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Gastroprotective effect of a flavone from *Lonchocarpus araripensis* Benth. (Leguminosae) and the possible mechanism

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Abstract

The gastroprotective effect of DDF (3,6-dimethoxy-6″, 6″-dimethyl-[2″, 3″ : 7, 8]-chromeneflavone) from *Lonchocarpus araripensis* Benth. (Leguminosae) on gastric damage induced by absolute ethanol (96%, 0.2 mL/mouse) and indometacin (30 mg kg^{-1}, p.o.) in mice was investigated. Intraperitoneally administered DDF at dose levels of 50, 100 and 200 mg kg^{-1} markedly reduced the gastric lesions in the ethanol model by 62, 72 and 96%, and in the indometacin model by 34, 70 and 75%, respectively, as compared with misoprostol (50 μg kg^{-1}, p.o.), the reference compound that caused lesion suppression by 67% in ethanol model and by 72% against indometacin-induced ulceration. The ED50 of DDF in reducing gastric lesions induced by ethanol and indometacin (dose of the DDF that reduced the gastric lesion area by 50% in relation to the control value) was 50.87 and 61.56 mg kg^{-1}, respectively. Mechanistic studies were carried out at 100 mg kg^{-1} DDF using the ethanol model. Compared with *N*-acetylcysteine (750 mg kg^{-1}, p.o.), a donor of sulfhydryls, DDF only partially replenished the ethanol-induced depletion of gastric mucosal NP-SH. Pretreatment with TRPV1 antagonist capsazepine (5 mg kg^{-1}, i.p.) or the non-selective cyclooxygenase inhibitor indometacin (10 mg kg^{-1}, p.o.) effectively blocked the gastroprotective effect of DDF (100 mg kg^{-1}) against ethanol damage. Furthermore, the effect of DDF was significantly reduced in mice pretreated with L-NAME, or glibenclamide, the respective inhibitors of nitric oxide synthase and K^+_{ATP} channel activation. These data provide evidence to show that DDF affords gastroprotection against gastric damage induced by ethanol and indometacin by different and complementary mechanisms, which include involvement of endogenous prostaglandins, nitric oxide release, the activation of TRPV1 receptor or K^+_{ATP} channels, besides a sparing effect on NP-SH reserve.

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Introduction

Lonchocarpus araripensis Benth. (syn. *Derris araripensis*) (Leguminosae), popularly known as angelim or sucupira branca, is a tree that largely grows in the North East of Brazil. Although *L. araripensis* has no folk medicinal usage, there are reports showing the anti-inflammatory activity (Alencar et al 2005; Napimoga et al 2007) and the inhibitory effect on gastric H^+, K^+-ATPase (Reyes-Chilpa et al 2006) of a few *Lonchocarpus*-related species. Chemical studies carried out on *L. araripensis* demonstrated the presence of flavonoids (Nascimento & Mors 1981), including 3,6-dimethoxy-6″, 6″-dimethyl-[2″, 3″: 7, 8]-chromeneflavone (DDF). Some flavones have been reported to possess clinically relevant properties, such as anti-allergic (Yano et al 2007), anti-inflammatory (Kim et al 2004; Lee et al 2007), antinociceptive (Rajendran et al 2000), antioxidant (Benedek et al 2006), anti-ulcerogenic (Takase et al 1994; La Casa et al 2000), antispasmodic (Gilani et al 2006), antiviral (Brinkworth et al 1992) and anti-tumoral (Cabrera et al 2007) actions.

However, to date no pharmacological study has been carried out on this flavone, DDF. Since flavonoid substances of plant origin in general are considered highly gastroprotective

against damage caused by necrotizants or NSAIDs, probably due to an improvement of gastric microcirculation (Zayachkivska et al 2005), this study examined whether DDF affords protection against gastric damage induced by ethanol and indometacin in mice, and further attempted to establish the possible mechanism.

