



The effect of cadmium on calretinin expression in the claustrum of the rat after consumption the beetroot/carrot juice



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Juice preparation

- ☐ washing and pressing
- ☐ pH ↓ to 4.0 (1.5 g of citric acid/100 cm³ of juice)
- ☐ sampled by 16 consumers who regularly consumed this type of product, a 9- point hedonic scale
- ☐ mixed Opolski beetroot juice and Nantejska carrot juice 80:20 v/v



→ betalaines, water-soluble nitrogen pigments

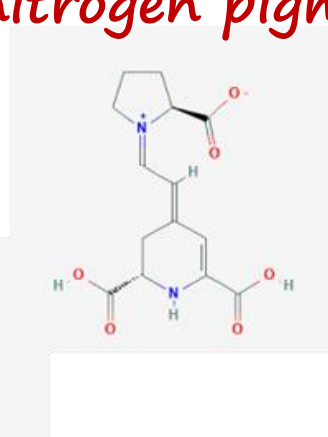
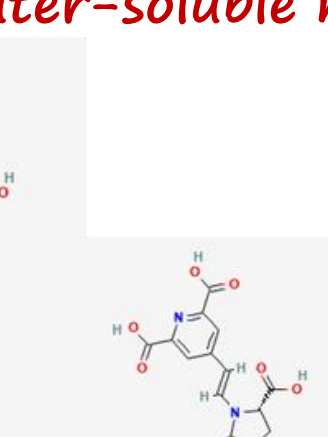
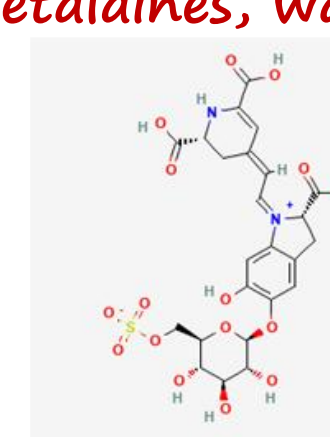
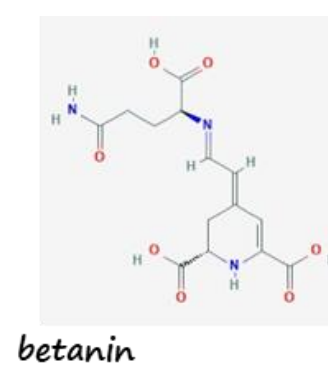


Table 1. Biological activities of Opolski/ Nantejska 80:20 v/v juice.

Biological property	Value
Anti-acetylcholinestase activity	73.0 ± 4.4 %
Anti-butyrylcholinestase activity	70.0 ± 2.0 %
Antioxidant activity (ABTS* *)	80.0 ± 2.0 %
Antioxidant activity (DPPH*)	87.0 ± 2.0 %
Inhibition of linoleic acid oxidation	13.0 ± 0.1 %
Ferric antioxidant power (FRAP)	22.03 ± 0.76 (µg Trolox/cm ³)
Cupric reducing antioxidant capacity (CUPRAC)	28.9 ± 1.3 %
Hydroxyl radical averting capacity (HORAC)	16.0 ± 0.6 %

