

Gastroprotective effect of alpha-pinene and its correlation with antiulcerogenic activity of essential oils obtained from *Hyptis* species

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Submitted: 05-05-2014

Revised: 12-06-2014

Published: 21-01-2015

ABSTRACT

Background: Alpha-pinene (α -pinene) is a monoterpene commonly found in essential oils with gastroprotective activity obtained from diverse medicinal plants, including *Hyptis* species. The genus *Hyptis* (Lamiaceae) consists of almost 400 species widespread in tropical and temperate regions of America. In the north and northeastern Brazil, some *Hyptis* species are used in traditional medicine to treat gastrointestinal disturbances. **Objective:** The present study has investigated the gastroprotective effect of purified α -pinene in experimental gastric ulcer induced by ethanol and indomethacin in mice. **Materials and Methods:** Gastric ulcers were induced in male Swiss mice (20-30 g) by oral administration of absolute ethanol or indomethacin 45 min after oral pretreatment with vehicle, standard control drugs or α -pinene (10, 30, and 100 mg/kg). One hour after the ulcerative challenges, the stomach were removed, and gastric lesions areas measured. The effects of α -pinene on the gastric juice acidity were determined by pylorus ligation model. The gastrointestinal motility and mucus depletion were determined by measuring the gastric levels of phenol red and alcian blue, respectively. Hematoxylin and eosin stained sections of gastric mucosa of the experimental groups were used for histology analysis. **Results:** α -pinene pretreatment inhibited ethanol-induced gastric lesions, reduced volume and acidity of the gastric juice and increased gastric wall mucus ($P < 0.05$). Furthermore, we showed an interesting correlation between concentration of α -pinene and gastroprotective effect of *Hyptis* species (P Pearson = 0.98). **Conclusion:** Our data showed that the α -pinene exhibited significant antiulcerogenic activity and a great correlation between concentration of α -pinene and gastroprotective effect of *Hyptis* species was also observed.

Key words: Alpha-pinene, antiulcerogenic, essential oil, gastric ulcer, *Hyptis*, terpenes

INTRODUCTION

Medicinal plants are important sources of therapeutic agents used in the treatment of different pathologies, including gastrointestinal disorders.^[1,2] Over the last years, an increasing number of studies has reported the gastroprotective effect of essential oils and purified compounds present in their chemical composition.^[3,4]

Alpha-pinene (α -pinene) is a bicyclic monoterpene widely found in the nature, acting as insect-repellent agent in plant defense.^[5] A variety of interesting pharmacological properties have been attributed to α -pinene, including anti-inflammatory, bronchodilator, hypoglycemic, sedative, antioxidant, and broad-spectrum antibiotic activities.^[6-8] In a recent study, Rufino *et al.* showed convincing evidences of the anti-inflammatory and anticatabolic effects of α -pinene by evaluating its ability to modulate inflammation and extracellular matrix remodeling in human chondrocytes. According to authors, α -pinene induced an inhibition of the interleukin-1-induced inflammatory and nuclear factor-KB and c-Jun N-terminal kinase activation accompanied by decrease of the

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Access this article online

Website:

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DOI:

10.4103/0973-1296.149725

Quick Response Code:



expression of the inflammatory (Inducible nitric oxide synthase) and catabolic (MMP-1 and -13) genes.^[9]

