

Study Antiulcer and Possible Gastroprotective Mechanisms of Musa Pulp ABB spp

María de los Ángeles Boffill Cárdenas^{1*}

María José Martín Calero²

Arianna Valido Díaz¹

Carmen Garrido Arce³

¹Universidad de Ciencias Médicas de Villa Clara, Unidad de Toxicología Experimental. Santa Clara, Cuba.

²Universidad de Sevilla, Facultad de Farmacia. Sevilla, España.

³Hospital Universitario "Arnaldo Milián Castro". Santa Clara, Cuba.

*Author for correspondence. E-mail: mariaabc@infomed.sld.cu

ABSTRACT

The objective of this work was to evaluate in a model of chronic ulcer the antiulcer effect and the possible mechanisms by which this fruit produces gastroprotection. The pulp of the green fruit was sliced, dried at 50 °C, ground and sieved. Ninety Male Wistar rats of 190 ± 10 g were used. The antiulcer effect of pulp was performed with the chronic model of ulcer by acetic acid, used 5 groups of 8 animal, the suspension of the pulp at 125; 250; 500 mg / kg BW were applied 72 hours after inducing the ulcer for 7 days. For the determination of the mechanisms, also 5 groups of 10 animal were used, the banana pulp was supplied in a dose of 125, 250 and 500 mg / kg of BW, for three days, before inducing the ulcers with indomethacin, the activity of the myeloperoxidase, the superoxide dismutase and the levels of prostaglandins in the gastric mucosa were determined. Treatment with the suspensions of the pulp in the chronic ulcer model reduced in a very highly significant way the damaged area in all the treated groups and this reduction was greater than that produced by ranitidine; there was a significant decrease in myeloperoxidase only with the highest dose tested and a significant increase of superoxide dismutase and prostaglandins level with all the doses used. We conclude that the pulp preparations provoked an intense anti-ulcer. The mechanism of gastroprotective action was mediated by an antioxidant effect and mucosal protection.

Keywords: Chronic ulcer; gastroprotective mechanism; myeloperoxidase activity; superoxide dismutase activity; prostaglandins.

INTRODUCTION

Gastric ulcers occur when there is an imbalance between the aggressive and defensive factors on the luminal surface of the epithelial cells. A large number of medicinal plants and their secondary metabolites have been reported, with potential activity against gastric ulcer and ulcerative colitis, with which satisfactory results have been obtained. In previous studies we have demonstrated experimentally that the green fruit of the *Musa* spp ABB, variety Burro CEMSA, is effective as a gastroprotective agent in a model of induction of acute ulcers by absolute alcohol and indomethacin.⁽¹⁾ The objective of this work was to evaluate in a model of chronic ulcer the antiulcer effect and the possible mechanisms by which the pulp of this fruit produces gastroprotection in a model of acute ulcer.

METHODS

The study was carried out in conventional male young Wistar rats, with a weight comprised between 190 ± 10 g, from CENPALAB. A quarantine period was established that lasted for 7 days keeping the animals in environmental conditions of 22 ± 2 °C of temperature, 40 - 70% of humidity and light-dark cycle of 12 x 12 hours. They were fed commercial ratonine and the drinking water was suitable for consumption, at free demand. At the end of the quarantine the animals were marked by tattoo on the ear, and then they were weighed and deposited in T-4 Techniplast grid bottom boxes, the experimental groups was conforming using a table of random numbers.

The pulp of the green banana was sliced, dried at 50 °C for 72 hours, ground and sieved. The antiulcer effect of pulp suspensions was performed with the model of chronic ulcer by acetic acid. To perform this technique, the animals were anesthetized with ketalar, an incision was made in the abdomen and the stomach was carefully exteriorized, injected with 0.05 mL of 20% acetic acid solution in the subserosa, after closing the abdomen the rats were maintained with antibiotic treatment for 48 hours. Five experimental groups of 8 rats each were used; group 1 negative control, group 2 positive control (ranitidine 50 mg / kg BW, groups 3, 4 and 5 that was given the suspension of the pulp at 125; 250; 500 mg / kg BW. The substances to be tested were applied 72 hours after inducing the ulcer by a period of 7 days. At the end of the administration period, the

animals were sacrificed and the damaged area was measured. Lesions are also evaluated by histopathological techniques to determine active ulcers.