Materials and Methods

Drugs

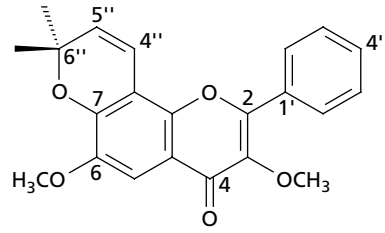
Capsaicin, indometacin, glibenclamide, diazoxide, L-arginine and N^{w}-nitro-L-arginine methyl ester (L-NAME) were purchased from Sigma-Aldrich (St Louis, MO). Tween 80 was from Merck AG (Darmstadt, Germany). Absolute ethanol was obtained from Synth (Brazil) and prostaglandin analogue 16,16-dimethyl PGE2 (misoprostol) was from Continental Pharma (Cytotec, Italy). All solvents used were of analytical grade. The 3,6-dimethoxy-6″, 6″-dimethyl-[2″, 3″:7, 8]-chromeneflavone (DDF) from *L. araripense*s was dissolved in 2% Tween 80 and diluted just before use in 0.9% saline.

Plant material

Lonchocarpus araripensis Benth. (syn. *Derris araripensis*) was collected from the plantation in Acarape County (Ceará State, Brazil), during February 2005 after its authentication by Prof. Edson P. Nunes. A voucher specimen (No. 11074) was deposited at the Herbário Prisco Bezerra (EAC) of the Departament of Biology, Universidade Federal do Ceará.

Extraction and isolation

The air-dried and ground root bark material of *L. araripensis* (1.9 kg) was extracted three times with hexane (9 L each) followed by ethanol (9 L each) at room temperature. The solvents were evaporated under reduced pressure to give 51.9 and 78.3 g of the crude hexanic and ethanolic extracts, respectively. The hexane extract was coarsely fractioned on silica gel by elution with *n*-hexane, *n*-hexane–CH_2Cl_2 1:1, CH_2Cl_2, EtOAc and EtOH to obtain the respective fractions. The CH_2Cl_2 fraction (3.6 g), a crystalline material, was subjected to recrystallization using *n*-hexane–EtOAc 9:1 to yield the pure compound **1** (1.02 g). The structure of **1**, which was established as 3,6-dimethoxy-6″, 6″-dimethyl-[2″, 3″ : 7, 8]-chromeneflavone (Figure 1), was determined by spectroscopic methods, including IV, EM and ^{1}H and ^{13}C NMR, and comparison with published data (Nascimento & Mors 1981; Arriaga et al 2000). The spectral details are as follows: 3,6-dimethoxy-6″, 6″-dimethyl-[2″, 3″ : 7, 8]-chromeneflavone (**1**): white crystals; mp 202–204°C; IR ν_{max} 3012, 1663, 1500, 1483, 1130,1069; EIMS *m/z* 364 [M]+ (80), 349 (100), 331 (10), 217 (30), 105 (25), 77 (28); ^{1}H NMR ($CDCl_3$) 8.07 (dd, J = 8.4 and 1.8 Hz, H-2′/H-8′), 7.50 (m, H-5, H-3′, H-4′, H-5′), 6.88 (d, J = 10 Hz, H-4″), 5.74 (d, J = 10 Hz, H-5″), 3.96 (s, MeO-3), 3.88 (s, MeO-6), 1.55 (s, CH_3-6‴/CH_3-6‴); ^{13}C NMR ($CDCl_3$) 174.4 (C-4), 154.6 (C-2), 147.5 (C-9), 147.2 (C-6), 146.6 (C-7), 141.2 (C-3), 131.6 (C-5″), 131.5 (C-1′), 130.5 (C-4′), 128.7 (C-5′/C-3′), 128.4 (C-6′/C-2′), 117.5 (C-10), 115.5 (C-4″), 104.6 (C-5), 78.4 (C-6″), 60.3 (MeO-3), 56.5 (MeO-6), 28.1 (C-6‴/C-6‴).



