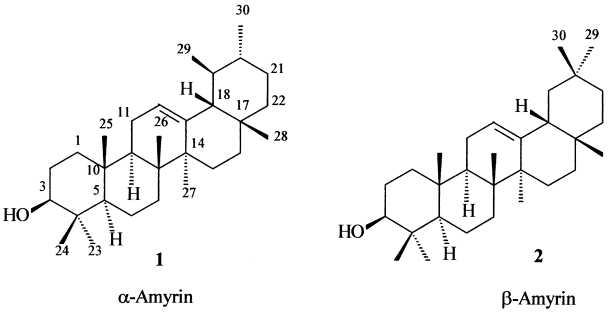
Gastroprotective Effect of the Mixture of α- and β-Amyrin from *Protium heptaphyllum*: Role of Capsaicin-Sensitive Primary Afferent Neurons

Francisco A. Oliveira[1], Gerardo M. Vieira-Júnior[2], Mariana H. Chaves[2], Fernanda R. C. Almeida[2], Kelcyana A. Santos[1], Fabrício S. Martins[1], Regilane M. Silva[1], Flavia A. Santos[1], Vietla S. N. Rao[1]

Abstract

This investigation evaluated the role of capsaicin-sensitive afferent neurons in the gastroprotective effect of α- and β-amyrin, a triterpenoid mixture isolated from *Protium heptaphyllum* resin. Gastric mucosal damage was induced in mice by intragastric ethanol and assessed by planimetry. Mice pretreated orally with the amyrin mixture (50 and 100 mg/kg) or capsaicin (2.5 and 5 mg/kg), the pungent principle from red hot peppers, showed a significantly lower intensity of ethanol-associated gastric mucosal damage, in relation to vehicle-treated controls. At higher doses both these agents produced either a diminished protection or no significant effect. The maximal gastroprotection that was observed at the dose of 100 mg/kg amyrin mixture was almost abolished in mice with their sensory afferents chemically ablated by a neurotoxic dose of capsaicin, suggesting that the gastroprotective mechanism of α- and β-amyrin mixture involves at least in part the activation of capsaicin-sensitive primary afferent neurons.

Many plant-derived triterpenoids have been shown to produce gastroprotective effects in experimental and clinical studies [1], [2]. *Protium heptaphyllum* March (Burseraceae), popularly known as almécega grows abundantly in the Amazon region and in various other parts of Brazil. The resinous exudate collected from the trunk wood of this plant in its natural form is a reputed folk remedy with anti-inflammatory, analgesic, expectorant and wound-healing actions [3]. Phytochemical studies on the resin revealed the presence of several monoterpenes and some pentacyclic triterpenes that include a mixture of α- and ß-amyrin [4]. Recently, we have reported that the crude resin from P. heptaphyllum had a gastroprotective property [5]. Since the resin offers gastroprotection and presents a much higher proportion of a mixture of α- and β-amyrin as major constituent, this study sought to verify whether these triterpenoid compounds could be responsible for the gastroprotection against ethanol-induced gastric damage in mice, and if so to verify a possible role of capsaicin-sensitive afferents in the gastroprotection.



The effects of oral pretreatments with the mixture of α- and β-amyrin (50 – 600 mg/kg) in comparison with capsaicin (2.5 – 20 mg/kg) on ethanol-induced gastric mucosal damage in mice are shown in Fig. **1A** and **1B**. Vehicle-treated control mice showed extensive gastric mucosal lesions in the form of hemorrhagic erosions in glandular segments only. Smaller doses of the mixture of amyrin (50 and 100 mg/kg) and capsaicin (2.5 and 5 mg/kg) suppressed the gastric injury in a significant and dose-dependent manner and at these doses, the extents of reduction in the respective groups of animals were in the order of 52 and 67 % for the amyrin and 79 and 83 % for capsaicin. Higher doses of amyrin and capsaicin, however, produced only a diminished

