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Inhibition of gastric acid secretion by a standardized aqueous extract of *Cecropia glaziovii* Sneth and underlying mechanism

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Abstract

Cecropia glazioui Sneth (Cecropiaceae) is used in folk medicine in tropical and subtropical Latin America as cardiotonic, diuretic, hypotensive, anti-inflammatory and anti-asthmatic. The hypotensive/antihypertensive activity of the plant aqueous extract (AE) and isolated butanolic fraction (BuF) has been confirmed and putatively related to calcium channels blockade in vascular smooth musculature [Lapa, A.J., Lima-Landman, M.T.R., Cysneiros, R.M, Borges, A.C.R., Souccar, C., Barreta, I.P., Lima, T.C.M., 1999. The Brazilian folk medicine program to validate medicinal plants – a topic in new antihypertensive drug research. In: Hostettman, K., Gupta, M.P., Marston, A. (Eds.), Proceedings Volume, IOCD/CYTED Symposium, Panamá City, Panamá, 23–26 February 1997. Chemistry, Biological and Pharmacological Properties of Medicinal Plants from the Americas. Harwood Academic Publishers, Amsterdam, pp. 185–196; Lima-Landman, M.T., Borges, A.C., Cysneiros, R.M., De Lima, T.C., Souccar, C., Lapa, A.J., 2007. Antihypertensive effect of a standardized aqueous extract of *Cecropia glaziovii* Sneth in rats: an *in vivo* approach to the hypotensive mechanism. Phytomedicine 14, 314–320]. Bronchodilation and antidepressant-like activities of both AE and BuF have been also shown [Delarcina, S., Lima-Landman, M.T., Souccar, C., Cysneiros, R.M., Tanae, M.M., Lapa, A.J., 2007. Inhibition of histamine-induced bronchospasm in guinea pigs treated with *Cecropia glaziovi* Sneth and correlation with the *in vitro* activity in tracheal muscles. Phytomedicine 14, 328–332; Rocha, F.F., Lima-Landman, M.T., Souccar, C., Tanae, M.M., De Lima, T.C., Lapa, A.J., 2007. Antidepressant-like effect of *Cecropia glazioui* Sneth and its constituents – *in vivo* and *in vitro* characterization of the underlying mechanism. Phytomedicine 14, 396–402]. This study reports the antiulcer and antisecretory gastric acid activities of the plant AE, its BuF and isolated compounds with the possible mechanism involved. Both AE and BuF were assayed on gastric acid secretion of pylorus-ligated mice, on acute models of gastric mucosal lesions, and on rabbit gastric H^+, K^+-ATPase preparations. Intraduodenal injection of AE or BuF (0.5–2.0 g/kg, i.d) produced a dose-related decrease of the basal gastric acid secretion in 4-h pylorus-ligated mice. At 1.0 g/kg, BuF decreased the volume (28%) and total acidity (33%) of the basal acid secretion, and reversed the histamine (2.5 mg/kg, s.c.)- or bethanecol (1.0 mg/kg, s.c.)-induced acid secretion to basal values, indicating inhibition of the gastric proton pump. Pretreatment of mice with the BuF (0.05–0.5 g/kg, p.o.) protected against gastric mucosal lesions induced by 75% ethanol, indomethacin (30 mg/kg, s.c.) or restraint at 4 °C. BuF also decreased the gastric H^+, K^+-ATPase activity *in vitro* proportionately to the concentration (IC_{50} = 58.8 μg/ml). The compounds isolated from BuF, consisting mainly of cathechins, procyanidins and flavonoids [Tanae, M.M., Lima-Landman, M.T.R., De Lima, T.C.M., Souccar, C., Lapa, A.J., 2007. Chemical standardization of the aqueous extract of *Cecropia glaziovii* Sneth endowed with antihypertensive, bronchodilator, antacid secretion

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and antidepressant-like activities. Phytomedicine 14, 309–313], inhibited the *in vitro* gastric H^+, K^+-ATPase activity at equieffective concentrations to that of BuF. The results indicate that *C. glazioui* constituents inhibit the gastric proton pump; this effect may account for the effective antisecretory and antiulcer activities of the standardized plant extract.

