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Note

Radiological determination of gastric motility in experimental model treated with *Rosmarinus officinalis* Linn. (Lamiaceae) extracts

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Among the biological properties of *Rosmarinus officinalis* Linn. (rosemary), the infusions and ethanolic extracts are popularly used to treat gastrointestinal disorders. However, the effects of these extracts on the gastric motility are unknown. Here, we evaluated influence of the rosemary extracts on gastric motility and its chemical composition. Forty-eight female Wistar rats with food restriction for 12 h were made into eight experimental groups (n=6, each) and given oral treatments (1 mL) of ethanolic and aqueous extracts at the doses of 125, 250 and 500 mg/kg, and distilled water (control) and metoclopramide (2 mg/kg, intramuscular). After one hour, five metal spheres (1 mm) were administered with barium contrast. A series of radiographs were taken every hour for six hours. Significant differences were shown in the gastric emptying of the metal spheres within three hours among the extracts and the control groups. By high-performance liquid chromatography (HPLC), both extracts presented kaempferol, carnosic acid and rosmarinic acid as major compounds, which would influence in the higher time for emptying. This study showed that the rosemary extracts increased the gastric emptying time in healthy rats, suppressing the gastric motility, and may be useful in treating digestive disorders, like diarrhea.

Keywords: Carnosic acid, Gastric emptying, Gastrointestinal motility, Kaempferol, Metoclopramide, Rosmarinic acid, Rosemary

Medicinal plants are known by pharmacological relevance and are used to treat numerous gastrointestinal disorders[1]. Issues with conventional drugs, such as side effects, resistance, high costs, etc. have encouraged researchers to look upon medicinal plants as potential alternative source. Also, in many geographic locations, medicinal plants are the only available therapeutic resource[2]. *Rosmarinus officinalis* Linn. (Lamiaceae family), popularly known as rosemary, is a perennial herb whose aromatic leaves are commonly used as a flavouring agent in food and cosmetic industry[3,4]. Earlier works on rosemary have confirmed its anti-inflammatory[5], antimicrobial[6,7], antiseptic and disinfectant[8,9], anti-nociceptive[10], anti-aging and neuroprotective[10,11], diuretic[12], hepatoprotective[13], antispasmodic[3] and antiulcer[14] properties. These biological properties are attributed to their different chemical constituents.

In the Brazilian traditional medicine, *R. officinalis* infusions are indicated to treat gastrointestinal disorders, such as flatulence, stomach aches, intestinal problems and cramps[4,15]. Further, aqueous and ethanolic extracts have shown activity proven relaxing gastrointestinal smooth muscle, as well as hepatoprotective, antispasmodic and stimulating secretion of bile, having a choleretic effect[3,13,15,16].

Gastrointestinal tract disorders are of significant concern in human and veterinary medicine, especially in cases of esophageal and gastric dysmotility, gastric emptying disorders, and digestive abnormalities[17,18]. In view of the empirical use of *Rosmarinnus officinalis* L. extracts in gastrointestinal disorders, here, we studied the chemical composition of ethanolic and aqueous extracts of its aerial parts, and also evaluated their influence on gastric motility in an experimental model.

Material and Methods

Plant Material

Dried leaves of *Rosmarinnus officinallis* L. were commercially acquired from Luar Sul - Indústria e Comércio de Produtos Alimentícios Ltda. (Santa Cruz do Sul/RS, Brazil, lot 001/2011).

Preparation of the extracts

The plant material was dissolved in ethanol P.A. (Synth) for obtaining the ethanolic extract (10% w/v) and in sterile distilled water (10% w/v) for the crude aqueous extract, according to the Waller *et al*.[7] with modifications. Both extracts were individually packaged into a volumetric flask and kept with constant shaking on a hot plate in an oil bath at a

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temperature of 65-70ºC. These conditions were maintained for 24 h in the ethanolic extract, which was subsequently filtered and subjected to rotatory evaporation to obtain the dry extract. For the aqueous extract, the product was maintained for 1 hour and, afterward submitted to a decantation process for 15 min, filtered and separated. Subsequently, 250 mL of sterile distilled water was added, and the content was subjected to heating and kept under shaking for 1 h. This technique was repeated up to obtain three extractions, which were homogenized, packed in metal trays and frozen at −70ºC in Ultra-Freezer. The aqueous extract was lyophilized for 48 h to obtain a pale-yellow crystalline powder and stored in sealed amber vials for subsequent use.