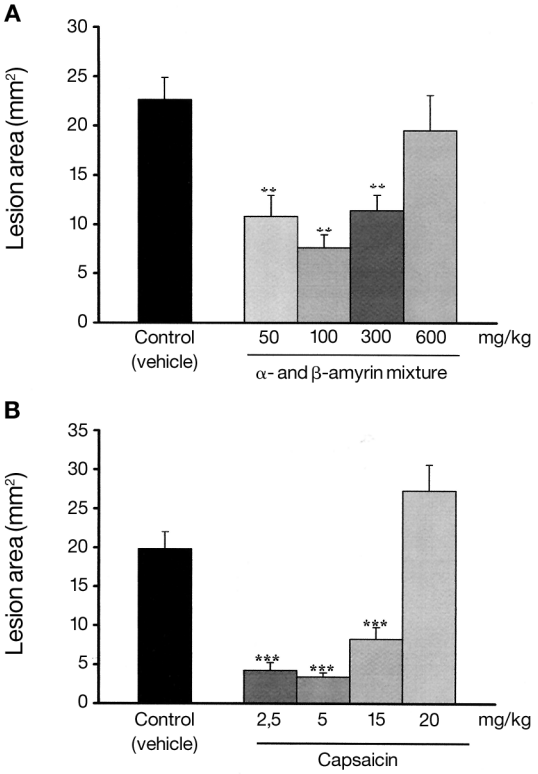


Fig. **1** Effects of oral pretreatments with a mixture of α- and β-amyrin (**A**) and capsaicin (**B**) on gastric lesions induced by ethanol in mice. Bars represent the mean ± S.E.M (n = 6 – 10). ** P < 0.01, *** P < 0.001 Significantly different from the respective controls (Mann-Whitney U test).

Affiliation: [1] Departamento de Fisiologia e Farmacologia, Universidade Federal do Ceará, Fortaleza, CE, Brasil · [2] Departamento de Química, Universidade Federal do Piauí, Brasil

Correspondence: Vietla S. N. Rao · Departamento de Fisiologia e Farmacologia · Universidade Federal do Ceara · Rua Cel Nunes de Melo-1127 · Caixa Postal-3157 · 60430–270 Fortaleza, CE · Brasil · Fax: +55-85-288-8333 · E-mail: vietrao@ufc.br

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or no significant gastroprotection, indicating a likely common mode of action. In capsaicin desensitized mice ethanol damage was more intense than that observed in normal controls that received only vehicle. As shown in Fig. **2**, desensitization of sensory afferents with capsaicin almost completely abolished the gastroprotective effect of the100 mg/kg mixture of amyrin.

In recent years, substantial evidence has been accumulated for the pivotal role of capsaicin-sensitive peptidergic sensory fibers in the maintenance of gastric mucosal integrity [6]. Orally administered capsaicin has been shown to protect against ethanol-induced gastric injury [7]. Therefore, the present study compared the gastroprotective effect of triterpene mixture, α- and β-amyrin with that produced by capsaicin. Similar to amyrin mixture, capsaicin protected mice against ethanol-induced damage but, however, both these agents showed a similar tendency of diminished efficacy at higher doses. The mechanism underlying the capsaicin-induced gastric protection has been described as being due to an increase in mucosal blood flow, increased secretion of mucus, and bicarbonate mediated by the activation of sensory neurons [8]. It is generally agreed that the protective effect of capsaicin results from the activation of sensory afferent neurons in the stomach. This concept has been confirmed in the present experiments that show the abolition of gastroprotection by chemical ablation of capsaicin-sensitive afferents in mice with a neurotoxic dose of capsaicin. In contrast with results of our previous study with crude resin, in which we observed 72% and 89% gastroprotection at the respective oral doses of 200 and 400 mg/kg [5], the present experiments with α- and ß-amyrin mixture displayed a maximal gastroprotection (67%) at the dose of 100 mg/kg and at higher doses a reduced trend was noticed. Apparently, the crude resin may have other triterpenoid components, which besides activating sensory afferents in the gastric mucosa may stimulate other protective mechanisms involving prostaglandins and or endogenous nitric oxide, as shown by Arrieta et al. with other triterpenoids [2]. In conclusion, the data obtained in this study confirm that the α- and ß-amyrin mixture possess gastroprotective property and suggest that it involves, at least in part, the activation of capsaicin-sensitive afferent neurons.

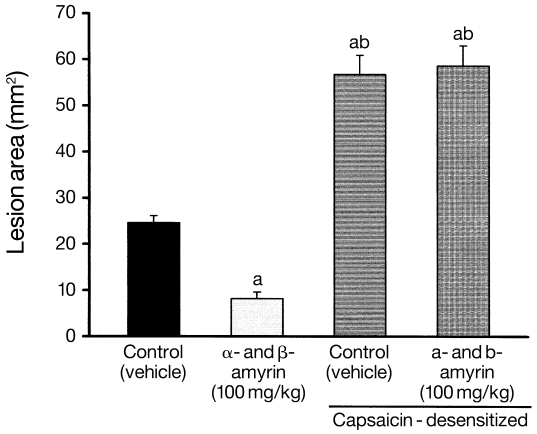

Fig. **2** Effect of oral pretreatment with a mixture of α- and β-amyrin on ethanol-induced gastric mucosal injury in normal and capsaicin-desensitized mice. Bars represent the mean ± S.E.M (n = 6 – 10). Significantly different from the respective controls (Mann-Whitney U test). [a] $P < 0.001$ vs. vehicle-treated control; [ab] $P < 0.01$ vs. vehicle-treated normal and a mixture of α- and β-amyrin-treated controls.