For the determination of the gastroprotective mechanisms, the study was carried out in conventional Wistar rats, males, young, with a weight comprised between 190 and 200 g, from the Vivarium of the Seville University; the animals were kept under ambient conditions of 22 ± 2 °C of temperature, 40-70% of humidity and light-dark cycles of 12 x 12 hours. They were fed commercial rat chow and the drinking water was suitable for consumption, at free demand. The animals were marked by tattoo on the ear, later they were weighed and deposited in boxes T-4 with bottom of grids; the experimental groups were formed using a table of random numbers. Five experimental groups of 10 animals each were formed; group 1 the negative control, group 2 the positive control (omeprazol 20 mg/Kg BW), groups 3, 4, and 5 to which the banana pulp was supplied in a dose of 125, 250 and 500 mg / kg BW for three days, by intubation with an intragastric cannula, before inducing the ulcers with indomethacin: at 40 mg / kg BW. The food was removed 15 hours before the induction of the ulcers, and the ulcerogenic agent was administered 24 hours after the last administration of the test substances.

The animals were sacrificed five hours after the induction of the ulcers; the stomachs, which were opened by the greater curvature, were extracted, washed with saline solution, spread on a sheet of filter paper, and the damaged area was quickly measured in mm². The gastric mucosa was taken from all the animals for the determination of the enzymatic activities and prostaglandins levels. The determination of myeloperoxidase activity (MPO), was by the technique proposed by Grisham MB et al, 1994, the determination of the superoxide dismutase (SOD) by the method described by McCord and Fridovich, 1969 and the determination of the prostaglandins E₂ (PGE₂) levels in the gastric mucosa was assessed by an enzyme-linked immunosorbent assay (ELISA), following the techniques described in the Experimental Techniques Manual used in the preclinical study of drugs with gastrointestinal activity.⁽²⁾

For the evaluation of the results, the statistical package SPSS was used. The mean for each group and the Mann-Whitney U were calculated to establish if the differences between the treated groups and the control were statistically significant. The comparison between several groups was made by the multidimensional Kruskal-Wallis test.

RESULTS

The table 1 shows the effect of the treatment with the suspensions of the pulp in the chronic ulcer model, this treatment reduced in a very highly significant way the damaged area in all the treated groups and this reduction was greater than that produced by ranitidine.

Table.1 Ulceration grade in Chronic Ulcer and Histopathologic

| GROUPS | ULCERATION GRADE (mm ²) | Histopathologic Result | | | | |
|-------------------------|-------------------------------------|------------------------|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| Group1 Negative Control | 27,125 ± 8,37 | 5 | 3 | - | - | - |
| Group 2 (Ranitidine) | 9,625 ± 7,89 (c) | 1 | 3 | 4 | 4 | - |
| Group 3 Pulp 125 mg/kg | 4,48 ± 8,44 (c) | 1 | 2 | 3 | 3 | |
| Group 4 Pulp 250 mg/kg | 0,348 ± 0,44(c) | - | - | 7 | 7 | 1 |
| Group 5 Pulp 500 mg/kg | 0,163 ± 0,35 (c) | - | - | 5 | 5 | 3 |

1. Active ulcer; 2. Chronic inflammatory infiltrated of mucosa; 3 Superficial gastric erosion; 4. Intense epithelial regeneration; 5. Normal mucosa. (c) p< 0,001 in relation with control

The histopathological result showed that the animals of groups 4 and 5 had more superficial gastric erosion, and intense epithelial regeneration in the ulcer zone that de negative control and the group to which the banana pulp was supplied in a dose of 125 mg /Kg BW. Moreover in the groups 3 and 4 appear Intense epithelial regeneration and in some animal normal mucosa in the ulcer zone.

The tale 2, show the effects of the banana pulp on the damage area, the enzymatic activity and the prostaglandin level in the gastric mucosa of rat.

a) p< 0,05 in relation with the negative control; b) p< 0,01 in relation with negative control; c) p< 0,001 in relation with negative control, NS not signification.

A highly significant decrease in lesion intensity was obtained with the use of all preparations of the banana pulp in the indomethacin ulcer induction model; there was a significant decrease in myeloperoxidase only with the highest dose tested and a significant increase of superoxide dismutase and prostaglandins level in the gastric mucosa with all the doses used.

We can see that only the group that received the dose of the pulp at 500 mg / kg BW was able to decrease the activity of the MPO, The superoxide dismutase SOD was significantly increased at the doses of 250 and 500 mg / kg BW and that all the doses of the suspensions of the Musa ABB pulp were able to increase the PGE 2 content of the gastric mucosa, the doses of 125 and 250 mg / Kg BW significantly and the 500 mg / Kg BW very highly significant in relation to the negative control. The group that was induced ulcers with indomethacin 40 mg / Kg BW and that did not receive treatment showed a very highly significant decrease in relation to the positive control group and all groups that were administrated the pulp of the green banana.