(1)

Figure 1 Chemical structure of 3,6-dimethoxy-6″, 6″-dimethyl-[2″, 3″:7, 8]-chromeneflavone (**1**).

Animals

Male Swiss mice, 20–25 g obtained from the Central Animal House of this University were used. Experimental groups consisted of 8 mice per group. They were housed at 24 ± 2°C under a 12-h light–dark cycle and had free access to standard pellet diet (Purina chow) and tap water. The mice were deprived of food for 15 h before experimentation, but had free access to drinking water. The institutional Ethics Committee on the Care and Use of Animals for experimentation approved the experimental protocols, and all experiments were performed in accordance with the guidelines of the National Institutes of Health, Bethesda, USA.

Gastric damage induced by ethanol

Groups of mice (n = 8) were treated with 3,6-dimethoxy-6″, 6″-dimethyl-[2″, 3″ : 7, 8]-chromeneflavone (DDF) (25, 50, 100 and 200 mg kg^{-1}, i.p.), misoprostol (50 μg kg^{-1}, p.o.), or vehicle (2% Tween 80 in saline in a volume of 10 mL kg^{-1}). Forty-five minutes after treatment, each mouse was given orally 0.2 mL of ethanol (96%) and they were sacrificed 30 min later (Robert 1979). 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 (area: 1 mm^2) placed on the glandular mucosal surface and expressed in percentage (%) in relation to total area of corpus.

Gastric damage induced by indometacin

Mice in groups (n = 8) were treated with DDF (25, 50, 100 and 200 mg kg^{-1}, i.p.), misoprostol (50 μg kg^{-1}, p.o.) or vehicle. Forty-five minutes after treatment, each mouse received an oral dose of 30 mg kg^{-1} indometacin and they were sacrificed 6 h later (Rainsford 1982). The stomachs were removed, immersed in 5% formalin for 30 min, and then opened along the greater curvature to register the incidence and extent of ulceration according to the following scale: 0 = no petichial haemorrhage or erosion; 1 = up to 5 petichial; 2 = up to 5 petichial with erosions of depth 1 mm; 3 = up to 10

petichial; 4 = up to 10 petichial with erosions of depth above 1 mm. The mean ulcer score for each mouse was calculated and compared between groups.

Effect of DDF on ethanol-induced depletion of non-protein sulfhydryls

To study the role of gastric mucosal non-protein sulfhydryls (NP-SH) in the gastroprotective effect of DDF, groups of mice (n = 8) were pretreated with vehicle, DDF (100 mg kg^{-1}, i.p.), *N*-acetyl-L-cysteine (NAC; 750 mg kg^{-1}, p.o.) alone, or in their combinations before the oral administration of 0.2 mL of ethanol (96%). DDF and NAC were administered 45 min and 60 min before ethanol, respectively. Thirty minutes after ethanol, the mice were sacrificed, stomachs excised and the glandular part of the stomach was homogenized in 5 mL ice-cold ethylenediaminetetraacetic acid (EDTA; 0.02 M; pH 8.9). Gastric mucosal NP-SH were measured according to a previously described method (Sedlak & Lindsay 1968). Volumes of 4 mL of the homogenates were mixed in 15 mL test tubes with 3.2 mL of distilled water and 0.8 mL of 50% trichloroacetic acid (TCA). The tubes were shaken intermittently for 10 min and centrifuged at 3000 rev min^{-1} for 15 min. Two millilitres of supernatant was mixed with 4 mL of 0.4 M Tris buffer at pH 8.9, 0.1 mL of 5, 5′-dithio-bis-(2-nitrobenzoic acid) (DTNB; 0.01 M) was added and the sample was shaken. The absorbance was measured within 5 min after addition of DTNB at 412 nm against a reagent blank with no homogenate. The absorbance values were extrapolated from a gluthathione standard curve and expressed as μg per g of stomach tissue (μg g^{-1}).