Previous studies have reported the presence of α -pinene in the composition of essential oils with gastroprotective activity obtained from aromatic plants, such as *Citrus aurantium*, *Syzygium aromaticum*, *Zanthoxylum rhoifolium* and *Croton zenthneri*.^[3,10-13] In particular, essential oils extracted from *Hyptis* species appear to have a great correlation between their antiulcerogenic activities and presence of α -pinene. According to McNeil *et al.*, α -pinene is present in various concentrations in all of the eighteen *Hyptis* plant species evaluated, being the dominant constituent in *Hyptis crenata* and *Hyptis emoryi*.^[14] Previous studies have shown that some *Hyptis* species (e.g. *Hyptis mutabilis*, *Hyptis martiusii*, *Hyptis suaveolens* and *Hyptis spicigera*) exhibited significant antiulcerogenic activity, acting by different mechanisms, such as a decrease of acid secretion, antioxidant, anti-*Helicobacter* effects, and improvement of gastric blood flow.^[15-19] In recent times, our group showed that the essential oil obtained from aerial parts of *H. crenata* reduced the rat gastric mucosa lesions and increased the mucus production and the gastrointestinal emptying.^[20] These results suggest that the genus *Hyptis* (Lamiaceae), comprised for more than 300 species widespread in tropical and temperate regions of America,^[21] might be an interesting natural source of novel gastroprotective agents.

Gastric ulcer is one of severe kinds of human ailments that causes maximum discomfort, high morbidity, and substantial economical burden on healthcare market. Only in the United State, there are incidence of 4 million patients with the cost of 10 billion dollars/year in their treatment.^[22-24] In addition of the incomplete effectiveness, the current therapeutics agents used in the treatment of peptic ulcers exhibit severe side effects, and high costs have limited their chronic use.^[25,26] Thus, the current study was performed to assess the gastroprotective properties of purified α -pinene in absolute ethanol and indomethacin models of gastric ulcer.

MATERIALS AND METHODS

Animals and drugs

Swiss mice (20-30 g) were obtained from the bioscience unit of the Unichristus University Center, Fortaleza, CE, Brazil. They were housed in standard conditions under free access to standard chow and water. The animals were kept at room temperature (22°C \pm 2°C) with a light/dark cycle of 12/12 h. All of the procedures described had prior approval from the local animal ethics committee (n° 07227619-3). α -pinene (98% purity) was purchased from Sigma Chemical Co. (St. Louis, MO, USA), Atropine Sulfate (Atropion®- Ariston indústria

química e Farmacêutica Ltda, São Paulo, SP, Brazil), Indomethacin (Indocid® - Merck Sharp and Dohme Farmacêutica Ltda, São Paulo, SP, Brazil), HCl (Merck, Germany). Ethanol (Merck, Germany) and tween 80 (Merck, Germany).

Alpha-pinene: Dose and vehicle

Absolute ethanol – and indomethacin-induced gastric ulcers, the following doses, were tested: 10, 30, and 100 mg/kg. The highest effective dose was used for all subsequent experiments. 0.1% tween-80 aqueous solution was used as a vehicle and all drug compounds were solubilized in this vehicle, including the positive controls.

Evaluation of gastric lesions

The gastric lesions were evaluated by measuring of the lesion area. This area was calculated using the Scion Image Program as described by Khan.^[27] Briefly, the stomach samples were crushed and carefully sandwiched between two layers of transparent plastic folder. Then, the specimens within the plastic folder were scanned using a scanner, and the captured image was saved as a TIFF file on the computer hard drive. The scanned image was analyzed to quantify the gastric lesion areas using the processing and analysis program Scion Image for Windows, Release Beta 4.0.2. The lesion areas (mm²) in a single animal were calculated using the Scion Image Program. Afterwards, the images were expressed as a percent of the total gastric areas.

Ethanol-induced gastric ulcers

Acute gastric lesions were induced according to the method described by Morimoto *et al.* (1991) with modifications.^[28] Briefly, male Swiss mice (20–30 g) fasted for 12 h and were randomly distributed into five groups ($n = 6$ /group). The animals were orally dosed with 0.5 ml of vehicle (0.1% tween-80 aqueous solution), ranitidine (40 mg/kg) or α -pinene (10, 30 and 100 mg/kg) dissolved in vehicle. After 1 h, 0.2 ml of absolute ethanol was then orally administered. 45 min after the ethanol administration, the mice were sacrificed by cervical dislocation, and their stomachs were removed. Thereafter, the stomachs were opened along the greater curvature and washed. The flattened stomachs samples were scanned, and the area of ulcer lesion (mm²) was measured as described previously.^[27]