Table 2. Damage area, Enzymatic Activity and PGE₂ Level in the Gastric Mucosa

| GROUPS | Ulcer induction | ENZIMÁTIC ACTIVITY | | PGE ₂ Level |
|-----------------------------|-----------------------------|-------------------------------|------------------------------------|------------------------|
| | Damage area mm ² | Myeloperoxidase U / mg mucosa | Superoxide dismutase U / mg mucosa | pg / mg mucosa |
| Group 1 Negative Control | 35,50 ± 5,49 | 100 ± 12,87 | 8,35 ± 0,37 | 4,58 ± 2,24 |
| Group 2 Positive Control | 2,60 ± 1,17 | 88,35 ± 14,14 NS | 8.31 ± 0.06 NS | 14,8 ± 1,57 (c) |
| Group 3 Pulp 125 mg/kg | 19,79 ± 7,53 (b) | 95,44 ± 5,93 NS | 8,94 ± 0,18 (a) | 10,02 ± 1,48 (b) |
| Group 4 Pulp 250 mg/kg | 15,25 ± 5,44 (b) | 94,25 ± 8,45 NS | 9,19 ± 0,11 (a) | 10,12 ± 2,25 (b) |
| Group 5 Pulp 500 mg/kg | 6,42 ± 2,90 (b) | 83,61 ± 7,75 (a) | 9,75 ± 0,09 (b) | 11,54 ± 1,78 (c) |

a) $p < 0,05$ in relation with the negative control; b) $p < 0,01$ in relation with negative control; c) $p < 0,001$ in relation with negative control, NS not signification

DISCUSSION

The study of the effects of new drugs on the appearance of ulcers is carried out with laboratory animals and through the use of different experimental models of ulcer induction.⁽³⁾ The effectiveness of the suspension of the Musa ABB pulp as antiulcer agent was proved in this work using the chronic ulcer model, which is the experimental model highly resemble human ulcers in terms of both pathological features and healing mechanisms.⁽⁴⁾

The treatment with the suspensions of the pulp reduced the damaged area in all the treated groups and this reduction was greater than that produced by ranitidine, speaking this in favor of the effectiveness of these preparations as antiulcer agents. It should be noted that the histopathological results showed that only in the groups that were treated with the highest doses of the Musa ABB there were intense epithelial regeneration and normal mucosa and some animals of these groups were totally cured, evidencing this great regenerative and healing effect of these preparations. Then we can state that these suspensions not only have gastroprotective effects but also are substances capable of reducing or eliminating ulcerative lesions more effectively than some of the synthetic drugs used for this purpose. These results coincide with

what has been reported regarding the healing effect on chronic ulcers induced with acetic acid with another variety of Musa.⁽⁵⁾

The use of indomethacin as a gastric ulcer inducer of acute ulcer is very frequent, since it inhibits the synthesis of prostaglandins and, in addition, produces free radicals, which are the critical biochemical processes in the pathogenesis of gastric ulceration.⁽⁶⁾

The most relevant finding of the phytochemical screening of the green fruit of the Musa ABB variety Burro CEMSA was the predominance of polyphenols and alkaloids,¹ so that the gastroprotective effect of the suspensions of this fruit could well be attributed to the antioxidant actions of these chemical compounds.

In this work we explored several mechanisms of the gastroprotective action that presented the preparations of the pulp of the green banana, for which the decrease of the activity of the MPO was evaluated as an indicator of the anti-inflammatory effect, the increase of the activity of the SOD as an indicator of the antioxidant activity and the increase content of PGE₂ as an indicator of the production of protective mucus in the gastric mucosa.

The effect of the preparations of the pulp of the green banana fruit on the MPO of the gastric mucosa only showed an evident effect of its decrease with the highest dose, so that the anti-inflammatory effect does not explain the gastroprotective action in the groups to which it is applied the lowest doses.

The increased of superoxide dismutase SOD when preparations of the Musa ABB pulps were administrated at the doses of 250 and 500 mg / kg BW could protect the mucosa against free radicals, as it was capable of kidnapping the RLOs that appear in the processes that occur with oxidative stress,⁽⁷⁾ free radicals and lipid peroxidation have been implicated in the pathogenesis of gastric mucosal lesions.⁽⁸⁾

The protective activity of prostaglandins -such as PGE₁, PGE₂, PGF₂ -, is demonstrated, since they act by inducing the formation of mucus and the production of dipalmitoyl phosphatidylcholine, which increases the hydrophobicity and thickness of the gel layer,⁽⁹⁾ It is noteworthy that endogenous prostaglandins act by activating K channels (ATP) and this mechanism partially mediates gastroprotection.⁽¹⁰⁾

CONCLUSIONS

Treatment with the suspensions of green Musa ABB pulp in the chronic ulcer model provoked an intense anti-ulcer effect, reduced the damaged area in all the treated groups and this reduction was greater than produced by ranitidine, The groups treatment with doses higher there were an intense epithelial regeneration and some animal of this groups had normal mucosa. All the suspension

from the pulp had a strong gastroprotective action in the acute ulcer model with indomethacin. The mechanism of action was mediated by an antioxidant effect and mucosal protection by increasing prostaglandin levels.

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