DARU Vol. 19, No. 4 2011

Gastroprotective activity of α-terpineol in two experimental models of gastric ulcer in rats

Souza RHL., Cardoso MSP., Menezes CT., Silva JP., De Sousa DP., *Batista JS.

Departamento de Fisiologia, Centro de Ciências Biológicas e da Saúde, Universidade Federal de Sergipe, 49100-000, São Cristovão (SE), Brazil

Received 22 March 2011; Revised 16 Aug 2011; Accepted 18 Aug 2011

ABSTRACT

Background and the purpose of the study: Several plant essential oils, as well as terpenes present in essential oils, have shown gastroprotective activity. The aim of the present work was to evaluate the gastroprotective activity of α-terpineol, a monoterpene alcohol which is present in essential oils of various plants.

Methods: The gastroprotective activity of α-terpineol was evaluated in rats by assessing the changes in ethanol and indomethacin-induced gastric ulcer scores and on gastric secretory volume and total acidity in pylorus-ligated rats. Alpha-terpineol was administrated orally at the doses of 10, 30, and 50 mg/kg one hour before administration of the ulcer inducing agents by the pylorus ligation procedure. The involvement of endogenous prostaglandins in the protective effect of α-terpineol in ethanol-induced gastric lesions test was assessed by administration of indomethacin (10 mg/kg, s.c.) 30 min before oral administration of α-terpineol at the dose of 50 mg/kg.

Results: α-terpineol presented gastroprotective activity against ethanol-induced ulcers at the doses of 10, 30, and 50 mg/kg. Epoxy-carvone at the dose of 10 mg/kg did not present gastroprotective activity against ulcer induced by indomethacin, but at the doses of 30 and 50 mg/kg it attenuated the gastric damages induced by this agent significantly. Pretreatment with indomethacin did not prevent the gastroprotective effect of α-terpineol on ethanol-induced ulcers. Alpha-terpineol also did not affect the gastric secretion in pylorus-ligated rats.

Major conclusion: The results suggest that α-terpineol presents gastroprotective action which does not involve either an increase in the synthesis of endogenous prostaglandin or a decrease in the gastric acid secretion.

Keywords: Monoterpenes, Essential oils, Gastroprotection.

INTRODUCTION

In folk medicine, as well as in phytotherapy, essential oils have been utilized as therapeutic agents to treat several diseases (1), and evidence for the biological effects of their components has been reported (2). Compounds such as α-terpineol (3, 4), carvone and (+)-pulegone (5) have shown significant pharmacological properties.

Peptic ulcer is one of the most common gastrointestinal diseases. In the recent years, a widespread search has been launched to identify new anti-ulcer drugs from natural sources. Essential oils of several plant, such as *Cryptomeria japonica* (6) and *Croton cajucara* Benth. (7) possess anti-ulcer activity. Some terpenes present in essential oils, such as monoterpene terpinen-4-ol and the sesquiterpene elemol isolated from the essential oil from the leaves of *Cryptomeria japonica* (6), have shown inhibitory activity on ulceration induced by different agents. Alpha-terpineol (Fig. 1) is a volatile monoterpenoid alcohol, present in essential oils of several species of plants (8, 9). Previous studies have demonstrated that α-terpineol possesses pharmacological activities, such as, anticonvulsant (3), sedative (4), antinociceptive (10), and hipotensive (11). As α-terpineol is an isomer of the monoterpene terpinen-4-ol which has anti-ulcer activity (6), it is possible that this monoterpene also presents anti-ulcer activity. In light of these reports, it was of interest to evaluate the α-terpineol activity in two classical models of gastric ulcer in rats.

MATERIAL AND METHODS

Animals

Wistar male rats (weighing 170-250 g), obtained from the Central Biotery of the Federal University of Sergipe, were used in this study. The animals were housed at a constant temperature of 25±2 °C for two days before the experiments, and were maintained

Correspondence: profjosemar@yahoo.com.br

under a 12 hrs light-dark cycle. The animals were fasted for 16 hrs before experiments, but were allowed free access to water. To avoid coprophagy, the rats were fasted in wire-bottomed cages. All experiments were performed in accordance with current guidelines for the care of laboratory animals and ethical guidelines for investigations of experimental animals, approved by the Animal Research Ethical Committee of the Federal University of Sergipe (protocol number 78/06).

