IV Incontro SYRP:
S.I.Fit. Young Researchers Project
Giovani Ricercatori in Fitoterapia

EFFECTS OF DIPLACONE AND CUDRAFLAVONE B ON GASTROINTESTINAL MOTILITY

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BACKGROUND

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder with high social and economic impact, characterized by abnormal gut motor function, enhanced visceral perception, abnormalities in central pain processing and neurochemical coding, and altered gut microbiota, besides psychosocial and genetic factors (De Palma et al., 2014). The etiology remains unclear and there is no causal treatment. The only available therapy have purely symptomatic effects and only limited efficacy. Several studies have shown the anti-inflammatory and antioxidant effects of flavonoids, making them potential candidates for treating inflammatory processes (Vochyanova et al., 2015). Recently, diplacone, a geranylated flavanone, has been shown to ameliorate dextran sulfate sodium-induced colitis in rats (Vochyanova et al., 2015) whereas cudraflavone B, a prenylated flavonoid, has been shown to exert anti-inflammatory and neuroprotective effects (Lee et al., 2014; Zelova et al., 2014).

CONCLUSION

Our study provides evidence that flavonoids, such as diplacone and cudraflavone B:

- ✓ influence enteric motor function;
- ✓ have potential clinical interest for treating intestinal dysmotility associated to IBS.

AIM

To evaluate the effects of diplacone and cudraflavone B on gastrointestinal neuromuscular function.

