



Investigation of the Mechanisms Underlying the Gastroprotective Effect of *Cymbopogon Citratus* Essential Oil

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ABSTRACT

Cymbopogon citratus is a medicinal plant popularly used in Brazil for the treatment of various diseases, and the research interest in this plant is justifiable because of its potential medicinal value in stomachache and gastric ulcer. This study was aimed to test the validity of this practice by using experimental models of gastric ulcer and to clarify the mechanisms of gastroprotection by *C. citratus* leaves essential oil (EOCC). EOCC was evaluated for the ability to protect the gastric mucosa against injuries caused by necrotizing agents (absolute ethanol and aspirin) in rodents. The results of this study revealed that EOCC possesses a dose-independent anti-ulcer effect against the different experimental models. EOCC pretreatment depicted a higher preventive index in ethanol-(88%) and aspirin-induced (76%) acute ulceration. On pretreatment of mice with indomethacin, the cyclooxygenase inhibitor slightly suppressed the gastroprotective effect of EOCC (48.5%). Furthermore, EOCC gastroprotection was not attenuated in mice pretreated with L-NAME (85.2%), glibenclamide (100%), or yohimbine (79.7%), the respective inhibitors of nitric oxide synthase, K⁺_{ATP} channel activation, and α_2 receptors. These results confirmed the traditional use of *C. citratus* for the treatment of gastric ulcer. Thus, we provide the first evidence that EOCC reduces gastric damage induced by ethanol, at least in part, by mechanisms that involve endogenous prostaglandins.

Key words: *Cymbopogon citratus*, essential oil, gastroprotection, mechanisms

INTRODUCTION

Gastric ulcers are caused due to imbalances between offensive and defensive factors of the gastric mucosa.^[1]

Oral administration of absolute ethanol in rats is noxious for the stomach, affecting the gastric mucosa topically by disrupting its barrier and provoking pronounced microvascular changes in few minutes after its application. Thus, rapid and strong vasoconstriction is accompanied by rapid and vigorous arteriolar dilation, and this combination of microvascular events induces damage in mucosal capillaries.^[2,3] Pathophysiology of ulcer is due to an imbalance between aggressive factors (acid, pepsin, *Helicobacter pylori*, and non-steroidal anti-inflammatory agents) and local mucosal defensive factors (mucus bicarbonate, blood flow, and prostaglandins).^[4] The

Access this article online	
Quick Response Code:	Website: www.jyoungpharm.in
	DOI: 10.4103/0975-1483.93578

lipid peroxidation mediated by reactive oxygen species is an important cause of destruction and damage to cell membranes, and it is involved in the pathogenesis of acute mucosal injury induced by ethanol, ischemia-reperfusion, and indomethacin.^[5,6]

Natural medicinal products have been used for millennia for the treatment of multiple ailments. Although many have been superseded by conventional pharmaceutical approaches, there is currently a resurgence of interest in the use of natural bioactive products by the general public, with many healthy subjects and patients taking them for the prevention and treatment of multiple conditions, including gastrointestinal disorders and postoperative recovery.^[7] Unfortunately, current evidence of the scientific validity of many of these traditional and commercial compounds is severely limited.^[6]

Cymbopogon citratus (DC.) Stapf, popularly known as citronella grass or lemongrass, belongs to the Poaceae family, which comprises approximately 500 genus and 8,000 herb species. The plant is a perennial grass that is widespread throughout the world, mainly in the tropical and savannah regions. The tea from its leaves has been widely used as an antiseptic, antifever, antidyspeptic, carminative, tranquilizer, and stomachic. Several investigations have demonstrated the sedative, central nervous system depressor, analgesic, antimicrobial, and fungistatic activities of *C. citratus* leaves. Neral, geranial, limonene, citronellal, myrcene, and geraniol were identified as major compounds in the essential oils of *C. citratus*.^[8] Previous results of acute oral toxicity showed *C. citratus* to be of low toxicity and as such could be considered relatively safe on acute exposure.^[9]

Although used in traditional folk medicine in Brazil for the treatment of gastric disturbances, there is no scientific evidence that establishes this activity. Here we report the effect of the essential oil extracted from *C. citratus* leaves on experimental gastric ulcer in mice.

MATERIALS AND METHODS

Plant material and oil isolation

Fresh leaves (1.750 g) of *C. citratus* were collected in the Medicinal and Aromatic Plant Garden at Pimenta Campus of Regional University of Cariri, Crato, CE, Brazil, in March 2007. Leaves were triturated and extracted by hydrodistillation for 2 h using a Clevenger-type apparatus. *C. citratus* leaves essential oil (EOCC) was collected and then dried using anhydrous sodium sulfate and subsequently stored under low light conditions at <10°C until analysis.

