Anti-ulcer Activity of Spray-dried Powders prepared from Leaf Extracts of Maytenus ilicifolia Martius ex Reiss

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SUMMARY. Anti-ulcerative activity of Maytenus ilicifolia spray-dried powders was tested per os in male Wistar rats, using indomethacin as ulcerogenic and cimetidine as control substances. An aqueous extract was prepared by infusion, using a plant:solvent ratio of 1:10 (w/v). The dry residue was 2.40% (w/w). Spray-dried extracts were prepared either without adjuvant addition (SDP1) or using 20 parts of colloidal silicon dioxide to each 80 parts of dry residue (SDP2). For comparison purposes a freeze-dried extract (FDE) was prepared additionally. The total tannin content was determined using a Folin-Denis modified technique. The SDP2 and FDE showed the best anti-ulcer activity, with about 77% lesion area decrease, while the SDP1 activity was not statistically significant (p = 0.05).

RESUMEN. “Actividad anti-ulcera de extractos secos nebulizados preparados a partir de extractos acuosos de hojas de Maytenus ilicifolia Martius ex Reiss”. La actividad anti-ulcera de extractos secos nebulizados de M. ilicifolia fue ensayada por vía oral en ratas machos Wistar. La indometacina fue usada como sustancia ulcerogénica y la cimetidina como sustancia control. Los extractos acuosos fueron preparados por infusión, manteniéndose una proporción planta seca : líquido extractivo de 1:10 (p/V). De este extracto, con un residuo seco igual al 2.40% (p/p), fueron preparados dos extractos secos por nebulización y un tercero por liofilización. El primer extracto seco nebulizado (ESN1) fue obtenido sin uso de adyuvantes del proceso. El segundo extracto nebulizado (ESN2) fue obtenido utilizando 20 partes de dióxido de silicio coloidal para cada 80 partes de residuo seco. El extracto liofilizado (ESL) fue preparado a través de la técnica clásica y sirvió para fines de comparación entre los extractos. El contenido de taninos totales fue calculado de acuerdo con el método de Folin-Denis, con algunas modificaciones. Los extractos ESN2 y ESL demostraron una buena actividad contra la-ulcera, ocurriendo una disminución del 77% de la superficie lesionada. De modo opuesto, con el extracto ESN1 no fue detectada una actividad que fuera estadísticamente significativa (p = 0.05). Se puede concluir que, si bien por un lado el uso del dióxido de silicio coloidal preserva la actividad anti-ulcera, por el otro enmascara parcialmente los resultados, ya que el mismo también posee una actividad protectora.

INTRODUCTION

Maytenus ilicifolia Martius ex Reiss (Celastraceae) is a medicinal shrub native of South America. In Brazil is popularly known as espinheira-santa, cancorosa and espinheira-divina, where the leaves are mainly used for the treatment of several gastrointestinal diseases, such as hyperacidity, stomach ulcer, functional dyspepsia, hyperchlorhydric gastralgias and other non specific gastrointestinal illnesses 1,2. According to several authors, the anti-ulcer activity is attributable to the condensed tannins, which belongs to the catechin group and are considered as the main aqueous and hydroethanolic leaf extracts constituents 3-5. The anti-ulcer activity was confirmed by pre-clinical assay in rats, using freeze-dried leaf extracts and doses ranging from 170 to 340 mg/kg, p.o. and i.p. The activity was equivalent to that obtained with a cimetidine dose range of 50 to 100 mg/kg. Similarly, the infusion of the leaves showed an inhibitory activity on the gastric secretion and acidity 4,5, as well as a significant reduction of the dyspepsia symptoms in human
In isolated stomach mucous membrane the leaf aqueous extract showed also an inhibitory effect on the gastric secretion induced by histamine. Studies on the adverse effects induced by chronic administration and exceptionally high doses (5.4 and 10.8 g/kg) of infusion and freeze-dried powders showed no toxic effects neither in laboratory animals nor in human beings.

Spray drying is one of the most important industrial processes for the preparation of vegetable dried extracts, in which the risks of thermal denaturation or pharmacological inactivation of labile vegetable active substances are particularly overcome. Further TLC studies on Maytenus ilicifolia spray-dried powders revealed that the tannins fingerprints of the aqueous extracts remained unmodified after spray drying. However, the conservation of the anti-ulcer activity was not surely confirmed.

In the present work, the influence of both the spray drying process and the presence of the adjuvant colloidal silicon dioxide (Aerosil® 200) on the anti-ulcer activity were studied in rats, after oral administration of M. ilicifolia spray-dried and freeze-dried powders.

MATERIAL AND METHODS

Aqueous extract preparation

Maytenus ilicifolia leaves were ground and extracted by infusion of 100 g of dried leaves with 1000 mL of water, during 15 min. The aqueous extract (AE) was filtered at room temperature and stored protected, all the time, from daylight.