Effects of capsazepine and indometacin pretreatments on DDF gastroprotection

Groups of mice (n = 8) were pretreated with vehicle, DDF (100 mg kg^{-1}, i.p.), capsaicin (0.3 mg kg^{-1}, p.o.) and misoprostol (50 μg kg^{-1}, p.o.), alone or in their combinations with capsazepine (5 mg kg^{-1}, i.p.) or indometacin (10 mg kg^{-1}, p.o.) before the oral administration of 0.2 mL of ethanol (96%). When given alone, DDF, capsaicin and misoprostol were administered 1 h before ethanol. Indometacin and capsazepine were administered 2 h and 30 min, respectively, before ethanol.

Role of nitric oxide (NO) on the gastroprotective effect of DDF

To investigate the influence of endogenous NO in the gastroprotective effect (Arrieta et al 2003), mice (n = 8 per group) were pretreated with vehicle, DDF (100 mg kg^{-1}, i.p.) or L-arginine (600 mg kg^{-1}, i.p.) alone or in their combinations with L-NAME (20 mg kg^{-1}, i.p.) before induction of gastric damage with ethanol (0.2 mL of ethanol, 96%); DDF was administered 45 min before and L-NAME and L-arginine were given 30 min before ethanol.

Role of K_{ATP} channels on the gastroprotective effect of DDF

To verify a likely activation of ATP-sensitive potassium channels in the gastroprotective effect (Peskar et al 2002), groups of mice (n = 8) were pretreated with vehicle, DDF (100 mg kg^{-1}, i.p.) or diazoxide (3 mg kg^{-1}, i.p.) alone or in their combinations with glibenclamide (5 mg kg^{-1}, i.p.) before the oral administration of 0.2 mL of ethanol (96%). DDF was given 45 min before, whereas diazoxide was administered 30 min before ethanol or glibenclamide. Glibenclamide was administered 30 min before DDF.

Statistical analysis

The results are presented as the mean ± s.e.m. of 8 mice per group. Statistical analysis was carried out using one-way analysis of variance followed by Tukey's test for parametric data and Kruskal–Wallis test followed by Dunn's test for nonparametric data. The ED50 (dose of the DDF that reduces the gastric lesion area by 50% in relation to the control value) was calculated by sigmoidal curve-fitting analysis by using GraphPAD software and expressed along with its 95% confidence limits. $P < 0.05$ was considered as indicative of statistical significance.

Results

DDF at the tested doses of 50, 100 and 200 mg kg^{-1} exhibited a dose-related protective effect against ethanol-induced gastric lesions (Table 1) and when compared to vehicle group, the extent of inhibition for the respective doses employed were 62, 72 and 96%. Against indometacin ulceration, the protection was significant only at higher doses (100 and 200 mg kg^{-1}) of DDF, with 70 and 75% decrease of gastric lesion scores, respectively. The respective ED50 values and the confidence limits (CL) for DDF gastroprotection against gastric damage induced by ethanol and indometacin were in the order of 50.87 (38.36–67.46) and 61.56 mg kg^{-1} (42.29–89.60). Misoprostol, the positive control included for the study, also offered significant protection of a similar magnitude (Table 1), against both ethanol (67% inhibition)- and indometacin (72% inhibition)-induced gastric lesions.

The level of NP-SH in the gastric mucosa of normal control rats (393.8 ± 26.7 μg g^{-1}) significantly decreased to 189.8 ± 14.1 μg g^{-1} following the administration of ethanol (Table 2). While pretreatment with NAC (750 mg kg^{-1}) almost completely replenished the ethanol-induced depletion of NP-SH (318.1 ± 18, 9 μg g^{-1}), DDF caused only a weak replenishment of NP-SH (273.9 ± 10.6 μg g^{-1}).