RESULTS

EFFECTS OF CUDRAFLAVONE B ON INTESTINAL MOTILITY

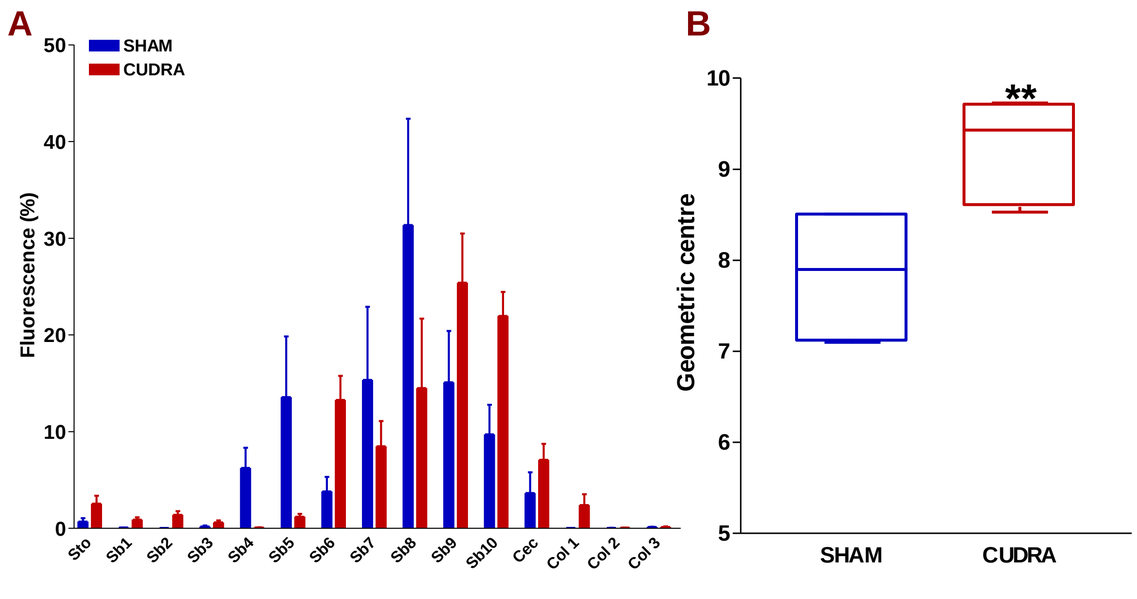


Figure 1. Impaired gastrointestinal transit in mice treated with cudraflavone B.
Panel A. Gastrointestinal (GI) transit was measured as % of nonabsorbable FITC-dextran distribution in 15 gut segments, comprising stomach (Sto), small intestine (Sb 1–10), cecum (Cec) and colon (Col 1–3), 30 minutes after oral administration in SHAM and CUDRA mice (N = 3). *Panel B.* Analysis of Geometric center (GC) demonstrated a significant alteration of GI transit in CUDRA mice compared to SHAM mice (GC_{CUDRA}= 9.4±0.2 vs GC_{SHAM}=7.8±0.2; N = 3; **p<0.01 vs SHAM). SHAM = mice treated with vehicle (1% methyl cellulose); CUDRA = mice treated with cudraflavone B (25 mg/kg suspended in 1% methyl cellulose).

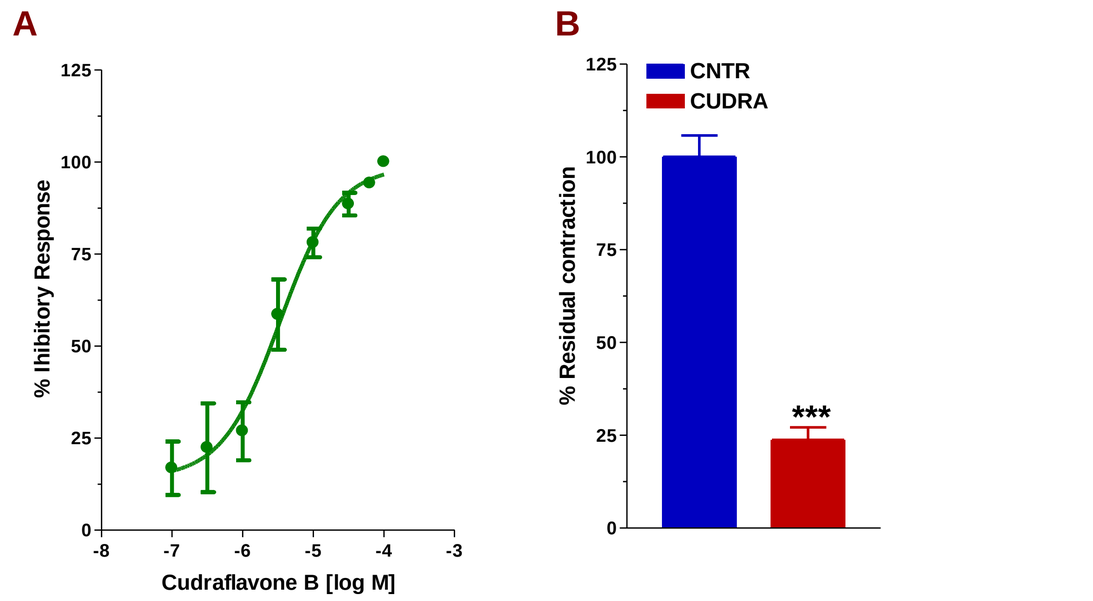
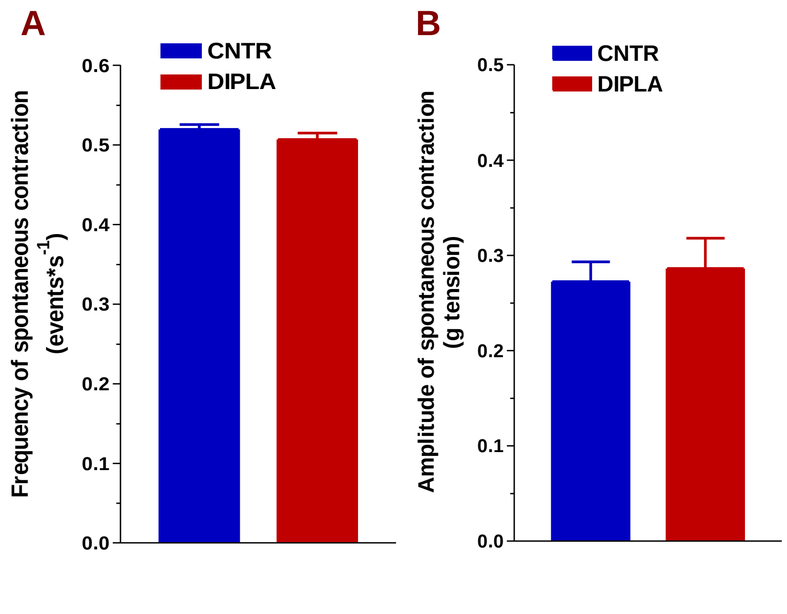