Spray-dried powders preparation

Two spray-dried powders were prepared from the AE using a Büchi 190 Mini-Spray Dryer provided with a pneumatic nozzle (0.7 mm φ i.d.) and functioning by co-current flow. The operational conditions were: inlet temperature 140 °C, outlet temperature 102 °C; 4 mL/min feed rate and 2 bar spraying pressure. The first spray-dried powder was prepared directly without addition of any drying adjuvant (SDP1). The other one was prepared using a mixture of 20 parts (w/w) of colloidal silicon dioxide (Aerosil® 200 - Degussa) to each 80 parts of dry residue (SDP2). The spray-dried powders were preserved in tight, light-resistant containers and stored in a glass desiccator over silica.

Freeze-dried extract preparation (FDE)

About 1000 g of AE was freeze-dried, using an Edwards Module 4K, under the following conditions: process time of 72 h; pressure of 1 kPa; freezing temperature - 60 °C.

Loss on drying

Spray-dried powders: 500 mg samples were accurately weighted and assayed according to the German Pharmacopoeia.

Tannin chromatographic assay

TLC Aluminum sheets coated with silica gel GF254, 0.1 mm thickness (SIL G/UV-Whatman) and a mobile phase consisting of ethyl acetate:formic acid:water (95:5:5, v/v) mixture were used. The distance from the starting line was 15 cm and the analysis was performed under saturated chamber conditions. The tannin detection was carried out using a vanillin hydrochloric solution 1% (w/v), followed by heating at 110 °C during 5 min., and anisaldehydesulphuric acid solution. The spots were observed under long-wave UV light and in daylight. (+)-Catechin (Sigma) and (-)-epicatechin (Sigma) were used as reference substances.

Total tannins content assay

The total tannins content was calculated as directed under the Krameria argentea roots monograph of the German Pharmacopoeia. The method was modified and, afterwards, validated.

Analysis solutions preparation

A 0.750 g leaves sample was extracted by decoction in 100.0 mL of water. The extractive solution volume was completed up to 250.0 mL with water and filtered (AS1). Spray-dried powders and freeze-dried powders 100.0 mg samples, were dissolved in 100.0 mL of water and filtered. The resulting solutions were coded as AS2, AS3 and AS4, respectively.

Total polyphenols content determination (TP)

The analysis solutions were obtained by dilution of 5.0 mL of the respective AS2, AS3 and AS4 up to 25.0 mL. Two milliliters aliquots were mixed with 2.0 mL Folin-Denis reagent and 16.0 mL of 20% (w/v) sodium carbonate solution. The absorbances were recorded exactly 2 min after the addition of the sodium carbonate solution, at 750 nm (Unicam 8625 UV/VIS spectrophotometer), using water as a blank.
Non-tanning polyphenol (NTP) content determination
An amount of 0.150 g of casein (Sigma) was stirred with 10.0 mL of the respective analysis solution during one hour. After filtration, the assay proceeds as directed under total polyphenols content determination and the non-tanning polyphenol calculated using the equation 2.

Total tannins (TT) contents determination
It corresponds to the difference between the total polyphenols (TP) and non-tanning polyphenol (NTP) contents expressed as gram of pyrogallol per100 g of sample (Equation 3).

\[
TP = \frac{A_1 \text{DF}}{(m-p) A_{1\%}}
\]

\[
NTP = \frac{A_2 \text{DF}}{(m-p) A_{1\%}}
\]

\[
TT = TP - NTP
\]

\[m = \text{mass of the drug (g); } p = \text{loss on drying calculated mass (g); } A_1, A_2 = \text{solutions absorbance (A.U.); } \text{DF} = \text{dilution factor; } TP = \text{total polyphenols content (g%); } TT = \text{total tannins content (g%); } NTP = \text{non-tanning polyphenol content (g%); } A_{1\%} = \text{pirogallol specific absorbance} = 1714.\]

Anti-ulcer Activity
Gastro-intestinal tolerance was assessed in male Wistar rats (200-250 g, Biotério Central UFRGS, Porto Alegre, Brazil). The groups (n = 9) were kept in separate cages at a temperature of 20 ± 1 °C, in 12/12 hours light/darkness cycles and the rats were allowed to drink ad libitum.

Positive control group
After a 24 h fasting period, the rats received per os 50.0 mg/kg of cimetidine. After 30 min, indomethacin (20.0 mg/kg) was injected subcutaneously. Then, after 3 h, the negative group received per os water (5 mL).

Negative control group
After a 24 h and 30 min fasting period, the rats received per os water. After 30 min, indomethacin (20.0 mg/kg) was injected subcutaneously. Then, after 3 h, the negative group received per os water (5 mL).

Treated groups
After a 24 h fasting period, the rats received per os 50.0 mg/kg of cimetidine. After 30 min, indomethacin (20.0 mg/kg) was injected subcutaneously. Then, after 3 h, the different groups were treated per os with 300.0 mg/kg of SDP1, 375.0 mg/kg of SDP2, 300.0 mg/kg of FDE and 75.0 mg/kg of Aerosil 200®.