Indomethacin-induced gastric ulcers

Male Swiss mice (20-30 g) were randomly distributed into five groups ($n = 6$ /group). The animals were water/chow deprived for 12 h prior to the experiments. Vehicle (0.5 ml of 0.1% tween-80 aqueous solution), ranitidine (40 mg/kg) or α -pinene (10, 30 and 100 mg/kg) dissolved in the vehicle were orally administered 45 min before the induction of gastric lesions by the oral administration of indomethacin (30 mg/kg,

in a volume of 0.2 ml). After 5 h, the mice were sacrificed by cervical dislocation and their stomachs were removed. Thereafter, the stomachs were opened along the greater curvature and washed.^[29] The stomachs samples were stretched out and scanned, and the area of ulcer lesion (mm²) was measured as described previously.^[27]

Determination of gastric wall mucus

The gastric wall content was determined according to the method previously described by Corne *et al.* with modifications.^[30] Briefly, vehicle (0.1% tween-80 in distilled water) or α -pinene (100 mg/kg) were orally administered to mice 1 h before absolute ethanol administration (0.2 ml/animal). The control group, which received only saline but not ethanol, was also included. 40 min later, the animals were sacrificed by cervical dislocation and their stomachs were removed. The glandular segments from the stomachs of the animals were removed and weighed. Each segment was transferred immediately to 1% Alcian blue solution (in 0.16 M sucrose solution, buffered with 0.05 M sodium acetate, pH 5) for 2 h. Then, the excess dye was removed by rinsing with a sucrose solution. The dye fixed to the gastric wall mucus was extracted with magnesium chloride. A 4 ml sample of the blue extract was then shaken with an equal volume of diethyl ether. The resulting emulsion was centrifuged at 3000 rpm for 10 min, and the absorbance of the supernatant was measured at 580 nm. The quantity of Alcian blue extracted per gram (net) of glandular tissue was then calculated (μ g per mg of wet tissue).

Evaluation of gastric juice parameters

The animals were randomly divided into five groups ($n = 6$ /groups) and fasted for 16 h with free access to a 5% glucose solution. For the pyloric ligature, the animals were anaesthetized (xylazine 6 mg/kg and ketamine 60 mg/kg, i.p), the abdomen was opened and the pylorus was ligated. Immediately after the ligature, α -pinene (100 mg/kg), vehicle (0.1% Tween-80 aqueous solution) and ranitidine (40 mg/kg) was administered intraduodenally. 4 h later, the animals were sacrificed by cervical dislocation. Then, their abdomens were opened, and their stomachs were removed. The gastric secretions were collected and centrifuged at 5000 rpm for 15 min. The final volume (ml) was determined after washing the mucosal side of the stomach with 2 ml of distilled water, and the pH values were measured using a digital pH meter. The total acidity of the gastric juices was determined by titrating it to pH 7.0 with 0.1 N NaOH using 2% phenolphthalein as indicator. The total concentration of acid was expressed as mEq.[H⁺]/mL/4h.^[31]

Gastrointestinal motility

Gastric emptying

The gastric emptying was measured according to the previous method described by Gupta and Brans with

modifications.^[32] In brief, male Swiss mice fasted for 12 h and were randomly divided into six groups ($n = 6$ /group). The animals were orally pretreated with 0.3 ml vehicle (0.1% tween-80 aqueous solution) or α -pinene (100 mg/kg) or subcutaneous atropine (0.3 ml, 3 mg/kg, positive control). After 75 min, each animal received (by gavage) 0.5 ml of phenol red (0.5 mg/ml). One group of animals was sacrificed by cervical dislocation immediately after the phenol red administration (sham group), and the other groups were sacrificed 40 min later. Thereafter, a ligature was made in the pylorus. Then, the stomachs were removed and opened along the greater curvature and washed with 5 ml of distilled water. The stomach contents were collected and centrifuged at 8000 rpm for 10 min. Measurement of the gastric emptying was assessed spectrophotometrically by measuring the absorbance of a solution formed by 1 ml of the sample supernatants added to 1 ml of 1 N NaOH at 560 nm. The results were expressed as a concentration (μ g) of dye retained in the stomach in relation to the control group.

