Journal of Ethnopharmacology 173 (2015) 134–138

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Ethnopharmacological communication

Gastroprotective effect of *Cymbopogon citratus* infusion on acute ethanol-induced gastric lesions in rats

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ARTICLE INFO

Article history:
Received 31 March 2015
Received in revised form
29 June 2015
Accepted 1 July 2015
Available online 6 July 2015

Keywords:
Cymbopogon citratus
Lemongrass
Infusion
Gastric ulcer
Gastroprotective

ABSTRACT

Ethnopharmacological relevance: Treatment of gastric ulcers with medicinal plants is quite common in traditional medicine worldwide. *Cymbopogon citratus* (DC) Stapf. leaves infusion has been used in folk medicine of many tropical and subtropical regions to treat gastric disturbances. The aim of this study was to assess the potential gastroprotective activity of an essential oil-free infusion from *C. citratus* leaves in acute gastric lesions induced by ethanol in rat.

Materials and methods: The study was performed on adult male Wistar rats (234.0 ± 22.7 g) fasted for 24 h but with free access to water. The extract was given orally before (prevention) or after (treatment) intragastric administration of absolute ethanol. Effects of dose (28 or 56 mg/kg of body weight) and time of contact of the extract with gastric mucosa (1 or 2 h) were also assessed. Animals were sacrificed, being the stomachs removed and the lesions were assessed by macroscopic observation and histopathology.

Results: *C. citratus* extract, given orally before or after ethanol, significantly ($P < 0.01$) reduced gastric mucosal injury compared with control group (vehicle + ethanol). The effect does not appear to be dose-dependent. Results also suggested that the extract is more effective when the time of contact with gastric mucosa increases.

Conclusions: The results of this assay confirm the gastroprotective activity of *C. citratus* extract on experimental gastric lesions induced by ethanol, contributing for the pharmacological validation of its traditional use.

1. Introduction

Peptic ulcer disease is a serious gastrointestinal disease that affects a considerable number of people in the world and has a significant impact on the quality of life (Yuan et al., 2006). Many drugs currently available for the treatment of peptic ulcers have decreased the morbidity rates, but limitations still exist, such as incidence of relapses and adverse effects (Borrelli and Izzo, 2000; Schmeda-Hirschmann and Yesilada, 2005). *Cymbopogon citratus* (DC) Stapf. (Poaceae), also known as lemongrass, is a perennial herb native to India and widely cultivated in other tropical and subtropical regions. Infusions and decoctions of *C. citratus* leaves have been used in popular medicine to treat a wide variety of health problems such as feverish conditions, nervous and gastrointestinal disorders (Contar et al., 1986). It is also recommended in folk medicine to treat gastric disturbances, stomachache, gastritis and ulcers (De Albuquerque et al., 2007; Novais et al., 2004; Tene et al., 2007). Studies on *C. citratus* leaves infusions and decoctions have shown antioxidant and anti-inflammatory activities (Carbajal et al., 1989; Cheel et al., 2005; Figueirinha et al., 2010, 2008; Francisco et al., 2013, 2011; Habana, 2001; Pereira et al., 2009) and revealed the presence of several polyphenols, including tannins, phenolic acids and flavonoids (Cheel et al., 2005; Figueirinha et al., 2008; Francisco et al., 2014; Marques and Farah, 2009; Pereira et al., 2009). Those properties may be indicative of the potential benefit in the gastrointestinal tract, mainly on gastric mucosal injury generated by oxidative damage. However, to our knowledge there are no pharmacological studies regarding the potential gastroprotective activity of this medicinal plant in literature.

This study was therefore carried out in order to assess the gastroprotective activity of an essential oil-free infusion from *C.*

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http://dx.doi.org/10.1016/j.jep.2015.07.001
0378-8741/

citratus dry leaves in acute gastric lesions experimentally induced by ethanol in rats.

2. Materials and methods

2.1 Plant material and extract preparation

Dry leaves of *C. citratus* (DC) Stapf. were acquired from Ervital® (Mezio, Castro Daire, Portugal). The plant was cultivated in the region of Mezio, Castro Daire (Portugal), in a greenhouse located 1000 m above sea level. The identity of the plant was confirmed by Prof. Dr. J. Paiva (Department of Botany, University of Coimbra, Portugal). A voucher specimen is deposited in the herbarium of the Faculty of Pharmacy, University of Coimbra (A. Figueirinha 0109).