Figure 2. In vitro inhibitory response mediated by cudraflavone B on isolated ileal segments.
Panel A. Concentration-response curve to cudraflavone B (0.1-100 µM) in isolated mouse ileal preparations (N = 3). Data are expressed as percentage of the decrease in the amplitude of the spontaneous contraction. The value 100% corresponds to 75% reduction of spontaneous contractions. CNTR = isolated ileal segments in absence of cudraflavone B; CUDRA = isolated ileal segments treated with increasing concentrations of cudraflavone B. *Panel B.* Percentage of residual contraction at the higher concentration of cudraflavone B (100 µM; N = 3) . ***p<0.001 vs CNTR.

EFFECTS OF DIPLACONE ON SMALL INTESTINE CONTRACTILITY



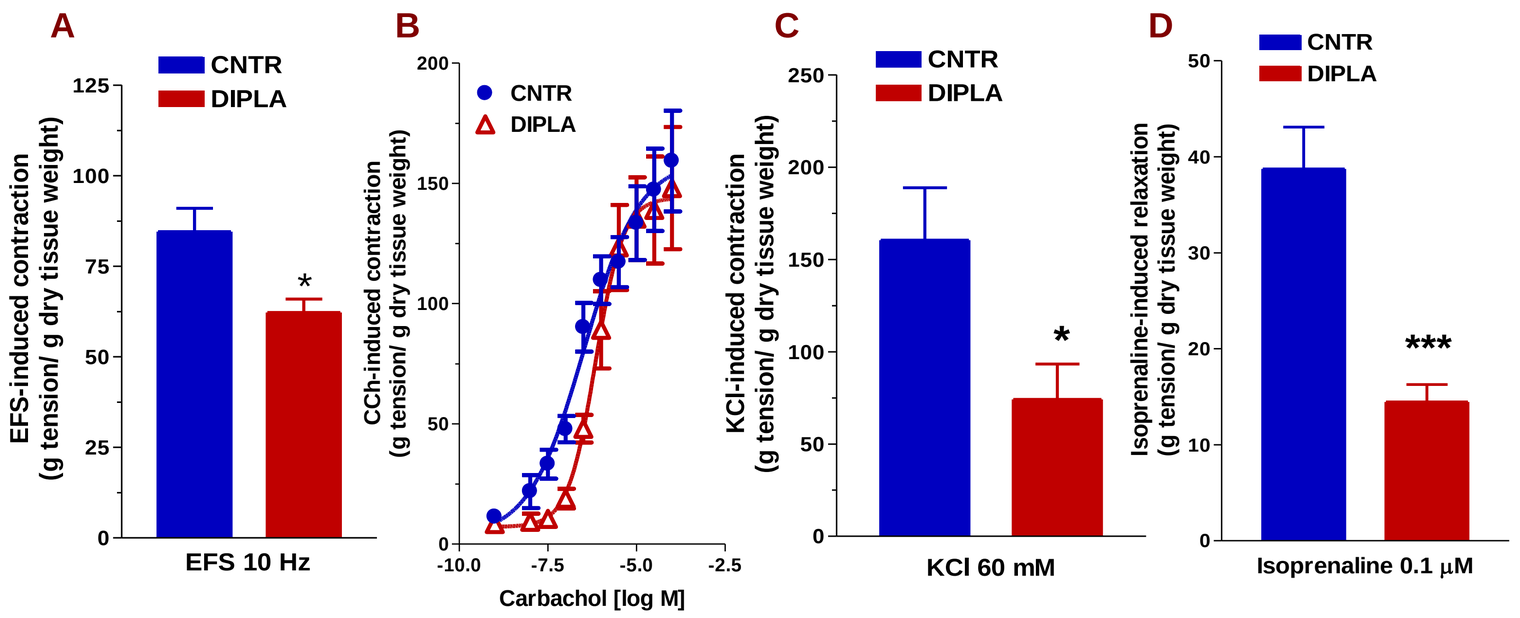


Figure 3. Effects of diplacone on the ileum spontaneous contractility.
Panels A and B. Diplacone (100 µM) does not alter the spontaneous contractility of ileum in frequency and amplitude (N = 4). CNTR = isolated ileal segments in absence of diplacone; DIPLA = isolated ileal segments pretreated with 100 µM diplacone.

Figure 4. Effects of diplacone on the excitatory and inhibitory contractile responses.
Panel A. Effect of electrical field stimulation (EFS; 10 Hz) on the excitatory response of ileal segments in presence or absence of 100 µM diplacone pretreatment. *Panels B and C.* Concentration–response curves to carbachol (0.001-100 µM) and excitatory effect elicited by KCl 60 mM in ileum segments in presence or absence of 100 µM diplacone pretreatment. *Panel D.* Inhibitory response to isoprenaline (0.1 µM) of ileal segments in presence or absence of 100 µM diplacone pretreatment. (N = 4). *p<0.05; ***p<0.001 vs CNTR. CNTR = isolated ileal segments in absence of diplacone; DIPLA = isolated ileal segments pretreated with 100 µM diplacone.

METHODS

- ✓ Diplacone was isolated from the unripe fruits of *Paulownia tomentosa* (Thunb.) Steud. (Paulowniaceae) as previously described (Vochyánová et al., 2015).
- ✓ Cudraflavone B was isolated from *Morus alba* L. (Moraceae) as previously described (Zelová et al., 2014).

Male mice C57BL/6J (8±1 weeks old; Charles River Laboratories, Italy) were used.