Six hours from the beginning of the experiment, the rats of all groups were sacrificed and the whole stomach removed from their body. In order to quantify gastric lesions, the stomachs were opened along the greater curvature and stretched out on a flat polystyrene surface. The lesions were observed with an entomological stereoscope (Bausch & Lomb) provided with graduated objective and the lesions area were measured. Since the ulcerous lesion induced by indomethacin occurred as disseminated areas and they overlapped, the lesion number criterion could not be applied. The experimental data were expressed in mm² of average area and statistically analyzed according to the Mann-Whitney test.

RESULTS AND DISCUSSION
The Maytenus ilicifolia leaves loss on drying was 8.54 ± 0.09% (w/w), the total polyphenols content of 8.72 ± 0.23 g%, the non-tanning polyphenol content was 5.61 ± 0.11 g% and the total tannin content was 3.11 g%.

The addition of 20% (w/w) colloidal silicon dioxide, a quantity which was calculated in relation to the extract dry residue, led to a substantial increase of the process yield, confirming previous results. The SDP1 and SPD2 loss on drying assay results could be considered as acceptable, when the spray dryer dimensions are considered (Table 1). Because of its high hygroscopicity the moisture residue of the freeze-dried extract was rather as it is normally expected (ca. 2.07%).

The presence of condensed tannins in M. ilicifolia leaves as well as in all dried products was confirmed throughout TLC-analysis. The chromatogram comparison revealed no detectable chromatographic differences between the spray-dried powders (SDP1, SDP2) and the freeze-dried extract (FDE), a fact which disagreed with the pharmacological results.

Spray-dried powders anti-ulcer activity
The ulcers were mostly located on the stomach great curvature, which is characteristic of some ulcer-causing agents as indomethacin.
The peritoneal fluid and the stomach of the animals treated only with indomethacin presented an anomalous odor and brownish yellow coloration. The gastric mucous membrane presented gastric perforation, edema and localized hemorrhage. Such appearance was not otherwise observed in the groups treated with cimetidine and dried preparations, in which a lesions area decrease could be clearly observed. In addition to those observations the gastric membrane showed no alterations, having a normal appearance and small mucus amounts. For SDP1, SDP2 and FDE, a yellowish mucus accumulation on the gastric mucous membrane agreed with further studies using freeze-dried extracts prepared from M. ilicifolia leaf aqueous infusion 4. It was supposed that these mucus accumulation is resultant from a possible reaction between tannins and mucopolysaccharides 21. The FDE presented a protection level of 73.5%, which was comparable to the literature data 4.

All of the groups FDE, SDP2, A (Aerosil® 200) and C+ (cimetidine) showed a significant difference of the estimated lesion area in comparison with the indomethacin group (C-) (p <0.05). The SDP1 group showed the weakest protection degree (40.1%) in relation to the other ones, while the FDE showed the highest anti-ulcer activity of all dried preparations (p <0.1). No animal of control group C(W) presented lesions (Figure 1).

The small differences between the total tannin contents of SDP2 (0.0206 mg) and FDE (0.0245 mg) extracts can be due to the different drying techniques used and the presence of Aerosil® 200 in SDP2. However, both preparations presented a similar gastric protective activity, namely, 76.7% for SDP2 and 73.5% for FDE. The SDP1 anti-ulcer activity was clearly different from the SDP2 (p <0.01); its lower activity is probably associated to a tannin denaturation, which is caused by the exposition of it to a high temperature drying process, even if it happens for a very short length time. In fact, the SDP1 total tannin content, which was spectrophotometrically determined, was about 27% lower than the FDE content.

Since SDP2 and A (Aerosil® 200) groups showed no significant activity difference and considering that SDP1 and SDP2 were prepared under similar experimental conditions, the colloidal silicon dioxide effect should be taken into account. Regarding the analytical results, colloidal silicon dioxide was not able to protect tannin against thermal denaturation and, consequently, the anti-ulcer activity improvement ob-

<table>
<thead>
<tr>
<th>Groups</th>
<th>TT (g%)</th>
<th>Loss on drying (mg/kg)</th>
<th>Dried extract doses (mg)</th>
<th>Tannin equivalent</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDP1</td>
<td>5.91</td>
<td>3.79</td>
<td>300</td>
<td>0.0177</td>
</tr>
<tr>
<td>SDP2</td>
<td>5.49</td>
<td>3.26</td>
<td>375</td>
<td>0.0206</td>
</tr>
<tr>
<td>FDE</td>
<td>8.16</td>
<td>5.32</td>
<td>300</td>
<td>0.0245</td>
</tr>
</tbody>
</table>

Table 1. Spray-dried and freeze-dried extracts total tannins contents and the respective doses used for the anti-ulcer activity evaluation. SDP1: spray-dried extract without drying adjuvant; SDP2: spray-dried extract containing Aerosil® 200 as drying adjuvant; FDE: freeze-dried extract; TT: total tannins contents.
served in SDP2 was due probably to the colloidal silicon dioxide (Aerosil® 200) presence. In fact, colloidal silicon dioxide is able to reduce the lesion area (group A, 80.8%). As a result of that, the colloidal silicon dioxide activity overlaps the SDP2 ones and a sure differentiation between both activities remain therefore unfeasible.

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REFERENCES