In mice pretreated with the vanilloid antagonist capsazepine, the gastroprotective effect of DDF (100 mg kg^{-1}) on ethanol damage was only weakly blocked, unlike that of capsaicin (0.3 mg kg^{-1}, p.o.), which was completely prevented (Figure 2A). Indometacin (10 mg kg^{-1}, p.o.) pretreatment completely abolished the protective effect of DDF as well as that of 50 μg kg^{-1} misoprostol (Figure 2B). These data indicate that the gastroprotective effect of DDF is possibly mediated by activation of capsaicin-sensitive

Table 1 Inhibition by 3,6-dimethoxy-6″, 6″-dimethyl-[2″, 3″ : 7, 8]-chromeneflavone (DDF) from *L. araripensis* on gastric damage induced by absolute ethanol and indometacin in mice

Treatment	Dose and route	Ethanol	Indometacin
		Lesion area (mm^2)	Lesion score
Control (vehicle)	—	21.42 ± 2.22	31.56 ± 3.45
DDF	$25\,mg\,kg^{-1}$, i.p.	21.20 ± 3.70	25.17 ± 1.78
	$50\,mg\,kg^{-1}$, i.p.	$8.21 \pm 2.19^{***}$	20.67 ± 2.15
	$100\,mg\,kg^{-1}$, i.p.	$5.88 \pm 1.06^{***}$	$9.43 \pm 2.19^{**}$
	$200\,mg\,kg^{-1}$, i.p.	$0.83 \pm 0.54^{***}$	$7.80 \pm 3.90^{**}$
Misoprostol	$50\,\mu g\ kg^{-1}$, p.o.	$7.02 \pm 2.61^{***}$	$8.69 \pm 0.84^{**}$

Data represent means ± s.e.m., n = 8. Mice were treated with DDF or misoprostol, 45 min and 60 min, respectively, before ethanol or indometacin administration. $^{**}P < 0.01$,$^{***}P < 0.001$ compared with control group (analysis of variance followed by Tukey test (ethanol model) or Kruskal–Wallis test followed by Dunn test (indometacin model))

Table 2 Effect of 3,6-dimethoxy-6″, 6″-dimethyl-[2″, 3″ : 7, 8]-chromeneflavone (DDF) from *L. araripensis* on the levels of non-protein sulfhydryls (NP-SH) in glandular stomach of mice treated with 96% ethanol

Treatment	Dose and route	NP-SH ($\mu g\ g^{-1}$)
Control (vehicle)	—	393.8 ± 26.7
Control (ethanol)	—	$189.8 \pm 14.1^{*}$
DDF	$100\,mg\,kg^{-1}$, i.p.	$273.9 \pm 10.6^{*\dagger}$
NAC	$750\,mg\,kg^{-1}$, p.o.	$318.1 \pm 18,9^{*\dagger}$

Data represent means ± s.e.m., n = 8. Mice were treated with DDF or *N*-acetylcysteine (NAC), 45 min and 60 min, respectively, before ethanol. $^{*}P < 0.05$ compared with control vehicle; $^{\dagger}P < 0.01$ compared with control ethanol (analysis of variance followed by Tukey test).

primary afferents and endogenous prostaglandins. L-NAME ($20\,mg\,kg^{-1}$, i.p.) significantly blocked the gastroprotection produced by DDF and L-arginine ($600\,mg\,kg^{-1}$, i.p.), suggesting the likely participation of NO (Figure 3A). The K^{+}_{ATP} channel blocker glibenclamide ($5\,mg\,kg^{-1}$, i.p.) was more efficient in abrogating the gastroprotective effect of DDF and also of diazoxide ($3\,mg\,kg^{-1}$, i.p.), indicating a potential role for K^{+}_{ATP} channels in the gastroprotection (Figure 3B).