Reagents and drugs

Ethyl alcohol p.a (Reagens), (±)-α-terpineol (Dierberger, Brazil), dissolved in 10% tween 80, p.a (VETEC), ranitidine chloridrate (oral solution 15 mg/ml-Ache, trade name Label), indomethacin (Sigma), formaldehyde p.a (VETEC) were used in this study. The indomethacin was dissolved in 5% sodium bicarbonate and then neutralized with an equal volume of 0.2 M HCl.

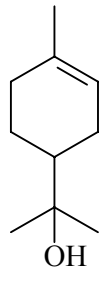

Figure 1. Chemical structure of α-terpineol.

Pharmacological assays

Acute gastric ulcer induction

Gastric ulcers were induced by oral administration of ethanol (12) or indomethacin (13). The animals were divided randomly into six groups of 10 animals each: the first group was treated with water (ranitidine vehicle), the second group was treated with 10% tween 80 (α-terpineol vehicle), and the third group was treated with ranitidine (50 mg/kg, positive control group). The three remaining groups were treated with α-terpineol at doses of 10, 30, and 50 mg/kg, respectively. All treatments were performed by oral route at the volume of 10 ml/kg body weight. One hour after administration of substances, all rats were treated orally (gavage) with 1 ml of 70% ethanol. Another six groups received the same treatments above, but ulcer induction was produced by oral administration of indomethacin (50 mg/kg, 5 ml/kg body weight). Thirty min after ethanol and 6 hrs after administration of indomethacin, the animals were killed by decapitation. Afterwards, the stomachs were removed and incised along the greater curvature, washed with tap water to remove gastric contents, and then fixed with 10% formalin for 15 min. The gastric surface was analyzed for the presence and severity of ulcerative lesions, which were measured with a ruler and magnifying glass (10X amplification) and expressed as ulcer index (UI) in millimeters (mm) and by ulcer inhibition percentage. The ulcer index was obtained by the sum of the lesion lengths of each stomach. The superficial ulcers in which widths were smaller than 1 mm, were multiplied by 1; those in which widths were between 1 and 2 mm were multiplied by 2; and the ones deeper and wider than 2 mm were multiplied by 3. In addition, each 5 petechial lesions were taken as equivalent to 1 mm of ulcer length.

The percentage of ulcer inhibition was determined as follows:

$$\text{Inhibition of ulcer (\%)} = [(\text{control UI} - \text{test UI}) / \text{Control UI}] \times 100$$

Ethanol-induced ulcer in indomethacin-pretreated rats

The ulcers were induced and measured as described above. To investigate the involvement of endogenous prostaglandins in the protective effect of α-terpineol, indomethacin (10 mg/kg, 5 ml/kg body weight, subcutaneous route) was administrated 30 min before oral administration of α-terpineol at the dose of 50 mg/kg. At this dose, indomethacin inhibits the synthesis of prostaglandins but does not induce ulcer formation.

Evaluation of α-terpineol effect in gastric secretion in the pylorus-ligation test

Pyloric ligation was carried out according to the method described by Shay et al. (14) with small modifications. Six groups of rats (n = 10), fasted for a 16 hrs period, were anaesthetized with ether. Then the stomach was incised, and the pylorus ligated. Water (10 ml/kg), ranitidine (50 mg/kg), 5% tween 80 (10 ml/kg), or α-terpineol (10, 30, and 50 mg/kg) were administered intraduodenally immediately after the pylorus ligation. Four hrs later, the rats were sacrificied with an overdose of ether, and the cardia sphincter was ligated. The stomachs were removed, and the gastric contents were collected and centrifuged at 3500 rpm for 30 min. The resulting supernatant was transferred to a test tube for the determination of gastric volume (ml), pH, and hydrogen ion concentration. The pH was measured with the aid of a digital pH meter, and the total acidity was determined by titration with 0.1 M NaOH using 2% phenolphthalein as indicator and expressed in mEq/ml/4 hrs.

Statistical analyses

The data were expressed as means±SEM and analyzed statistically using one-way analysis of variance (ANOVA) followed by Tukey's test. P values less than 0.05 were considered significant.

Table 1. Effects of α-terpineol, ranitidine, and vehicles (controls) on ethanol-induced gastric lesions.

Treatment	Dose (mg/kg)	Animals number	Ulcer index (mm)	Ulcer inhibition percentage
Water	10 ml/kg	10	79.0 ± 14.3	-
10% Tween 80	10 ml/kg	10	69.9 ± 10.1	-
Ranitidine	50	10	0 ± 0[a]	100
α-terpineol	10	10	23.3 ± 8.3*	66.7
α-terpineol	30	10	13.3 ± 4.8**	81.0
α-terpineol	50	10	4.1 ± 3.7***	94.1

* p<0.05; ** p<0.01 and *** p<0.001 in relation to control group treated with 10% tween 80, a p<0.001 in relation to control group treated with water (One way ANOVA followed Tukey post test). Ulcer index represents the mean ± SEM

Table 2. Effects of α-terpineol, and ranitidine on indomethacin-induced gastric lesions.