Materials and Methods

The resinous exudate from the trunk wood of *Protium heptaphyllum* March. was collected from the municipal areas of Timon, Maranhão State of Brazil, after its identification by botanist Roseli Farias de Melo Barros. A voucher sample (#18 247) has been deposited at the Herbarium Graziela Barroso of the Federal University of Piauí, Teresina, Brazil. The crude resin (410 g) was dissolved in methanol/dichloromethane (4 : 1), filtered and the solvent evaporated in a rotavapor to obtain 408 g (99.5%) of amorphous white resin. A part of this material (12 g) was chromatographed on silica gel (280 g) column (5.5 × 38 cm), eluted with hexane (100%, 625 mL); hexane-AcOEt (98 : 2, 875 mL); (95 : 5, 1000 mL); (9 : 1, 3750 mL); (8 : 2, 3225 mL); (7 : 3, 1750 mL) and AcOEt (100%, 500 mL). The fractions eluted with hexane-AcOEt (95 : 5) afforded 5.43 g (45%) of the α- and β-amyrin mixture (**1** + **2**). TLC using an authentic sample identified the presence of these constituents in this mixture. The structural identification of these substances was confirmed by ^{1}H- and ^{13}C-NMR spectral analysis, based on the method developed by Olea and Roque [9] and comparison with literature data [10]. The ratio of α- and ß-amyrin in this mixture is 63 : 37, calculated by ^{1}H-NMR, dividing the signal area of olefinic hydrogens δ = 5.14 (α-amyrin) and δ = 5.20 (ß-amyrin) by signal area in δ = 3.24 (dd, J = 11 and 5 Hz), attributed to H-3 in the two triterpenes and multiplied by 100. The determined optical rotation for the mixture was $[\alpha]_D^{20}$ + 92.5 (c 0.5 in $CHCl_3$).

The gastroprotective effect of α- and β-amyrin mixture was evaluated in comparison with capsaicin using male Swiss mice (20 – 25 g) deprived of food for 15 – 18 h before experimentation, but had free access to drinking water. The local Animal Care and Use Committee of the Federal University of Ceará approved the experimental protocols in accordance with the ethical guidelines of IASP. In the first series of experiments, mice in groups (6 – 10 per group) were treated orally either with α- and β-amyrin mixture (50, 100, 300 and 600 mg/kg), capsaicin (Sigma, MO, USA; 5, 10, 15 and 20 mg/kg), or vehicle in a volume of 10 mL/kg. The vehicle used for the mixture of α- and ß-amyrin was 3% Tween 80 in 0.9% saline and for capsaicin, it was a combination of absolute ethanol, Tween 80 and 0.9% saline (1 : 1 : 8). One hour following the treatments, gastric damage was induced in each animal by intragastric administration of 0.2 mL of ethanol (96%), and the animals were killed 30 min later [11]. The stomachs were excised, opened along the greater curvature, rinsed with saline (0.9%) and the mucosal lesion area (mm^2) was measured by planimetry using a transparent grid (1 mm^2 area) placed on the glandular mucosal surface [12] and was expressed in percentage (%) in relation to total area of corpus. In the second series of experiments, the possible involvement of capsaicin-sensitive afferent neurons in the gastroprotective effect of amyrins (100 mg/kg, *p.o.*) against ethanol-induced gastric mucosal damage was investigated in capsaicin-desensitized mice. Mice were anaesthetized with sodium pentobarbital (50 mg/kg, *i.p.*) and treated with capsaicin (2 × 25 mg/kg, *i.p.* for 3 days. *s.c.*) as a modification of the method described previously [13]. To counteract any respiratory impairment associated with capsaicin treatment, the animals were pretreated with aminophylline (10 mg/kg, *i.m.*) before the capsaicin injection. After 8 days, the efficiency of capsaicin pretreatment was verified by monitoring the wiping reflex to ocular instillation of a drop of 10 μg/mL capsaicin solution. Fail-

ure of the wiping reflex was considered as effective ablation of primary sensory afferents. In these experiments, none of the capsaicin-treated rats showed a wiping response, indicating effective ablation of primary sensory afferent neurons, whereas the wiping response was intact in vehicle-treated controls.

Data are presented as the mean ± SEM from 6 – 10 mice per group. For statistical analysis, the non-parametrical Mann-Whitney U test was used. A $P < 0.05$ was considered statistically significant.

Acknowledgements

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