Gastrointestinal transit ratio

After 12 h of fasting, the mice were randomly divided into six groups ($n = 6$ /group). The animals were orally pretreated with 0.3 ml vehicle (0.1% tween-80 aqueous solution) or α -pinene (30 mg/kg) or subcutaneous atropine (0.3 ml, 3 mg/kg). After 75 min, each animal received 0.5 ml of phenol red (0.5 mg/ml) given by gavage. Forty-five minutes after administering the marker, the animals were sacrificed, and the small intestines were quickly removed. The distance over which the phenol red had traveled and the total length of the small intestine were measured. The gastrointestinal transit ratio was expressed as the percentage of the distance traveled by the phenol red relative to the total length of the small intestine.^[33]

Histomorphological analysis

For the histomorphological analysis, gastric tissue samples were fixed in 10% buffered formalin for 24 h. Thereafter, the formalin-fixed tissues were dehydrated through graded alcohol series, cleared in xylene series and embedded in paraffin. Serial sections of 5 μ m thickness from the paraffin embedded tissue were cut by microtome and stained with hematoxylin and eosin (H and E). The slides were examined microscopically, in a blind manner, by counting of pathomorphological alterations such as congestion, hemorrhage, edema, and erosions per field of six samples of each group. An arbitrary scale was used for severity assessment of these changes.

Correlation: Alpha-pinene and gastroprotection of *Hyptis* species

In order to evaluate the correlation between α -pinene concentrations and gastroprotective effects of essential

oils obtained from *Hyptis* species, the relation between both parameters of four *Hyptis* species was analyzed by Pearson correlation and linear regression tests. It was evaluated the correlation between α -pinene concentrations (%) and the effect of oral treatment, at dose of 100 mg/kg, of essential oils obtained from *H. martiusii*,^[15] *H. suaveolens*,^[16,17] *H. spicigera*^[19] and *H. crenata*^[20] on inhibition of ethanol-induced gastric lesion area (%).

Statistical analysis

All the results were expressed as the mean \pm standard error of the mean. The significance ($P < 0.05$) of the results was assessed by One-way analysis of variance, followed by Bonferroni's multiple comparison test. The correlation between α -pinene and antiulcerogenic activities was calculated using the Pearson coefficient (ρ) and linear regression.

RESULTS

Effect of alpha-pinene on an absolute ethanol-induced ulcer and the indomethacin-induced ulcer

Pretreatment of animals with α -pinene reduced the gastric mucosa lesion induced by ethanol. As illustrated in the Table 1, α -pinene-pretreated animals showed a reduced gastric mucosa lesion area (17.58 ± 2.63 , 19.21 ± 4.74 and 19.81 ± 3.44 mm², at doses of 10, 30 and 100 mg/kg, respectively) when compared to the control group, vehicle (34.23 ± 4.68 mm²). The gastroprotective effect provided by α -pinene was similar to ranitidine (40 mg/kg). Ranitidine-pretreated animals showed a gastric mucosa lesion area of 16.32 ± 3.89 mm². The percentage of mucosa lesion area was approximately 15% in the α -pinene and ranitidine groups compared to 28% of vehicle-treated animals, corresponding to approximately 50% of gastroprotection. No significant difference within α -pinene treated groups and between the α -pinene and ranitidine groups and observed in the ethanol – induced gastric ulcer. In the indomethacin-induced ulcer model, oral pretreatment with α -pinene did not produce any

significant gastroprotective effect. No significant difference was observed between the gastric ulcer area of α -pinene- and vehicle-pretreated animals. Ranitidine group showed a significantly lower ulcer area compared to others experimental groups [Table 1].