Discussion

This study for the first time demonstrated the gastroprotective property of 3,6-dimethoxy-6″, 6″-dimethyl-[2″, 3″ : 7, 8]-chromeneflavone (DDF), a novel flavone isolated from *Lonchocarpus araripensis*. DDF pretreatment at doses of 50, 100 and $200\,mg\,kg^{-1}$ markedly reduced the gastric damage induced in mice by both ethanol and indometacin in a dose-related manner and the ED50 values obtained indicate a slightly higher potency of DDF against ethanol damage ($50.87\,mg\,kg^{-1}$) than against indometacin damage ($61.56\,mg\,kg^{-1}$). This finding is consistent with earlier reports on other flavones and flavonoids in general that show gastroprotective activity at doses in the range 5–500 mg orally or 3–$100\,mg\,kg^{-1}$ intraperitoneally (Goel et al 1988; Rao et al 1997; La Casa et al 2000; Zayachkivska et al 2005; Olaleye & Farombi 2006). Flavonoids in general possess a cytoprotective property (Robert 1979) and DDF simulated it. Cytoprotection in the stomach, consisting of mucus secretion, mucus circulation intensification and bicarbonate secretion to the gastric lumen, is highly dependent on the products of the arachidonic acid pathway and peroxidative–antioxidative balance. It has been firmly established that oxidative stress and impaired prostaglandin synthesis contribute to gastric mucosal damage in experimental models of gastric lesions induced by both ethanol and indometacin (Kwiecien et al 2002; Chattopadhyay et al 2006; Robertson et al 2007). However, antioxidants and prostaglandin analogues could attenuate gastric mucosal lesions in either of these models (Brzozowski et al 2005; Valcheva-Kuzmanova et al 2007). Accordingly, we tested the gastroprotective efficacy of DDF in comparison with misoprostol in both models of gastric injury. The results obtained clearly show that the natural flavone DDF and prostaglandin analogue misoprostol are equally effective in affording gastroprotection, possibly implicating a common cytoprotective mechanism.

Flavonoids may have powerful antioxidant effects when tested in-vitro but their poor oral bioavailability is a major limitation. However, they may be able to help protect the gastrointestinal tract against reactive species present in foods or generated within the stomach and intestines (Halliwell 2007). In this study, intraperitoneally administered DDF showed a significant effect, but it was much less sparing than that of *N*-acetyl-L-cysteine (NAC), a replenisher of sulfhydryls on gastric NP-SH content depleted by ethanol, suggesting that it has only a weak antioxidant action and possibly other mechanisms also participate in its gastroprotection.