Treatment	Dose (mg/kg)	Animals number	Ulcer index (mm)	Ulcer inhibition percentage
Water	10 ml/kg	10	32.2 ± 4.5	-
10% tween 80	10 ml/kg	10	30.5 ± 2.4	-
Ranitidine	50	10	2.9 ± 1.2[a]	91.0
α-terpineol	10	10	22.6 ± 4.0	25.9
α-terpineol	30	10	11.0 ± 1.2*	63.9
α-terpineol	50	10	5.7 ± 1.4*	81.3

* p<0.01 in relation to group treated with 10% tween 80 (control group of α-terpineol); a p<0.001 in relation to group treated with water (control group of ranitidine). One way ANOVA followed Tukey post test. Ulcer index represents the mean ± SEM

RESULTS

The oral administration of α-terpineol reduced the ethanol and indomethacin-induced gastric ulcer in rats. In the ethanol-induced ulcer model, α-terpineol reduced gastric lesions at all tested doses compared to its control group (10% tween 80). The percentages of ulcer inhibition obtained at the doses of 10, 30, and 50 mg/kg were 66.7, 81.0, and 94.1, respectively (Table 1). No significant difference was observed between the groups treated with water and 10% tween 80 (vehicle used for dissolution of ranitidine and Alpha-terpineol, respectively). In addition, the H_2-antagonist ranitidine (50 mg/kg) prevented ulcer formation in this test (Table 1).

Alpha-terpineol also reduced the gastric lesions induced by indomethacin, but significant inhibition ($p<0.001$) was only observed at the doses of 30 and 50 mg/kg. The ulcer index values observed in the rats treated with 10% tween 80 and α-terpineol at the doses of 10, 30, and 50 mg/kg were 30.5 ± 2.4, 22.6±4.0, 11.0±1.2, and 5.7±1.4 mm, respectively (Table 2). Gastroprotection was also obtained with ranitidine ($p<0.001$), which inhibited the ulcer formation by 91%.

Subcutaneous treatment with indomethacin (10 mg/kg) did not produce ulcers up to six hrs after its administration (data not shown). Pretreatment with indomethacin (10 mg/kg, s.c., 30 min before α-terpineol) did not inhibit the gastroprotective effect of α-terpineol at the dose 50 mg/kg (Fig. 2).

The volume of gastric content in animals treated with α-terpineol at the doses of 10, 30, and 50 mg/kg did not differ significantly from control animals (Table 3). On the other hand, the antisecretory drug ranitidine decreased the gastric volume value of 5.1± 0.4 ml observed in control animals treated with water to 2.2±0.3 ml. Moreover, the pH and proton concentration values of the gastric content were also unmodified by α-terpineol.

DISCUSSION

In the present study, the gastroprotective activity of the monoterpene α-terpineol in the two ethanol- and indomethacin-induced ulcer models in rats was studied. In the ethanol-induced ulcer model, oral administration of α-terpineol reduced gastric lesions at all doses tested in a dose-dependent manner (Table 1).

Gastric ulcers are generally caused by a disruption in the balance between aggressive factors (pepsin and hydrochloric acid) and mucosal defensive factors, such as blood flow, and mucus and bicarbonate secretion. Agents that present gastroprotection against ethanol-induced gastric lesions act mainly by stimulation of defense mechanisms (cytoprotective effect) rather than inhibition of aggressive factor production or release (antisecretory effect). Hence, it is possible that the gastroprotection presented by α-terpineol in this experiment occurs through one or more mechanisms responsible for gastric defense.

Oral treatment with α-terpineol also reduced the

Table 3. Effects of α-terpineol, ranitidine, and vehicles (controls) on the rat gastric secretion in the pylorus-ligation test.