Effect of the alpha-pinene on the gastric secretion parameters in the pylorus ligation model

Table 2 illustrates the effect of intraduodenal administration of α -pinene (100 mg/kg) on pH, total acidity and gastric volume of mice after 4 h of the pylorus ligation. Ranitidine-treated (40 mg/kg) animals showed a significant increase of pH (2.84 ± 0.05) and decrease of total acidity (2.84 ± 0.32 mEq/mL/4h) and gastric volume (0.21 ± 0.02 ml) compared to vehicle-treated mice. Similarly, α -pinene-treated mice showed higher pH (2.86 ± 0.03) and lower total acidity (2.44 ± 0.15 mEq/L/4h) and gastric volume (0.20 ± 0.02 ml) than vehicle-treated mice (2.58 ± 0.05 , 4.22 ± 0.24 mEq/mL/4h, 0.36 ± 0.02 ml, respectively). No significant difference on the gastric secretion parameters was observed between ranitidine and α -pinene groups.

Effect of the essential oil extracted from alpha-pinene on gastric mucus production

Figure 1 shows the gastric mucus production, which was determined by measurement of the gastric concentration of Alcian blue. Pre-treatment with α -pinene (100 mg/kg) significantly increased the amount of mucus adhering to the gastric mucosa (1360 ± 127.15 mg of alcian blue/g wet tissue), compared to either vehicle control group (476.16 ± 31.18 mg of alcian blue/g wet tissue) or ranitidine group (877.07 ± 136.23 mg of alcian blue/g wet tissue).

Effect of the essential oil extracted from alpha-pinene on gastrointestinal motility

No significant difference between the concentrations of phenol red present in the stomachs of α -pinene group (3.40 ± 0.04 μ g/ml) and vehicle group (3.340 ± 0.02 μ g/ml), indicating that α -pinene treatment (100 mg/kg) did not alter gastric emptying. Atropine- treated animals (3 mg/kg, s.c) showed a

Table 1: Effect of oral administration of α -pinene on gastric lesion area

Treatment (n=6/group)	Dose (mg/kg)	Ethanol-induced gastric ulcer		Indometacin-induced gastric ulcer	
		Ulcer area (mm ²)	Ulcer area Total area (%)	Ulcer area (mm ²)	Ulcer area Total area (%)
Vehicle	-	34.23 \pm 4.68	28.03 \pm 1.35	20.63 \pm 2.30	34.12 \pm 4.35
Ranitidine	40	16.32 \pm 3.89*	12.97 \pm 3.86*	8.95 \pm 1.46*	13.93 \pm 4.05
α -pinene	10	17.58 \pm 2.63*	13.06 \pm 1.63*	27.77 \pm 3.74	38.22 \pm 4.73
	30	19.20 \pm 4.74*	14.11 \pm 3.69*	26.91 \pm 4.43	32.11 \pm 3.82
	100	19.81 \pm 3.44*	17.13 \pm 2.67*	15.02 \pm 3.24	24.33 \pm 6.07

* $P < 0.05$ significantly different from negative control group treated with vehicle

significant increase of the concentration of phenol red ($3.54 \pm 0.05 \mu\text{g/ml}$) compared to both experimental groups. Similarly, α -pinene treatment did not produce any significant effect on the intestinal transit compared to vehicle-treated animals [Table 3].

Histology of gasitric lesions

Figure 2 illustrates the H and E-stained sections of ethanol-induced gastric mucosal lesions from untreated normal mice [Figure 2a], mice subject to pretreatment with vehicle [Figure 2b], ranitidine [Figure 2c] and α -pinene, at dose of 10 [Figure 2d], 30 [Figure 2e] and 100 [Figure 2f] mg/kg. It was observed substantial damages of the gastric mucosa, necrotic lesions penetrated deeply into the mucosa as well as edema and leukocyte infiltration into the submucosa layer in H and E-sections of

vehicle-pretreated mice [Figure 2b] compared to untreated normal animals [Figure 2a]. As seen in Figures 2d-f, mice pretreated with α -pinene showed significant protection of the gastric mucosa, evidenced by decreasing ulcer area, edema and gastric structure approached of healthy tissue [Figure 2a].