Chemical analysis

The chemical composition of the extracts was performed by liquid chromatography-high performance (HPLC) using Varian equipment with DAD detector, in column of C-18 reverse phase (25 cm × 4.6 mm × 5 µm, Phenomenex® Gemini, Torrance, United States), with flow rate of 1.0 mL/min and the injection volume of 10 µL. The identification of phenolic compounds was made by comparing retention times and mass spectra with the following authentic standard compounds prepared in methanol: rosmarinic acid, caffeic acid, ferulic acid, carnosol, *p*-coumaric acid, vanillic acid, syringic acid, vanillin , sinapic acid, rutin, carnosic acid, luteolin, apigenin, kaempferol and quercetin.

Experimental design

Forty-eight female Wistar rats (*Rattus norvergicus*) weighting 300±14 g, 7 weeks of age at the start of the experiment were purchased from the Central Vivarium of the Federal University of Pelotas (Pelotas, RS, Brazil). The animals were housed in controlled conditions of humidity (air-conditioned room at 22ºC), the light-dark cycle of 12/12 h, fed with commercial diet according to weight, and drinking water *ad libitum*. This study was approved by the ethics committee of the Federal University of Pelotas.

The animals were conditioned to daily manipulation for two weeks before the treatments and radiographic examination. After this period, the animals were randomly allocated into eight experimental groups, composed of six animals in each group, and subjected to food restriction for 12 h. The experimental groups were identified according to the type of treatment received, which were ethanolic extracts administered orally at dosages of 125 mg/kg (Et_{125}), 250 mg/kg (Et_{250}) and 500 mg/kg (Et_{500}); aqueous extracts administered orally at dosages of 125 mg/kg (Aq_{125}), 250 mg/kg (Aq_{250}) and 500 mg/kg (Aq_{500}); metoclopramide (Met) administered intramuscularly (2 mg/kg) and sterile distilled water as negative control (Cont). For groups treated with *R. officinalis* L., the extracts were previously weighed, diluted in sterile distilled water and administered with the aid of an orogastric tube (1.0 mL). In the remaining groups (Met and Cont), 0.1 mL of sterile distilled water was administered by orogastric tube. Furthermore, due to intramuscular administration in the Met group, all animals received the administration of 0.1 mL of sterile distilled water intramuscularly as control.

After one hour of treatment, all animals received the administration of five metal spheres (1 mm in diameter), via orogastric tube, totalling 30 spheres per group, along with 2 mL of radiological contrast of 10% barium sulfate ($BaSO_4$). Then, radiographic projections were performed every hour in order to evaluate the gastric emptying time. The first radiography was performed at zero time, and the last was performed six hours after the treatment administration, totaling seven radiographs per animal. The evaluation of the gastric motility was determined by the route of the metal spheres in the gastrointestinal tract, and the emptying of the same in the stomach outlet was considered as the observational point of the study.

Statistical analysis

For evaluation of results, Analysis of Variance (ANOVA) was performed using the Fischer test (LSD – Least Square Difference) and the statistical differences were determined by the value of $P < 0.05$, considered significant between the treatment means.