Essential oil analysis

Analysis of EOCC was performed using Gas Chromatography-Mass Spectroscopy (GC/MS QP5050A, Shimadzu, Kyoto, Japan). The instrument was equipped with a GC-17A gas chromatograph supplied with DB-5HT capillary column (30 m × 0.251 mm, 0.1 μm film thickness); carrier gas, helium 1.0 mL/min; column inlet pressure, 56.7 kPa; column flow, 1.0 mL/min; linear velocity, 36.5 cm/s; total flow, 32 mL/min; injector temperature, 250°C; detector temperature, 270°C; column temperature, 60 (2 min) to 180°C (4 min) at 4°C/min, then 180 to 260°C at 10°C/min (10 min); mass spectrometer operating conditions, 70 eV ionization energy. Identification of individual components was based on their mass spectral fragmentation using two computer library MS searches (wiley 229), retention indices, and comparison with literature data. EOCC represented 0.65% of their total fresh weight. Chromatographic analysis permitted the identification and quantification of 99.34% of the volatile components of the oil, including geranial (45.47%), neral (37.42%), and myrcene (12.44%), as presented in Table 1.

Chemicals and drugs

The following drugs and chemicals were used: absolute ethanol (EtOH; BDH, UK); N-G-nitro-L-arginine methyl ester (L-NAME), yohimbine, and glibenclamide (Sigma Chemical Company, St. Louis, MO); and indomethacin (Indocid[®], Merck Sharp and Dohme, Brazil). All other chemicals used were of analytical reagent grade (Merck). EOCC was first dissolved in Tween 80 and subsequently diluted in distilled water, and the resulting solutions of EOCC did not exceed concentration of Tween 80 beyond 2%. Other drugs were dissolved either in physiological saline or distilled water. Drug concentrations were adjusted for treatment to give a volume of 10 mL/kg. In pilot studies, 2% Tween 80 (vehicle) demonstrated no *per se* effect or influence on the ulcerogenicity of ethanol.

Table 1: Chemical composition (%) of the leaf oil of *C. citratus* leaves

Component	RT (min)	KI _{exp} *	Leaf oil (%)
Myrcene	4.4	990	12.44
Trans-β-ocimene	5.4	1047	0.24
Linalool	7.0	1100	0.66
Borneol	8.7	1162	0.67
Terpinen-4-ol	9.3	1180	1.14
Neral	11.1	1241	37.42
Trans-geraniol	11.6	1257	1.6
Geranial	12.2	1273	45.17

*Retention indices experimental. *n*-Alkanes were used as reference points in the calculation of relative retention indices

Animals

Male Swiss albino mice (20–25 g) obtained from the Central Animal House of Medicine Faculty of Juazeiro do Norte were used. They were housed in environmentally controlled conditions (22°C, 12-h light–dark cycle), with free access to standard pellet diet (Purina do Brasil LTDA, São Paulo, Brazil) and water *ad libitum*. Animals were kept in cages with raised floors to prevent coprophagy. They were fasted over a period of 15 h and were habituated to the test environment for 2 h before the experimentation. The experimental protocols were approved by the Animal Care and Use Committee of the Regional University of Ceará in accordance with the ethical guidelines of National Institute of Health, Bethesda, MD.

Gastric damage induced by ethanol

Acute gastric lesions were induced in mice ($n=8$ /group) by intragastric administration of absolute ethanol (96%) in a volume of 0.2 mL (using orogastric metal tube), EOCC dissolved in Tween (2% in distilled water as vehicle) was administered at oral doses of 50–400 mg/kg, 60 min before ethanol application. Vehicle (2% Tween 80)-treated group was included as negative control. Thirty minutes after ethanol administration, the animals were killed by cervical dislocation, stomachs were removed, opened along the greater curvature, and the area of gastric lesions was measured by planimetry, using a transparent grid. The lesion area in each animal was measured in mm² and was expressed in % in relation to total area of corpus.

Gastric damage induced by aspirin

Mice in groups ($n=8$ /group) were treated with EOCC (200 and 400 mg/kg, *per oros* [p.o.]) or vehicle (2% Tween 80 in a volume of 10 mL/kg). One hour after treatment, each animal received an oral dose of 500 mg/kg aspirin and they were killed 4 h later. 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. The mean ulcer score for each animal was calculated and compared between groups.