The extract was prepared as previously described by Figueirinha and co-workers (Figueirinha et al., 2008). Briefly, an infusion was obtained by adding 150 mL of boiling water to 5 g of the powdered plant material and left to stand at room temperature for 15 min. The infusion was filtered under vacuum and the volume was made up to 150 mL with the same solvent. Afterwards, an essential oil-free infusion was obtained by repeatedly washing the infusion with *n*-hexane to remove the less polar compounds; the aqueous phase was concentrated in a rotavapor and then freeze-dried (extraction yield: 14%). Before the experiment, the extract was solubilized in water (vehicle) and given to rats, by gavage, at a dose of 28 mg/kg of body weight (*d*1) or 56 mg/kg of body weight (*d*2). These doses were calculated by extrapolation from the human dose recommended in traditional medicine (2 g of dried leaf/150 mL of water) (Contar et al., 1986).

2.2 Animals

Adult male Wistar rats (234.0 ± 22.7 g) were purchased from Charles River Laboratories (Barcelona, Spain). The rats were housed at least for 7 days before experiments in the local bioterium under standard laboratory conditions, which include a temperature of 22 ± 1 °C, relative humidity of about 50–60% and a controlled 12 h light cycle beginning early in the morning. Rats were deprived of food for 24 h prior to experiments, but allowed free access to water. During the period of starvation animals were housed in wire-bottom cages to prevent coprophagy. Animal experimentation in this study was conducted in accordance with the European guidelines for laboratory animal use and care (86/609/EEC) and the project was approved by the Portuguese Veterinary General Division (Reference no. 99–0420/000/000, 9/11/2009).

2.3 Ethanol-induced gastric lesions assay

Rats were divided into nine experimental groups ($n=6$–9). Absolute ethanol administered orally by gavage at a single dose of 1 mL/rat was used as ulcerogenic agent. Group I received vehicle (water) orally one hour before ethanol (positive control). The animals were sacrificed one hour after ulcer induction. Animals from group II received orally only vehicle, and one hour later they were sacrificed (negative control). Animals from group III received orally only extract, and one hour later they were sacrificed (negative control). Animals from groups IV, V and VI were given extract orally prior to ulcer induction (prevention groups). Animals from group IV received dose *d*1 of the extract one hour before ethanol administration. Animals from group V received dose *d*2 of the extract one hour before ethanol administration. Animals from group VI received dose *d*1 of the extract two hours before ethanol administration. One hour after ulcer induction the animals from groups IV–VI were sacrificed. Animals from groups VII, VIII and IX received extract orally after ulcer induction by ethanol (treatment groups). One hour after ethanol administration, the animals received a dose d1 (groups VII and IX) or *d*2 (group VIII) of the extract. They were sacrificed one hour (groups VII and VIII) or two hours (group IX) after extract administration. After the animals were sacrificed, each stomach was removed, opened along the greater curvature, rinsed with physiological saline and photographed. The gastric mucosa was carefully examined, macroscopically and by histopathology, and scored.

2.4 Macroscopic assessment of gastric mucosal lesions

Gastric mucosa was examined macroscopically and each lesion was graded and scored based on its incidence and severity according to an arbitrary grading system. The grade of severity was attributed differently in each parameter: loss of mucosal folds (0, 1, 2, 3); hemorrhagic areas (0, 1, 2, 3); necrotic aereas/ulcers (0, 2, 4, 6); perforation (0, 10). The ulcer index (U.I.) for each stomach was the sum of scores of all lesions. The U.I. for each experimental group was reported as median (minimum–maximum). The significance of differences between groups was assessed by Kruskal–Wallis test, followed by Mann–Whitney *U* test, when appropriate. $P<0.05$ *versus* control was taken as significant.