Results and Discussion

The radiographic evaluation at different times showed that the number of eliminated contrasted spheres from the stomach within three hours were different among the experimental groups. In animals treated with *R. officinalis* L. ethanolic extract, 76.6% (23/30), 60% (18/30) and 63.3% (19/30) of the spheres were emptied after the administration of the doses 125 mg/kg (Et_{125}), 250 mg/kg (Et_{250}) and 500 mg/kg (Et_{500}), respectively, whereas 93.3% (28/30) and 100% (30/30) of the spheres were emptied in the

groups receiving water (Cont) and metoclopramide (Met), respectively (Fig. 1). Animals from metoclopramide group (Met^{A}) showed a gastric emptying time faster in comparison to those treated with ethanolic extracts (Et_{125}^{B}, Et_{250}^{B} and Et_{500}^{B}), differing statistically (P <0.05), whereas the animals treated with distilled water did not differ from any group ($Cont^{AB}$).

Similarly, animals treated with aqueous extracts also presented influence on the gastric emptying time. The oral administration of 125 mg/kg (Aq_{125}), 250 mg/kg (Aq_{250}) and 500 mg/kg (Aq_{500}) resulted in the emptying of 46.6% (14/30), 50% (15/30) and 86.6% (26/30) of the spheres within three hours, respectively, showing that the increased concentration of the *R. officinalis* aqueous extract was proportional to the acceleration of gastric emptying (Fig. 2). The statistical analysis showed a difference among the lower concentrations of the aqueous extract (Aq_{125}^{B}, Aq_{250}^{B}) and metoclopramide (Met^{A}) groups (P <0.05), whereas the higher concentration of this aqueous extract (Aq_{500}^{AB}) did not differ (P >0.05). Animals treated with distilled water also did not differ from any other groups ($Cont^{AB}$).

In general, the gastric emptying time was similar for all groups treated with *R. officinalis* L. extracts (P >0.05), although it was faster in the groups treated with the highest concentration of aqueous extract (Aq_{500}) and the lowest concentration of ethanolic extract (Et_{125}). However, when compared to groups treated with metoclopramide and distilled water, the aqueous and ethanolic extracts of *R. officinalis* L. decreased the gastric motility, possibly due to the active compounds in the chemical composition. The analysis by HPLC-DAD outlined the profile of the main compounds. Kaempferol, carnosic acid and rosmarinic acid were the major compounds in decreasing order for both rosemary extracts (Table 1).

R. officinalis L. extracts acted in the decreasing of gastric emptying time in rats. Although studies are scarce about the gastrointestinal properties of this plant[14], the antispasmodic activity of the ethanolic extract was shown on guinea pig[3]. These studies corroborate with our experiment, because the depressant activity in the gastric motility was

Table 1—Quantitative determination of phenolic compounds identified in ethanolic and aqueous extracts of *Rosmarinus officinalis* L. by HPLC with DAD detector

Chemical compound	*Rosmarinus officinalis* Linn.	
	Ethanolic extract (µg/mL)	Aqueous extract (µg/mL)
Caffeic acid	34	–
Carnosic acid	126	121
p-Coumaric acid	–	12
Rosmarinic acid	87	153
Apigenin	50	45
Kaempferol	240	251
Carnosol	28	26
Luteolin	35	32
Quercetin	76	83

– Nil

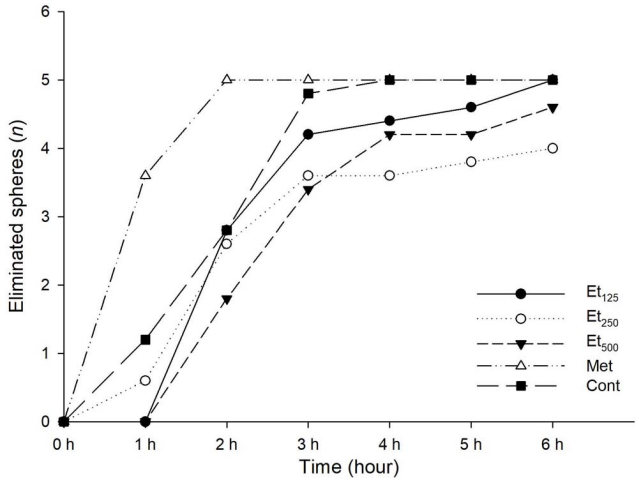


Fig. 1 — Evaluation of the gastric motility in experimental animals treated with ethanolic extracts of *Rosmarinus officinalis* L. at the doses of 125 mg/kg (Et_{125}), 250 mg/kg (Et_{250}), and 500 mg/kg (Et_{500}), metoclopramide (Met) and sterile distilled water as control (Cont), according to the number of metallic spheres eliminated and analyzed by radiographies in different times (hours).