Effects of yohimbine, indomethacin, L-NAME, and glibenclamide pretreatments on EOCC gastroprotection

In mechanistic studies, separate experiments were realized to examine the role of α_2 -receptors, prostaglandins, nitric oxide, and K⁺_{ATP} channel activation on the gastroprotective effect of EOCC (200 mg/kg), using appropriate antagonists, yohimbine (2 mg/kg, intraperitoneal [i.p.],

indomethacin (10 mg/kg, p.o.), L-NAME (10 mg/kg, i.p.), and glibenclamide (5 mg/kg, p.o.) before the oral administration of 0.2 mL of ethanol (96%). The dose selection for these agonists and antagonists were based on our pilot experiments and on literature findings. In each case, animals were pretreated with the specific antagonist 30 min before the use of EOCC.

Statistical analysis

The data are presented as mean \pm SEM of eight animals per group, and the statistical significance between groups was analyzed by one-way analysis of variance followed by Student–Newman–Keul’s test. The differences between groups were regarded as significant at $P<0.05$.

RESULTS AND DISCUSSION

The effects of orally administered EOCC on gastric damage induced by absolute ethanol and aspirin are shown in Table 2. Orally administered absolute ethanol (0.2 mL/animal) and aspirin (500 mg/kg) induced severe gastric mucosal damage. EOCC at the tested doses of 200 and 400 mg/kg exhibited a dose-unrelated protective effect against ethanol-induced gastric lesions and compared with vehicle group, and the extent of inhibitions for the respective doses used was 88 and 85%. Against aspirin ulceration, the protection was significant at both doses of EOCC, with 76 and 67% decrease of gastric lesion scores, respectively.

In mice pretreated with α_2 -antagonist yohimbine, the gastroprotective effect of EOCC (200 mg/kg) on ethanol-induced injury persisted (79.7%) [Figures 1 and 2]. On the other hand, indomethacin (10 mg/kg, p.o.) pretreatment almost abolished the protective effect of EOCC (48.5%). Pretreatment with L-NAME (10 mg/kg, i.p.) or glibenclamide (5 mg/kg, i.p.) did not reduce the gastroprotection produced by EOCC (85.2 and 100%, respectively). These data indicate that the gastroprotective effect of EOCC could be mediated by endogenous prostaglandins.

Table 2: Effect of *C. citratus* on gastric damage induced by absolute ethanol and aspirin in mice

Treatment	Dose (p.o.)	Ethanol lesion area (%)	Aspirin lesion score
Control	—	15.99 \pm 2.71	10.63 \pm 1.91
EOCC	50	14.37 \pm 3.45	—
	100	13.75 \pm 2.24	—
	200	1.88 \pm 1.05***	2.50 \pm 0.65***
	400	2.25 \pm 1.02***	3.42 \pm 0.97**

Data are presented as mean \pm SEM of eight animals in each group. Gastric damage was induced by oral administration of either absolute ethanol (96%, 0.2 mL/animal) or aspirin (500 mg/kg), 1 h after completion of pretreatment with vehicle or EOCC. ** $P<0.01$ and *** $P<0.001$ vs control (one-way analysis of variance followed by Student–Newman–Keul test)

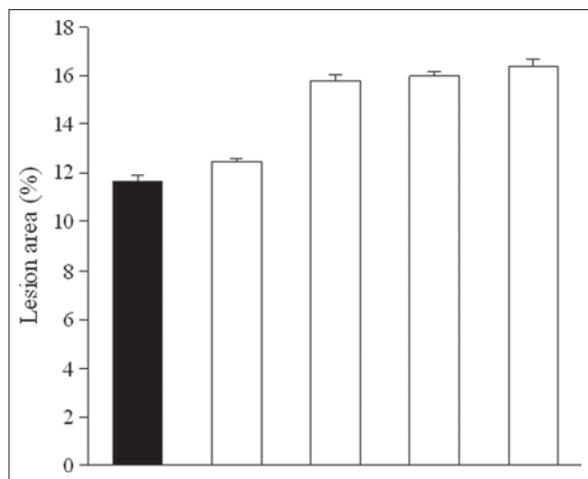


Figure 1: Effect of yohimbine 2 mg/kg (YO), indomethacin 10 mg/kg (INDO), L-NAME 10 mg/kg, and glibenclamide 5 mg/kg (GLIB) in mice on gastric mucosal injury induced by absolute ethanol. The results are shown as mean \pm SEM of eight animals per group (One-way analysis of variance and Student–Newman–Keul's test)

In the recent past, several reports documented the gastroprotective activity of latex, constituents that include flavonoids, oleo-resins, terpenes, xanthones, saponins, alkaloids, and tannins.^[10] This study describes for the first time that *C. citratus* has the gastroprotective function. EOCC significantly prevented gastric damage induced by ethanol and aspirin. A lower doses (50 and 100 mg/kg) of EOCC did not cause gastroprotection against ethanol injury. 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 ethanol.^[11]