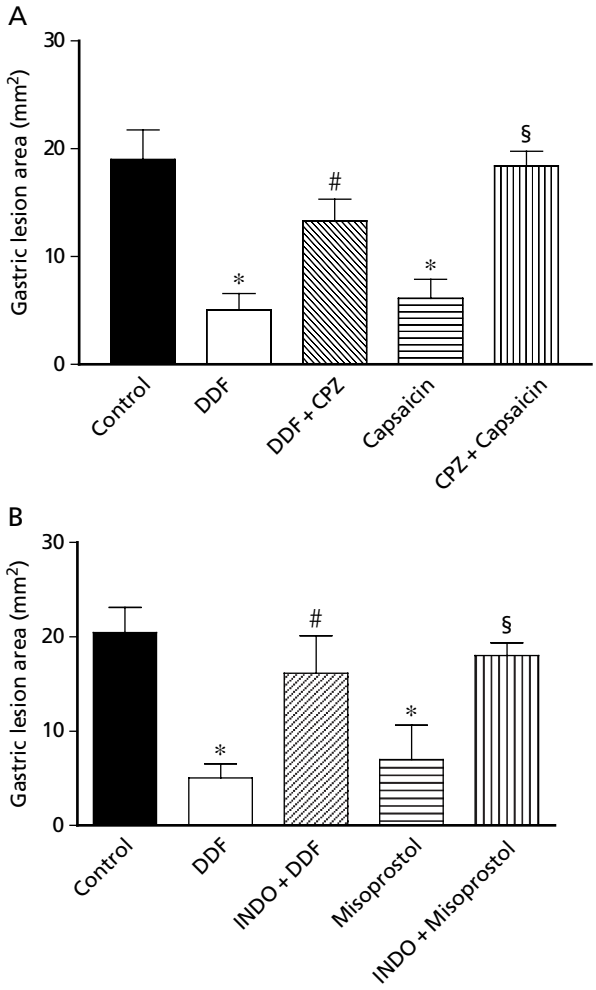


Figure 2 Role of capsaicin-sensitive sensory afferents (A) and prostaglandins (B) in the gastroprotective effect of DDF against ethanol-induced gastric damage in mice. A. Mice were pretreated with vehicle (control), DDF alone (100 mg kg^{-1}, p.o.), capsazepine (CPZ, 3 mg kg^{-1}, i.p) + DDF, capsaicin alone (0.3 mg kg^{-1}, p.o.) or capsazepine + capsaicin. B. Mice were pretreated with vehicle (control), DDF alone (100 mg kg^{-1}, i.p.), indometacin (INDO, 10 mg kg^{-1}, p.o.) + DDF, misoprostol alone (50 μg kg^{-1}, p.o.) or indometacin (INDO) + misoprostol. Data are presented as mean ± s.e.m. from 8 mice. $^{*}P < 0.05$ compared with vehicle (control) group; $^{\#}P < 0.05$ compared with DDF group; $^{\S}P < 0.05$ compared with corresponding control (analysis of variance followed by Tukey test).

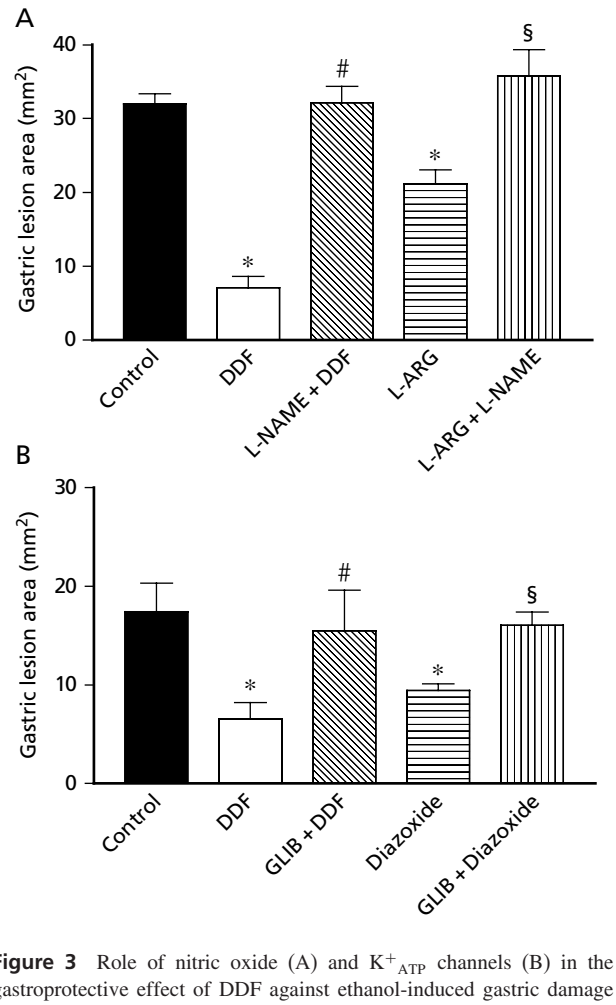


Figure 3 Role of nitric oxide (A) and K^{+}_{ATP} channels (B) in the gastroprotective effect of DDF against ethanol-induced gastric damage in mice. A. Mice were pretreated with vehicle (control), DDF alone (100 mg kg^{-1}, p.o.), L-NAME (20 mg kg^{-1}, i.p) + DDF, L-arginine alone (L-ARG, 600 mg kg^{-1}) or L-arginine + L-NAME. B. Mice were pretreated with vehicle (control), DDF alone (100 mg kg^{-1}, i.p.), glibenclamide (GLIB, 5 mg kg^{-1}, i.p.) + DDF, diazoxide alone (3 mg kg^{-1}, i.p.) or diazoxide + glibenclamide. Data are presented as mean ± s.e.m. from 8 mice. $^{*}P < 0.05$ compared with vehicle (control) group; $^{\#}P < 0.05$ compared with DDF group; $^{\S}P < 0.05$ compared with corresponding control (analysis of variance followed by Tukey test).