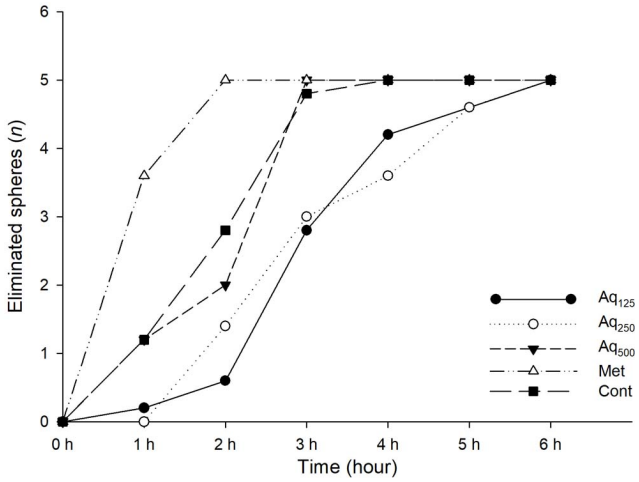


Fig. 2 — Evaluation of the gastric motility in experimental animals treated with aqueous extracts of *Rosmarinus officinalis* L. at the doses of 125 mg/kg (Aq_{125}), 250 mg/kg (Aq_{250}), and 500 mg/kg (Aq_{500}), metoclopramide (Met) and sterile distilled water as control (Cont), according to the number of metallic spheres eliminated and analyzed by radiographies in different times (hours).

observed. Although Luchese *et al.*[19] have demonstrated the inefficiency of the administration of 1 mL/kg of essential oil on piglets with neonatal diarrhea, the present study demonstrated the performance of plant extracts on the gastrointestinal tract of experimental animals.

Similar findings were shown in rats orally treated with aqueous extracts of *Braccharis trimera* (Asteraceae) or *Maytenus ilicifolia* (Celastraceae) at the same dose (50 mg/kg)[20], which delayed gastric emptying. Another plant with similar effect to that observed in *R. officinalis* was the aqueous extract of *Melia azedarach* (Meliaceae), that showed gastric emptying inhibitory effect when intra-gastrically administered at the dose of 400 mg/kg[21]. Although *R. officinalis* belong to the Lamiaceae family, not all plants in the same botanical family have identical activities. An example is *Thymus vulgaris*, which the oral administration of the aqueous extract (100-200 mg/kg) facilitated the emptying process in healthy rats[22], differentiating from our study findings.

The methodology employed was effective in evaluating gastric motility in rats using extracts of *R. officinalis* L., as described in the literature[23], as well as the safe use of barium contrast with the metal spheres[24], which allowed us to determine the exact time of gastric emptying of the animal with consciousness. Different from other techniques with duodenal cannulas[25], activated carbon and plastic balls[26] and stomach analysis[21,22], euthanasia was not necessary for this study, in which the animals remained healthy after the procedure, indicating the security of this technique.

Regarding to the speed, the average time of emptying with metal spheres was 3.5 h[24], corroborating the experimental model, which the passage of the same in the negative control (Cont) lasted 3 h. As a positive control, metoclopramide (Met) was used because it is an antiemetic drug, prokinetic and stimulating the gastrointestinal peristalsis[27]. Animals from the metoclopramide group showed increasing gastric motility because the gastric emptying time occurred in two hours, differing from the groups treated with both *R. officinalis* L. extracts (P <0.05).

Similarly, other medicinal plants showed activity on gastrointestinal system, altering motor function, such as aqueous extracts of *Salvia officinalis* L.[1], which also belong to the Lamiaceae family. The compounds of both plants belonging to this botanical species are similar and were investigated for biological properties on gastrointestinal disorders, like irritable bowel syndrome[28] and infectious diarrhea[29]. In this way, the increased time of gastric emptying showed in the aqueous and ethanolic extracts in the healthy rats may be useful for treating disorders that increase the gastric motility, like diarrhea.