In previous studies, *C. citratus* demonstrated an antioxidant activity in 2,2-Di-Phenyl-1-Picryl-Hydrazyl assay^[12] and in the current study; it offered cytoprotection against gastric mucosal damage induced by ethanol and aspirin. By increasing mucosal permeability and release of vasoactive products, ethanol causes vascular damage, and gastric cell necrosis which, in turn, leads to ulcer formation. 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 nonsteroidal anti-inflammatory drugs.^[10]

Some plant-derived substances have been shown to attenuate ethanol- and stress-induced gastric lesions via activation of prostaglandin, nitric oxide, and sensory nerve pathways and thus improving the microcirculation.^[13,14] Prostaglandins are of particular importance for the maintenance of gastric mucosal integrity when neuronal defense mechanisms are impaired.^[15]

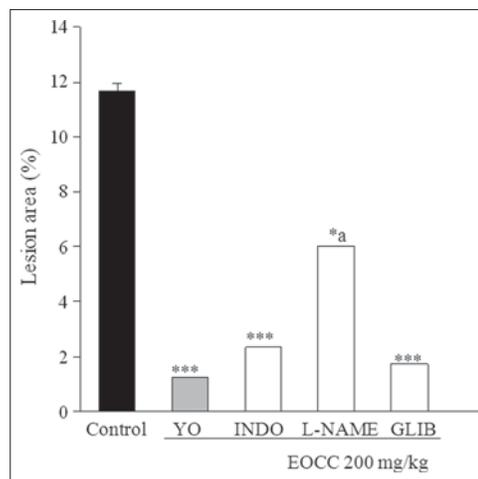


Figure 2: Effect of yohimbine 2 mg/kg (YO), indomethacin 10 mg/kg (INDO), L-NAME 10 mg/kg, and glibenclamide 5 mg/kg (GLIB) on effect of EOCC in mice on gastric mucosal injury induced by absolute ethanol. The results are shown as mean \pm SEM of eight animals per group. * $P < 0.05$ and *** $P < 0.01$ vs control; * $P < 0.05$ vs EOCC (one-way analysis of variance and Student–Newman–Keul's test)

The ability of EOCC to protect against aspirin-induced gastric ulcers may be due to enhanced synthesis of prostaglandins. On the basis of the results obtained, showing that EOCC exerts a gastroprotective effect against ethanol-induced lesions, we studied the mechanisms involved in such activity. In order to verify the role of prostaglandins in the gastroprotection afforded by EOCC, mice were pretreated with indomethacin, a non-selective cyclooxygenase inhibitor.

Plant extracts are attractive sources of new drugs and have been shown to produce promising results in the treatment of gastric ulcers.^[16] Natural medicinal products have been used for millennia for the treatment of multiple ailments. Although many have been superseded by conventional pharmaceutical approaches, there is currently a resurgence of interest in the use of natural bioactive products by the general public, with many healthy subjects and patients taking them for the prevention and treatment of multiple conditions, including gastrointestinal disorders and postoperative recovery.^[17] Previous reports indicate the gastroprotective effect of *Anisomeles indica*,^[18] *Lippia nodiflora*,^[19] *Alpinia calcarata*,^[20] *Camellia sinensis*,^[21] *Achillea wilhelmisii*,^[22] *Neregamia alata*,^[23] *Cissus quadrangularis*,^[24] and *Gymnosporia rotbiana*.^[25]

The results reveal that the gastroprotection by EOCC against ethanol-induced mucosal injury is vulnerable to indomethacin, suggesting a role for endogenous prostaglandins in gastroprotection.

In conclusion, the results of this study indicate a

cytoprotective role of EOCC affording gastroprotection against gastric damage induced by ethanol and aspirin, which is possibly mediated, in part, by endogenous prostaglandins and suggest that it might serve as a lead compound for future development of novel therapies that combat nonsteroidal anti-inflammatory drug-associated gastropathy.

ACKNOWLEDGEMENTS

This study was supported by grants from CNPq and FUNCAP. The authors are grateful to Faculty of Juazeiro do Norte for animal supply and CNPq-INCT for excitotoxicity and neuroprotection.

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How to cite this article: Fernandes CN, De Souza HF, De Oliveria G, Costa J, Kerntopf MR, Campos AR. Investigation of the mechanisms underlying the gastroprotective effect of *Cymbopogon citratus* essential oil. *J Young Pharmacists* 2012;4:28-32.

Source of Support: Grants from CNPq and FUNCAP, **Conflict of Interest:** None declared.

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