Correlation between alpha-pinene concentration and gastroprotective effects of essential oils obtained from Hyptis species

Figure 3 shows the correlation between published concentrations of α -pinene present in the chemical composition of *H. martiussi*, *H. suaveolens*, *H. crenata*

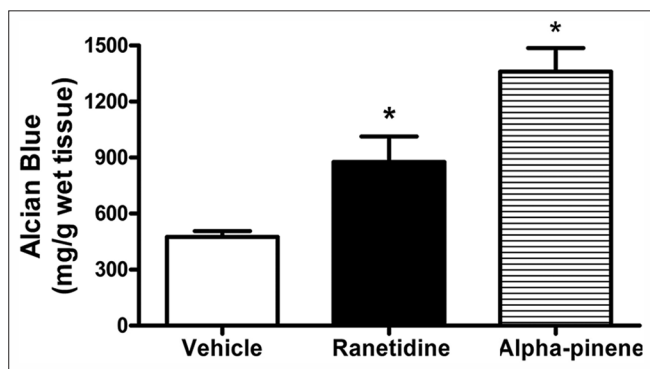


Figure 1: Effect of oral administration of the α -pinene (100 mg/kg) on gastric volume (a), total acidity (b), pH (c) and mucus production (d). In the graph, the ordinates express the ulcer area (mm^2) 45 min after ingestion of the ethanol. The results are expressed as the mean \pm standard error of the mean $n = 6$ animals for each group. *Significantly different from the vehicle group (analysis of variance followed by Bonferroni's multiple comparison test, $P < 0.05$)

Table 2: Effect of intraduodenal administration of α -pinene (100 mg/kg) on gastric secretion parameters in pylorus-ligated mice

Treatment (n=7/group)	Dose (mg/kg)	Gastric volume (mL)	Gastric pH	Total acidity mequiv[H ⁺]/mL/4h
Vehicle	-	0.36 \pm 0.02	2.58 \pm 0.05	4.22 \pm 0.24
Ranitidine	40	0.21 \pm 0.02*	2.84 \pm 0.05*	2.84 \pm 0.32*
α -pinene	30	0.20 \pm 0.02*	2.85 \pm 0.04*	2.44 \pm 0.15*

* $P < 0.05$ significantly different from negative control group treated with vehicle

Table 3: Effect of oral administration of α -pinene (100 mg/kg) on gastrointestinal motility

Treatment (n=8/group)	Dose (mg/kg)	Gastric emptying (phenol red [$\mu\text{g/ml}$])	Gastrointestinal transit ratio (%)
Time zero	-	4.5 \pm 0.06	0
Vehicle	-	3.34 \pm 0.02	48.81 \pm 6.92
Atropine	3	3.54 \pm 0.05*	38.72 \pm 4.41
α -pinene	30	3.44 \pm 0.04	38.92 \pm 6.45

* $P < 0.05$ significantly different from negative control group treated with vehicle