2.5 Microscopic assessment of gastric mucosal lesions

After macroscopic examination of the stomach, gastric tissue samples from the lesion sites were prepared for histopathological studies. The fragments were fixed in 4% neutral phosphate-buffered formalin, embedded in paraffin blocks, cut in 3 μm thick sections and stained with hematoxylin and eosin. The slides were examined under microscope and assessed for histopathological changes such as congestion, edema, hemorrhage and necrosis.

3. Results

The severity of gastric mucosal damage in the experimental groups, as measured by U.I., is listed in Table 1. Rats treated only with vehicle and *C. citratus* extract (groups II and III) showed a normal morphology of the gastric mucosa. In the positive control group, treated orally with vehicle and absolute ethanol, severe and

Table 1
Effect of essential oil-free infusion from *Cymbopogon citratus* leaves on gastric lesions induced by ethanol in rats.

Experimental group	Administered product (s)	Dose (mg/kg)	Time of contact (h)	Ulcer Index (U.I.) [median (min–max)]
Control groups:				
Group I	Vehicle, ethanol	–	–	9.0 (5–12)
Group II	Vehicle	–	–	0.0
Group III	Extract	28	1	0.0
Prevention groups:				
Group IV	Extract, ethanol	28	1	3.5 (2–4)*
Group V	Extract, ethanol	56	1	4.0 (3–5)*
Group VI	Extract, ethanol	28	2	2.5 (1–4)*
Treatment groups:				
Group VII	Ethanol, extract	28	1	4.0 (3–5)*
Group VIII	Ethanol, extract	56	1	4.0 (3–6)*
Group IX	Ethanol, extract	28	2	3.0 (1–4)*

* $P<0.01$, compared to positive control (group I) (Kruskal–Wallis test, followed by Mann–Whitney *U* test).

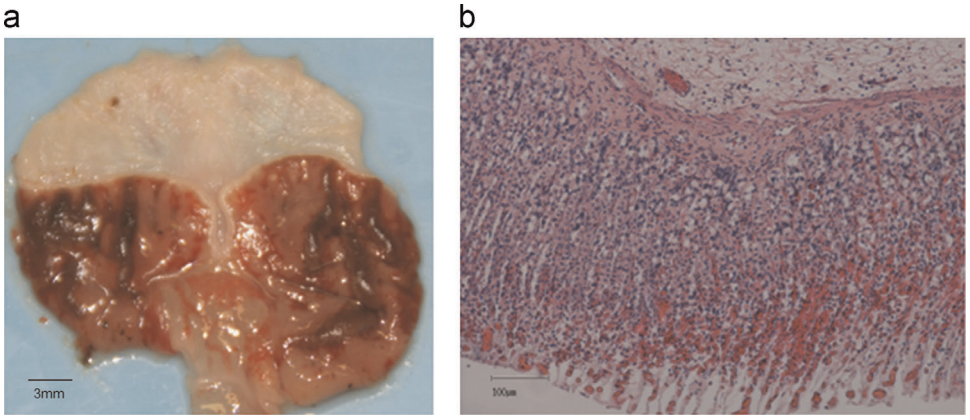

Fig. 1. Ethanol-induced gastric injury in rats. (a) Representative macroscopic aspect of the stomach, showing severe and widespread damage of the glandular mucosa one hour after ethanol administration. (b) Histological section of gastric mucosa, showing hemorrhagic areas, mucosal atrophy and presence of inflammatory infiltrate (HE staining).

widespread damage of the glandular mucosa was observed macroscopically and the U.I. was found to be very high (median: 9.0). Ethanol administration produced the expected characteristic hemorrhagic and necrotic mucosal lesions, consisting of elongated bands usually parallel to the long axis of the stomach, submucosal edema and loss of epithelial cells (Fig. 1a). In accordance with the macroscopic assessment, the histological examination of the gastric tissue samples revealed hemorrhagic areas, mucosal atrophy and presence of inflammatory infiltrate (Fig. 1b).