Keywords: *Cecropia glazioui*; Antiulcer; Gastric acid secretion; H^+, K^+-ATPase; Medicinal plant; Phytomedicine

Introduction

Cecropia species are fast-growing and short-lived trees native to tropical Central and South America regions. About 75 Cecropia species have been described in South and Latin America, some of them were reported to present hypotensive, vasorelaxant (Ramos Almeida et al., 2006), diuretic (Vargas Howell and Ulate Montero, 1996), hypoglycaemic (Andrade-Cetto and Heinrich, 2005), cardiotonic, sedative (Consolini et al., 2006), wound healing (Nayak, 2006), analgesic, anti-inflammatory (Perez-Guerrero et al., 2001) and anti-microbial (Rojas et al., 2006) properties.

The species *Cecropia glazioui* Sneth (Cecropiaceae), in particular, is reputed in Latin America folk medicine as cardiotonic, diuretic, hypotensive, anti-inflammatory and to relieve respiratory ailments (asthma, bronchitis, whooping coughs) (Braga, 1960, Lorenzi and Matos, 2002). Previous studies in our lab confirmed the antihypertensive activity of the plant aqueous extract (AE) and its purified butanolic fraction (BuF) (Lima-Landman et al., 2007), the latter mainly constituted by cathechins, procyanidins, and flavonoids (Tanae et al., 2007). The effect did not involve inhibition of the plasma angiotensin I converting enzyme activity (Ninahuaman et al., 2007), but was related to a blockade of calcium influx in the vascular smooth musculature (Lapa et al., 1999). Repeated treatment with the plant extract induced anxiolytic and antidepressant-like effect in both rats and mice (Rocha et al., 2002), possibly related to a blockade of monoamines uptake in the CNS (Rocha et al., 2007). The same extract was also shown to protect against histamine-induced bronchospasm in guinea-pig *in vivo* (Delarcina et al., 2007), confirming the reputed bronchodilator activity of *C. glazioui*.

This study describes the *in vivo* antacid/antiulcer activity of *C. glazioui* detected during the pharmacological screening of the plant AE and BuF; the effect was putatively attributed to isolated constituents that inhibited the proton pump activity *in vitro*.

Materials and methods

Plant extract

C. glazioui was cultivated at the Pluridisciplinary Center of Chemical, Biological and Agronomic Studies of the University of Campinas in the State of São Paulo (Magalhães, 2000), and the AE standardized as detailed before (Tanae et al., 2007). Briefly, the AE of the plant aerial parts (2% w/v) was partitioned with *n*-butanol yielding a BuF (yield = 20%) endowed with pharmacological activity 5–10-fold greater than that of AE. After evaporation of the organic solvent, BuF was freeze-dried and fractionated in preparative HPLC columns yielding ten purified compounds chemically identified as procyanidins (B2, B3, B5, and C1), cathechin, epicathechin, and flavonoids (orientin, isoorientin, and isovitexin) (Tanae et al., 2007).

Animals

Male Swiss albino (25–30 g) mice were used for testing the antiulcer activity, while F1 mice, a hybrid from a cross between inbred C57Bl/6 female and Balb/c male, were used for the pylorus ligature experiments because the little bleeding under surgery of these mice minimized interferences with acid titration of gastric secretion. Similar results were, however, obtained with the plant extracts in either species. All animals were housed under a controlled dark/light cycle and temperature ($22 \pm 2\,^{\circ}C$), with free access to standard chow diet and tap water. Solid food was withdrawn 15–18 h before the experiments. The experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the USA NIH and were approved by the institutional Animal Investigation Ethical Committee (CEP No. 005/00; 1106/03).

Gastric acid secretion

Pylorus ligature was performed in fasting mice under light ether anesthesia as previously described (Shay et al., 1945). The AE, BuF (0.5–2.0 g/kg) or the positive control ranitidine (0.05 g/kg) were injected into the duodenal lumen (i.d.) and the abdominal wall was sutured. Control animals received corresponding volumes of saline. After 4 h the animals were euthanized by ether inhalation, the gastric secretion was collected and its final volume and pH were determined. Total acidity of the gastric juice was titrated with 0.01 N NaOH, using 2% phenolphthalein as indicator. The BuF (1 g/kg) was also tested on gastric secretion stimulated by bethanecol (1.0 mg/kg, s.c.) or histamine (2.5 mg/kg, s.c.), at doses selected in preliminary dose-