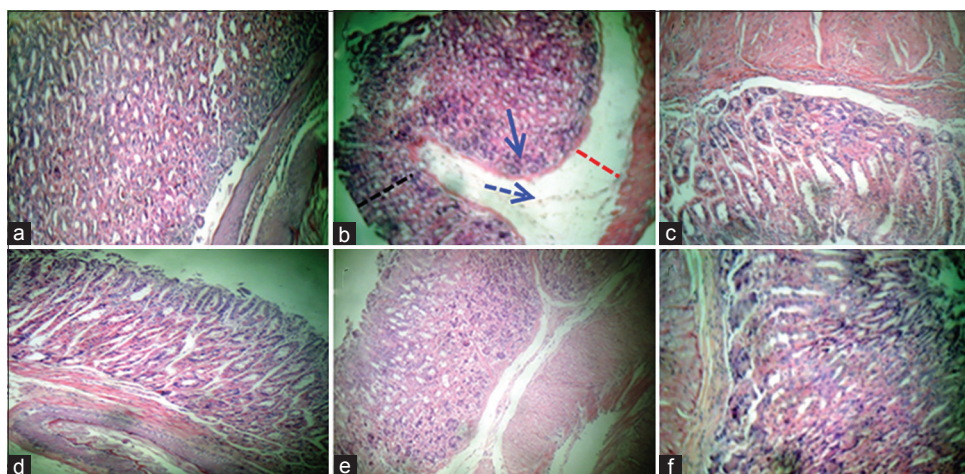


Figure 2: Hematoxylin and eosin – stained sections of the gastric tissue in mice (20 \times). (a) normal and (b) vehicle-pretreated mice. Large reduction in the gastric epithelium (black line) accompanied by intense edema in the submucosal layer (red line) and leukocyte infiltration (red arrow). (c) mice pretreated with ranitidine (40 mg/kg). Mild disruption of the surface epithelium mucosa, with no leukocyte infiltration and mucosal damage. (d-f) mice pretreated with 10, 30 and 100 mg/kg α -pinene, respectively. Gastric structure approached of normal control group (a)

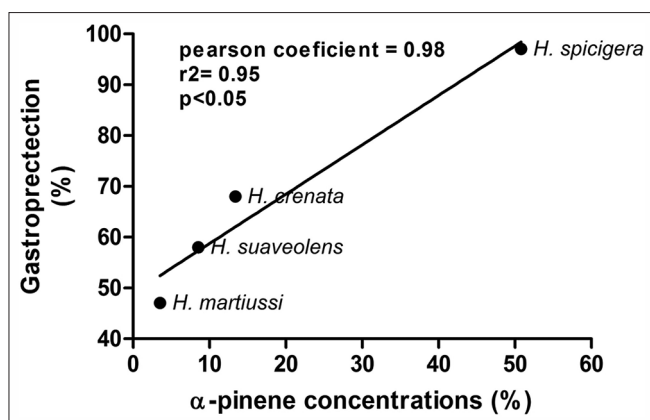


Figure 3: Correlation between concentration of alpha-pinene and gastroprotective effect (%/%) of essential oils obtained from four *Hyptis* species (*Hyptis martiussim*, *Hyptis suaveolens*, *Hyptis crenata* and *Hyptis spicigera*). Pearson correlation ($P = 0.98$) and linear regression ($R^2 = 0.95$) were performed to assess the significant correlation ($P < 0.05$)

and *H. spicigera* and their respective effects on inhibition of ethanol-induced gastric lesion area. It was observed significant Pearson correlations (Pearson coefficient = 0.98; $P < 0.05$) as well as the linear correlation showed a $R^2 = 0.95$ ($P < 0.05$), indicating a significant correlation between both parameters in the *Hyptis* species analyzed.

DISCUSSION

The present study showed for the first time that the α -pinene displays a significant antiulcerogenic activity, reducing the gastric mucosa lesion induced by ethanol. Although α -pinene has been recognized as the most widely encountered monoterpene in nature and various studies have reported its presence in chemical composition of essential oils with antiulcerogenic activity,^[3,8,12,14,33] no experimental study concerning its gastroprotective effect is found in the literature.

Our data show that oral administration of α -pinene, at doses of 10, 30 and 100 mg/kg, inhibited approximately 50% of the gastric mucosal lesions induced by ethanol, without any significant effect on the indomethacin-induced ulcer [Table 1]. In general, our findings suggest that the gastroprotective effect of the α -pinene appears to be associated with an increase of gastroprotective factors and decrease of ulcerative factors.^[34-36] For example, we observed that α -pinene, at dose of 100 mg/kg, reduced the gastric wall mucus depletion, protecting the gastric mucosa against acidity, bacterial colonization and mechanical force of proteolytic digestion.^[34] In addition, pylorus-ligated mice subjected to α -pinene pre-treatment showed a decrease of total acidity and reduced gastric juice volume [Table 2]. We

also observed a decrease of gastric mucosal lesions and edema in gastric mucosal slices stained with H and E of α -pinene – pretreated mice, supporting its antiulcerogenic activity [Figure 2].