Treatment	Dose	n	Gastric volume (ml)	Proton concentration (mEq/ml/4h)	pH
Water	10 ml/kg	10	5.0 ± 0.4	21.2 ± 1.5	1.6 ± 0.2
Ranitidine	10 ml/kg	10	2.2 ± 0.3*	13.3 ± 1.2*	4.0 ± 0.6*
Tween	50 mg/kg	10	5.4 ± 0.6	22.0 ± 0.9	1.4 ± 0.1
α-terpineol	10 mg/kg	10	3.1 ± 1.0	23.7 ± 4.3	1.7 ± 0.6
α-terpineol	30 mg/kg	10	3.4 ± 1.5	22.9 ± 5.5	1.4 ± 0.4
α-terpineol	50 mg/kg	10	4.5 ± 1.6	25.6 ± 4.5	1.2 ± 0.3

* $p < 0.05$ in relation to control group treated with water (One way ANOVA followed Tukey post test). Ulcer index represents the mean ± SEM

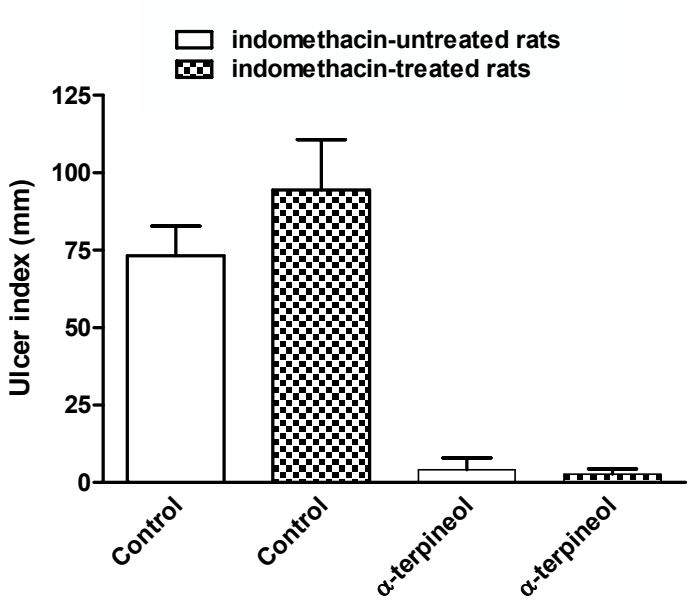

Figure 2. Effect of pretreatment with indomethacin (10 mg/kg, s.c., 30 min before oral administration of tween 80 and, α-terpineol) on gastric lesions induced by ethanol. Control groups were treated orally with 10% tween 80 and, α-terpineol groups were treated orally with α-terpineol at the dose of 50 mg/kg. Bars represent the means with SEM (n=10)

gastric lesions induced by indomethacin, but significant inhibition ($p<0.01$) was only observed at the doses of 30 and 50 mg/kg (Table 2). These results indicate the existence of a dose-dependent relationship in the gastroprotective effect of α-terpineol. Gastroprotection was also obtained with ranitidine, which inhibited ulcer formation by 91%.

Gastric acid is involved in the indomethacin-induced gastric mucosal lesion formation. Moreover, Gerkens et al. (15) suggested that the reduction of gastric mucosal blood flow contributes to the lesion formation induced by indomethacin. Thus, it is possible that alterations of gastric secretion and blood flow also contribute to α-terpineol gastroprotection.

The gastroprotective action of α-terpineol in the two ulcer models indicates the importance of this compound in the development of new anti-ulcer drugs. Moreover, antiulcerogenic effects have been shown by alcohol terpenes, such as 4-terpineol and elemol (7), which suggest the existence of a relationship between alcohol terpenes and antiulcerogenic effects. Nevertheless, more experiments will be necessary for better evaluation of this relationship.

In order to investigate the possible involvement of endogenous prostaglandins in the gastroprotective action of α-terpineol, indomethacin (10 mg/kg, s.c.) was administrated 30 min before oral administration of α-terpineol (50 mg/kg) in the alcohol-induced ulcer test. This pretreatment with indomethacin (10 mg/kg) did not inhibit the gastroprotective action of α-terpineol (Fig. 2). This result suggests that the gastroprotective action of α-terpineol at the dose of 50 mg/kg does not involve increase in prostaglandin synthesis.

The main drugs used currently to treat ulcers, are antagonists of histamine H_2 receptor and proton pump

inhibitors, which act by inhibition of the secretion of gastric acid. However, in the present experiments the gastric volume, pH, and proton concentration values were not altered by α-terpineol, which suggest that its gastroprotective action does not involve gastric acid secretion inhibition. Therefore, it is possible that the gastroprotective activity of α-terpineol occurs by cytoprotective mechanisms. However, the clinical potential of cytoprotective compounds for the treatment of ulcers is still poorly explored.