- ✓ In vitro contractility was assessed in full-thickness distal ileum segments isolated from control (CNTR) mice (N = 4), mounted vertically in organ baths containing 10 mL of oxygenated (95% O_2 and 5% CO_2) and heated (37°C) Krebs solution. Changes in muscle tension following carbachol (CCh; 0.01–100 µmol/L) or KCl (60 mmol/L) or isoprenaline (0.1 µmol/L) stimuli in presence or absence of pretreatment with diplacone (DIPLA; 100 µM) were recorded by isometric transducers. Neuronal-mediated contractions were evoked by electrical field stimulation (EFS; 10 Hz; 40 V). The effect was performed in presence of diplacone (DIPLA; 100 µM). In a second series of experiments concentration-response curve to cumulative addition of cudraflavone B (CUDRA, 0.1 µM-100 µM) was performed to assess changes in spontaneous contractility (Brun et al., 2013).
- ✓ Gastrointestinal transit was obtained by measuring the distribution of nonabsorbable fluorescein isothiocyanate labeled dextran (FITC-dextran, 70 KDa) from the stomach to the colon. A cudraflavone B sospension (25 mg/kg in 1% methyl cellulose) or a 1% methyl cellulose bolus was administered by gavage to CUDRA and SHAM mice, respectively, 2 hours before administering FITC-dextran. Each segment of the bowel of CUDRA and SHAM mice (N = 3) was examined separately and luminal contents was collected and clarified by centrifugation (12,000 rpm, 10 min, 4°C). Fluorescent intensity of the supernatant was measured at 492/521 nm using a fluorometer (Victor, PerkinElmer). Data were expressed as % of fluorescence per segment and gastrointestinal transit was calculated as the geometric center (GC) of distribution of the fluorescent probe (Brun et al., 2013).
- ✓ Statistical analysis was carried out using GraphPad Prism version 3.03 (San Diego, Ca-USA). Data are expressed as means±SEM; N refers to number of animals per group. Data were analyzed for statistical significance by unpaired Student's t-test or by one-way ANOVA followed by Newman-Keuls multiple comparison post-test. P values <0.05 were considered statistically significant.

REFERENCES

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