In the prevention groups, treated orally with *C. citratus* extract prior to ethanol, an important reduction in both number and severity of gastric lesions was observed (Fig. 2). The U.I. significantly decreased in all prevention groups ($P < 0.01$) compared to the positive control group. Similarly, when administered after ethanol administration (treatment groups), *C. citratus* extract also produced a significant reduction in the U.I. ($P < 0.01$) compared to the positive control group. However, the protective effect of the extract does not seem to be dose-dependent, as no significant differences in the U.I. were found across the doses used, both in prevention (group IV *versus* V; $P = 0.143$) and treatment groups (group VII *versus* VIII; $P = 0.154$). Although not statistically significant, the gastroprotective effects of the extract were more

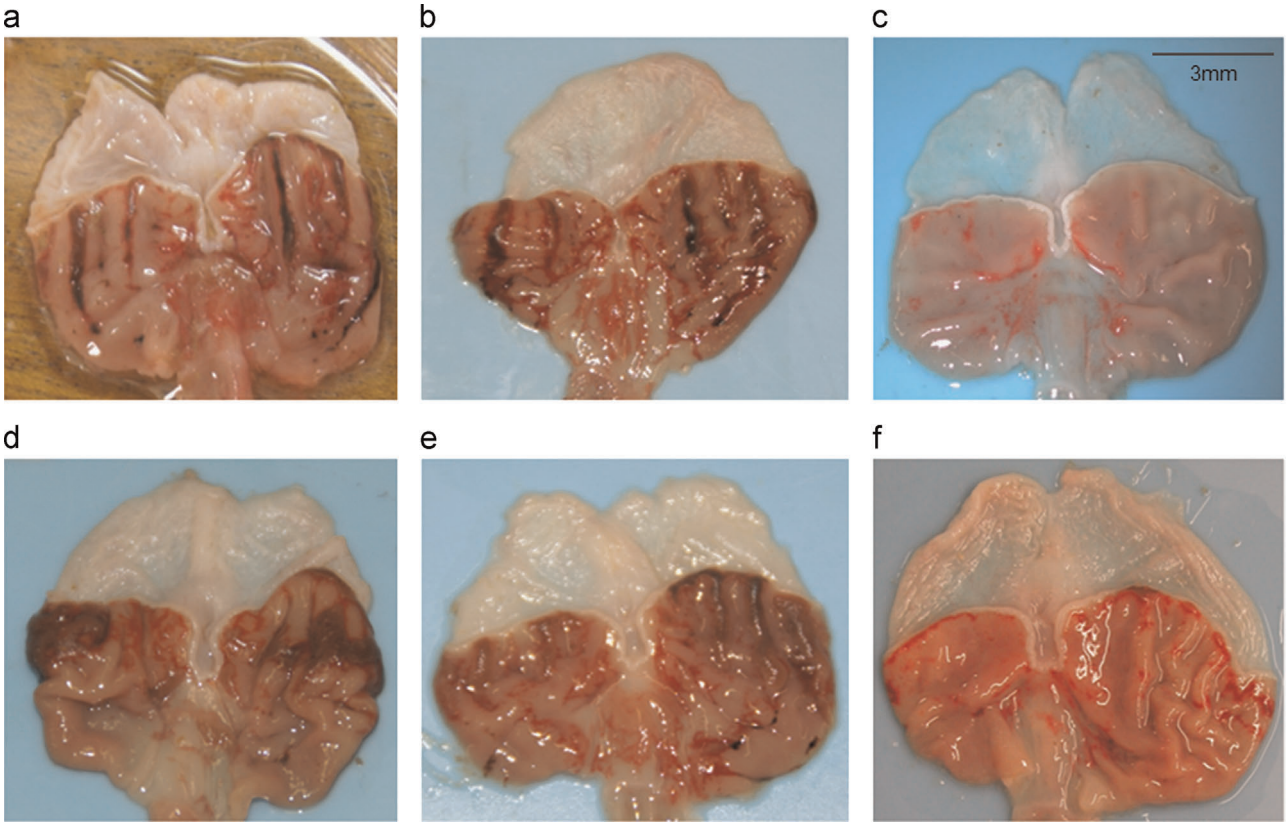

Fig. 2. Representative macroscopic aspect of the effects of *Cymbopogon citratus* extract on ethanol-induced gastric lesions in rats. (a) Stomach treated with extract, dose 28 mg/kg, one hour before ethanol (group IV). (b) Stomach treated with extract, dose 56 mg/kg, one hour before ethanol (group V). (c) Stomach treated with extract, dose 28 mg/kg, two hours before ethanol (group VI). (d) Stomach treated for one hour with extract, dose 28 mg/kg, after ethanol administration (group VII). (e) Stomach treated for one hour with extract, dose 56 mg/kg, after ethanol administration (group VIII). (f) Stomach treated for two hours with extract, dose 28 mg/kg, after ethanol administration (group IX).

pronounced when time of contact with gastric mucosa increased to 2 h (group VI and IX) compared to 1 h (groups IV and VII).