Previous studies have shown that α -pinene exhibits bactericide activity against both Gram-positive and Gram-negative bacteria^[6,7,18] and high antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl radicals.^[37] Taking into account that *Helicobacter pylori* infection and generation of free radicals of oxygen and nitrogen are among the main factors involved in the pathogenesis of gastric ulcers,^[38,39] the bactericide and antioxidant properties of α -pinene might be also closely linked to the action mechanisms by which α -pinene promotes gastroprotection.

In final years, experimental studies have reported the gastroprotective effect of some *Hyptis* species, supporting their popular use for treatment of gastrointestinal disturbances in traditional medicine from different countries, including Brazilian folk medicine.^[15-21] In addition, the presence of α -pinene has been reported in diverse *Hyptis* plant species,^[14] suggesting a possible relation between α -pinene and gastroprotection. In this context, we have investigated the relation between inhibition of ethanol-induced gastric lesion (%) produced by oral treatment of essential oil obtained from different *Hyptis* species (100 mg/kg) and their concentrations of α -pinene (%), respectively.^[15-20] As illustrated in Figure 3, it was observed the following relation: *H. martiussi* (3.5%:47.1%), *Hyptis suaveolens* (8.5%:58.0%), *H. crenata* (13.4%:68.0%) and *H. spicigera* (50.8%:97.1%).^[15-20] These relations have provided significant Pearson and linear correlations (Pearson coefficient = 0.9758; $R^2 = 0.9523$; $P < 0.05$) between magnitude of α -pinene concentration and gastroprotective effects of *Hyptis* species.

Of note, it is known that the biologic effects of essential oils are associated with the composition and concentration of monoterpenes and sesquiterpenes present in their chemical composition, being often related to a synergism among these terpenoids.^[40-42] This explains, at least in part, the moderate gastroprotective effect (approximately 50%) of purified α -pinene (100 mg/kg) compared to gastroprotective effect of whole compounds, such as essential oil obtained from *H. spicigera* (100 mg/kg), whose concentration of α -pinene in the chemical composition and gastroprotection in ethanol-induced ulcer model were 50.8% and 97.1%, respectively.^[15]

Prior findings have reported the α -pinene – containing essential oils with gastroprotective effects, such as essential oils obtained from *Hyptis* genus. To corroborate, our data

showed the antiulcerogenic effect of purified α -pinene and the correlation between concentration of α -pinene present in essential oils from some *Hyptis* species and their gastroprotective effects. Taking together, it is plausible to speculate that α -pinene might contribute to the screening process and quality control of essential oils with gastroprotective effect obtained from *Hyptis* genus, a promising source of scientific research for novel agents to treat gastric ulcers.^[43]

In summary, we showed that the α -pinene exhibits a significant inhibition of gastric mucosal lesions induced by ethanol, which might be associated, at least in part, with an increase of mucus secretion and reduction of gastric H⁺ secretion. Further pharmacological studies will be undertaken in order to provide more precise elucidation of the action mechanism involved in this antiulcerogenic activity. Moreover, we observed a straight correlation between gastroprotective effect and concentration of α -pinene present in the essential oils obtained from *Hyptis* species. Thus, our finding contribute to pharmacological knowledge of α -pinene and researches of new gastroprotective compounds originated from the little-studies species, such as *Hyptis* species.

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Cite this article as: Pinheiro Md, Magalhães RM, Torres DM, Cavalcante RC, Mota FS, Oliveira Coelho EM, *et al.* Gastroprotective effect of alpha-pinene and its correlation with antiulcerogenic activity of essential oils obtained from Hyptis species. *Phcog Mag* 2015;11:123-30.

Source of Support: Nil, **Conflict of Interest:** None declared.