It is known that many drugs used in diarrhea management face contraindication, as well as resistance of some bacterial strains, when diarrhea is infectious[29], which increases the need of new alternative and more effective drugs. In addition to activity on the gastric system, the antimicrobial effect[6–8,30,31] of this plant is recognized, helping the body restore physiological conditions.

Relating to the chemical compounds, the carnosic acid and rosmarinic acid were highlighted in the phenolic fraction, which has been described as the main compounds found in other studies[32-34]. The antioxidant and neuroprotective activities of rosmarinic acid have been reported, as well as its high absorption in the gastrointestinal tract[35]. Caffeic acid was only detected in ethanolic extract, whereas p-coumaric was in the aqueous extract. In the group of flavonoids, apigenin, carnosol, luteolin and quercetin were identified in both extracts, as observed by Bai *et al.*[33] and Borrás-Linares *et al.*[34]. Kaempferol was the most abundant compound from the flavonoids detected and its presence has promoted anti-proliferative activity in pancreatic tumor cells[36].

These identified compounds showed various biological properties, such as anticancer[36], antioxidant[37] and antispasmodic[3], in addition to inhibiting the acetylcholinesterase and butyryl-cholinesterase enzymes in degrading the remaining acetylcholine in the synaptic space of muscles[38,39]. This inhibitory activity on the mentioned enzymes has the effect of reducing the work of the smooth and striated musculature[40], contributing to the lower gastric peristalsis observed in the groups treated with aqueous and ethanolic extracts of *R. officinalis* L.

Although not analyzed in this study, the presence of other compounds in the extract should not be ruled out, because chlorogenic acid, carnosolic acid, rosmanol, ursolic acid, oleanolic acid, among others, have been detected in aqueous and ethanolic extracts of *R. officinalis* L.[33,34,41,42]. In the chromatographic analysis, it was not detected the compounds rutin, vanillin, ferulic acid, vanillic acid, syringic acid and sinapic acid in the studied polar extracts, which

discards the influence of these compounds in the gastric motility of the animals treated with ethanolic and aqueous extracts of rosemary.

Although some compounds present in aqueous extract may be lost during gastrointestinal digestion and colonic fermentation, such as rosmarinic acid[31], the absorption and bioavailability of others may be high, as showed in carnosic acid and rosmanol in a study evaluating the human intestinal absorption in Caco-2 cell monolayers model[43]. Interestingly, the carnosic acid was the second compound found in greater quantities in the ethanolic extract, and the third in the aqueous extract.

Considering the pharmacokinetic, the compounds carnosic acid, carnosol and rosmanol reach their peak concentration within 0.2 to 1 h after oral administration of ethanolic extract, as demonstrated in a study evaluating the plasmatic concentration of them in murine model, which kept the serum levels after 12 hours of ingestion[44]. Relating to our study, this finding demonstrated that the biological effects of chemical compounds remain active in the body, mainly due to the decreased gastric motility that the rosemary extracts cause in the host. Furthermore, biological properties are conferred, such as antioxidant at the hepatic, cerebral, stomach[30] and intestinal[45] levels. None of the experimental animals showed an adverse effect, and these findings in the literature reinforce the biological properties of the polar extracts of rosemary.

Conclusion

The aqueous and ethanolic extracts of *Rosmarinus officinalis* L. decreased the gastric motility in the experimental model in rats, indicating its use *in vivo* in disorders where gastric motility is accelerated.

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Animal Rights

The procedures used in this study were approved by the Ethics Committee on Animal Experimentation (*Comissão de Ética em Experimentação Animal* – CEEA, process no. 23110.006538/2010-66), of the Federal University of Pelotas (Pelotas, RS, Brazil).

Conflict of interest

Authors declare no conflict of interests.

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