4. Discussion

The formation of gastric mucosal lesions following ethanol administration seems to occur both directly and indirectly through several mechanisms. Gastric mucosal damage induced by ethanol has been reported to involve a depletion of gastric defensive mechanisms, including disruption of the mucosal barrier, gastric mucus depletion, reduced gastric blood flow and alterations in permeability, that contribute to the development of the hemorrhagic and necrotic aspects of tissue injury (Glavin and Szabo, 1992). Furthermore, some studies suggest that oxygen-derived reactive species and lipid peroxidation are associated with gastric damage induced by ethanol. This data suggest that antioxidant compounds could be active in this experimental model, producing gastroprotective effects (Casa et al., 2000; Glavin and Szabo, 1992).

Essential-oil free infusion from *C. citratus* leaves, administered before (prevention) or after (treatment) ethanol, reduced significantly the incidence and severity of gastric lesions and consequently the U.I. These results indicate that *C. citratus* extract, at doses equivalent to the human dose recommended in traditional medicine, display a gastroprotective effect against ethanol-induced ulcer.

A previous phytochemical study of the essential oil-free infusion from *C. citratus* used in this study has indicated the presence of tannins, phenolic acids (caffeic and *p*-coumaric acid derivatives) and flavonoids (Figueirinha et al., 2008). In the flavonoid fraction, representing 6.1% of the extract, several *O*- and *C*-glycosylflavones of apigenin and luteolin were identified (Francisco et al., 2014). Many compounds from these chemical classes have been shown to possess anti-ulcer properties (Borrelli and Izzo, 2000). Tannins have radical-scavenger properties and, at low concentrations, are known to create a layer in the mucosa and to increase the resistance to chemical and mechanical injury or irritation (Borrelli and Izzo, 2000). Recently, a different group of flavanic dimmers have been identified in lemongrass infusion, which have been correlated with a pronounced anti-radical activity (Costa et al., 2015). Phenolic acids, such as caffeic, *p*-coumaric, ferulic and hydroxycinnamic acids, display anti-ulcer activity in several acute gastric ulcer models (Barros et al., 2008). It is well known that many flavonoids have antisecretory and cytoprotective properties in different experimental models of gastric ulcer (Casa et al., 2000; Mota et al., 2009). In particular, several plants with anti-ulcer activity which contain luteolin and luteolin *O*- and *C*-glycosides have been reported (Batista et al., 2004; Coelho et al., 2009, 2006; Min et al., 2006; Yesilada et al., 2000). Therefore, the polyphenolic chemical composition of the extract may contribute, at least in part, to the gastroprotective effects observed in this study.

Literature reports that many polyphenols have antiulcer activity probably because of their antioxidant properties, which could prevent the formation of free radicals in the body and also minimize injuries by oxidative reactions (Casa et al., 2000; Repetto et al., 2002). In previous studies our group demonstrated the *in vitro* antioxidant and anti-inflammatory activity of the essential oil-free infusion from *C. citratus* used in this study and of different fractions obtained from it (Figueirinha et al., 2010, 2008), providing a possible mechanism to explain the protection produced by the plant extract against ethanol-induced gastric lesions.

5. Conclusions

In conclusion, the results of this study suggest a gastroprotective activity of *C. citratus* extract against ethanol-induced gastric lesions, contributing to the pharmacological validation of its traditional use. In the future, further pharmacological evaluations are required to identify and isolate the active gastroprotective compounds in the plant as well as elucidating their mechanisms of action.

Acknowledgments

We thank Ervital® for providing the plant material, and Dr. J. Paiva (Life Sciences Department, University of Coimbra, Portugal).

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