Some plant-derived substances have been shown to attenuate ethanol- and stress-induced gastric lesions via activation of prostaglandin, NO and sensory nerve pathways and thus improve the microcirculation (Zayachkivska et al 2004; Brzozowski et al 2005). In the experiments aimed to establish the possible mechanism of DDF gastroprotection, various agonists and antagonists were examined on an ethanol model of gastric damage. These agonists (capsaicin, misoprostol, L-arginine and diazoxide) and antagonists (capsazepine, indometacin, L-NAME and glibenclamide), employed in this study when tested alone may manifest some per-se reductions or enhancements on ethanol-induced gastric injury. We have chosen the smallest doses possible to minimize these per-se effects and at the same time not losing their agonistic or blocking effects. To verify the role of prostaglandins and TRPV1 in the gastroprotection afforded by DDF, mice were pretreated with indometacin, a non-selective cyclooxygenase inhibitor, and capsazepine, an antagonist of TRPV1. The results reveal that the gastroprotection by DDF against ethanol-induced mucosal injury was more vulnerable to indometacin than to capsazepine, suggesting a limited role for the capsaicin-sensitive afferents rather than endogenous prostaglandins in its gastroprotection. NO also appears to be a key mediator of gastrointestinal mucosal defence. NO, produced via activity of NO-synthase (NOS), appears to be one of the major factors, involved in the regulation of the gastric blood flow (GBF) and gastric microcirculation

(Wallace 2006). NO-releasing drugs protect against ethanol-induced gastric lesions and, conversely, inhibition of NO synthesis increases the susceptibility of the stomach to ethanol injury (Kawano & Tsuji 2000). In this study, L-arginine ($600\,mg\,kg^{-1}$)- and DDF ($100\,mg\,kg^{-1}$)-induced gastroprotection was reversed by L-NAME, a non-selective NOS inhibitor, suggesting that the gastroprotective effect of DDF is mediated, in part, by NO. It has been shown that prostaglandins mediated gastroprotection involving, at least in part, the activation of K^+_{ATP} channels (Peskar et al 2002). In our study, glibenclamide, a blocker of K^+_{ATP} channels, very potently antagonized the gastroprotective effect of DDF and diazoxide. Since the protection afforded by DDF is additionally indometacin-sensitive, we assume that endogenous prostaglandins act as activators of K^+_{ATP} channels and thus might contribute to enhanced gastric microcirculation.

Conclusion

The results of this study indicate a cytoprotective role for 3,6-dimethoxy-6″, 6″-dimethyl-[2″, 3″ : 7, 8]-chromeneflavone from *L. araripensis*, affording gastroprotection against gastric mucosal damage induced by ethanol and indometacin. DDF gastroprotection is possibly mediated by plurimechanisms that include an antioxidant effect preventing the depletion of gastric NP-SH, stimulation of endogenous prostaglandins and nitric oxide release, activation of capsaicin-sensitive gastric afferents and K^+_{ATP} channels opening, and these effects are likely to be accompanied by an increase in gastric microcirculation. Because of such an interesting wide spectrum of activity, future studies should address the likely synergistic effect of DDF with other gastroprotective agents like omeprazole or misoprostol. Such synergism may have an advantage in combating the undesirable side effects of these specific agents. From the clinical point of view, DDF may well be more suitable for adjunctive therapy to combat chemotherapy or NSAID-associated gastropathies.

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