfate. The results are very similar to those obtained after lyophilized cladodes administration (Galati *et al.*, 2001; Galati *et al.*, 2002).

From the above description, it appears that the preventive antiulcer activity of cladodes is due essentially to the mucilages, whose administration blocks the effects of ethanol and gives rise to a cytoprotective response, by preventing the mucus dissolution induced by ethanol and by decreasing the folding of the mucosa.

Further assays are necessary to optimize the above described extraction process and eventually to design a pilot-plant for extracting both mucilages and pectin. The first should be utilized for pharmaceutical use, the second as a jelly agent, as is the pectin extracted from apples and lemon peels. Furthermore the solid wet marc obtained after extraction of mucilages and pectin, could be dried and utilized as feedstuff for ruminants.

REFERENCES

- Burret F, Lebreton PH, Voirin B. 1982. Les aglycones flavonique de cactees: distribution, signification. *J Nat Prod* **45**: 687–693.
- Cardenas A, Higuera-Ciapara I, Gooicoolea FM. 1997. Rheology and aggregation of Cactus (*Opuntia ficus indica*) mucilage in solution. *J PACD* **2**: 152–159.
- Del Soldato P, Foschi D, Varin L, Daniotti S. 1984. Comparison of the gastric cytoprotective properties of atropine, ranitidine and PGE2 in rats. *Eur J Pharmacol* **106**: 53–58.
- Galati EM, Mondello MR, Monforte MT, Galluzzo M, Miceli N, Tripodo MM. 2003a. Effect of *Opuntia ficus indica* (L.) Mill. cladodes in the wound healing process. *J PACD* **5**: 1–16. Available in: <http://www.jpacd.org>
- Galati EM, Monforte MT, Tripodo MM, d'Aquino A, Mondello MR. 2001. Antiulcer activity of *Opuntia ficus indica* (L.) Mill. (Cactaceae): ultrastructural study. *J Ethnopharmacol* **76**: 1–9.
- Galati EM, Monforte MT, Tripodo MM, Trovato A. 2000. Antiinflammatory activity of *Opuntia ficus indica* (L.) Mill. cladodes. *X Congresso SIPHAR, Assisi (Italy)*, Abstracts, 13.
- Galati EM, Pergolizzi S, Miceli N, Monforte MT, Tripodo MM. 2002. Study on the increment of the production of gastric mucus in rats treated with *Opuntia ficus indica* (L.) Mill. cladodes. *J Ethnopharmacol* **83**: 229–233.
- Galati EM, Tripodo MM, Trovato A, d'Aquino A, Monforte MT. 2003b. Biological activity of *Opuntia ficus indica* (L.) Mill. (Cactaceae) note II: effect on experimental hypercholesterolemia in rats. *Pharm Biol* **41**: 175–179.
- Karawya MS, Wassel GM, Baghdadi HH, Ammar NM. 1980. Mucilages and pectins of *Opuntia*, *Tamarindus* and *Cydonia*. *Planta Med* (suppl.) 68–75.
- Lee JC, Kim HR, Jang YS. 2002. Antioxidant properties of an ethanol extract of the stem of *Opuntia ficus indica* var. *saboten*. *J Agric Food Chem* **50**: 6490–6496.
- Magistretti MJ, Conti M, Cristoni A. 1988. Antiulcer activity of an anthocyanidin from *Vaccinium myrtillus*. *Arzneimittelforsch* **38**: 686–690.
- McManus JFA. 1946. Histological demonstration of mucin after periodic acid. *Nature* **158**: 202.
- Meyer BN, Mc Laughlin JL. 1981. Economic use of *Opuntia*. *Cactus Succulent J (US)* **53**: 107–112.
- Salt TA, Tocker JA, Adler JH. 1987. Dominance of Δ^5 -sterols in eight species of the Cactaceae. *Phytochemistry* **26**: 731–733.
- Tutin TG, Heywood VH, Burges NA *et al.* 1968. *Flora Europea*, vol II. Cambridge University Press: London, 299–300.