In conclusion, the results of this study show that the monoterpene α-terpineol presents gastroprotective activity in two widely used models for the evaluation of antiulcerogenic drugs. Furthermore, the data also indicate that its gastroprotective activity does not involve gastric acid secretion inhibition or increase in prostaglandin synthesis.The following steps of this research will be the evaluation of the involvement of endogenous nitric oxide and free radical scavenger property in the gastroprotective activity of α-terpineol.

ACKNOWLEDGEMENTS

The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for supplying the Fellowship and to Fundação de Amparo à Pesquisa de Sergipe (FAPITEC-SE) for financial support.

REFERENCES

1. Lawless J. The encyclopedia of essential oils. London: Thorsons, 2002, 226p.
2. Arruda TA, Antunes RMP, Catão RMR, Lima EO, De Sousa, DP, Nunes XP, Pereira MSV, Barbosa-Filho JM, Cunha EVL. Preliminary study of the antimicrobial activity of Mentha x villosa Hudson essential oil, rotundifolone and its analogues. Rev Bras Farmacogn, 2006; 16: 307-311.
3. De Sousa DP, Quintans JL, Almeida RN. Evaluation of the Anticonvulsant Activity of alfa-Terpineol. Pharm Biol, 2007; 45: 69-70.
4. De Sousa DP, Raphael E, Brocksom U, Brocksom TJ. Sedative effect of monoterpene alcohols in mice: a preliminary screening. Z Naturforsch C, 2007; 62: 563-566.
5. De Sousa DP, Junior EVM, Oliveira FS, De Almeida RN, Nunes XP, Barbosa-Filho JM. Antinociceptive Activity of Structural Analogues of Rotundifolone: Structure-Activity Relationship. Z Naturforsch C, 2007; 62: 39-42.
6. Matsunaga T, Hasegawa C, Kawasuji T, Suzuki H, Saito H, Sagioka T, Takahashi R, Tsukamoto H, Morikawa T, Akiyama T. Isolation of the anti-ulcer compound in essential oil from the leaves of Cryptomeria japonica. Biol Pharm Bull, 2000; 23: 595-598.
7. Hiruma-Lima CA, Gracioso JS, Bighetti EJB, Grassi-Kassisse DM, Nunes DS, Brito ARMS. Effect of essential oil obtained from Croton cajucara Benth. on gastric ulcer healing and protective factors of the gastric mucosa. Phytomedicine, 2002; 9: 523-529.
8. Golshani S, Karamkhani F, Monsef-Esfehani HR, Abdollahi M. Antinociceptive effects of the essential oil of Dracocephalum kotschyi in the mouse writhing test. J Pharm Pharm Sci, 2004; 7: 76-79.
9. Raina VK, Kumar A, Srivastava SK, Syamsundar KV, Kahol AP. Essential oil composition of "kewda" (Pandanus odoratissimus) from India. Flavour Fragr J, 2004; 19: 434-436.
10. Quintans-Júnior LJ, Oliveira MGB, Santana MF, Santgana MT, Guimarães AG, Siqueira JS, De Sousa DP, Almeida RN. α-terpineol reduces nociceptive behavior in mice. Pharm Biol, 2011; 49: 583-586.
11. Ribeiro TP, Porto DL, Menezes CP, Antunes AA, Silva DF, De Sousa DP, Nakao LS, Braga VA, Medeiros IA. Unravelling the cardiovascular effects induced by alpha-terpineol: a role for the nitric oxide-cGMP pathway. Clin Exp Pharmacol Physiol, 2010; 37: 811-816.
12. Robert A, Nezamis JE, Lancaster C, Hanchar AJ. Cytoprotection by prostaglandins in rats. Prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl, and thermal injury. Gastroenterol, 1979; 77: 433-443.
13. Rainsford KD, Fox SA, Osborne DJ. Comparative effects of some non-steroidal anti-inflammatory drugs on the ultrastructural integrity and prostaglandin levels in the rat gastric mucosa: relationship to drug uptake. Scand J Gastroenterol, 1984; 19: 55-68 Suppl. 101.
14. Shay H, Komarov SA, Fels SS, Meranze D, Gruenstein M, Siplet H. A simple method for the uniform production of gastric ulceration in the rat. Gastroenterol, 1945; 5: 43-61.
15. Gerkens JF, Shand DG, Flexner C, Nies AS, Oates JA and Data JL. Effect of indomethacin and aspirin on gastric blood flow and acid secretion. J Pharmacol Exp Ther, 1977; 203: 646-652.