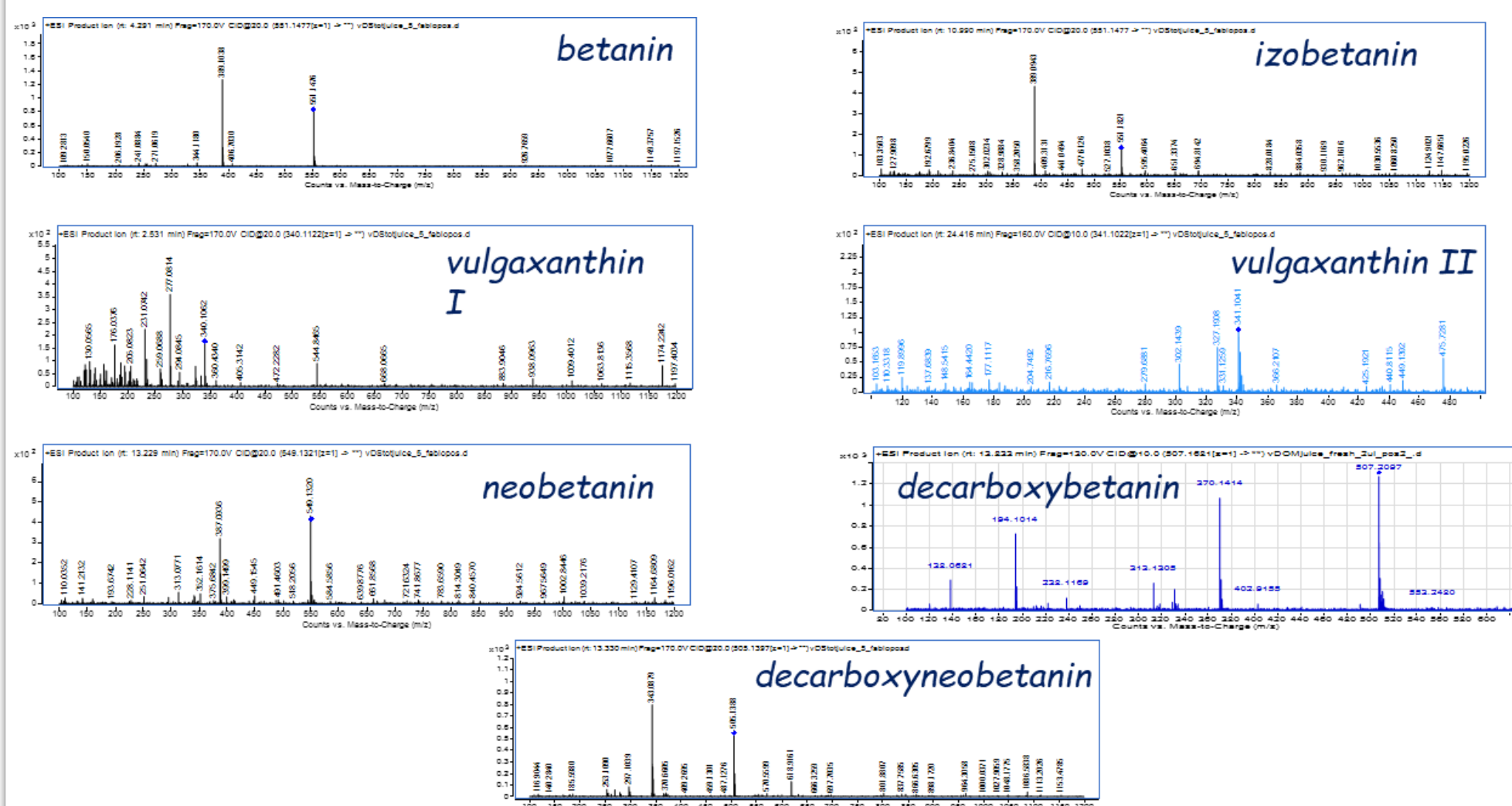
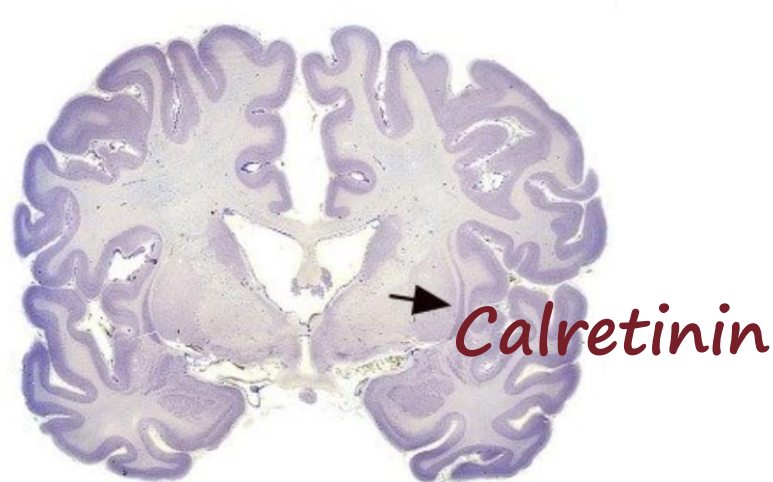


Fig. 1. MS/MS spectra of the identified components of the extract after fragmentation.



- ✓ localization - interneurons (GABA_ergic)
- ✓ buffering properties - calcium homeostasis
- ✓ buffering protein - parvalbumin, calbindin d28k, calretinin

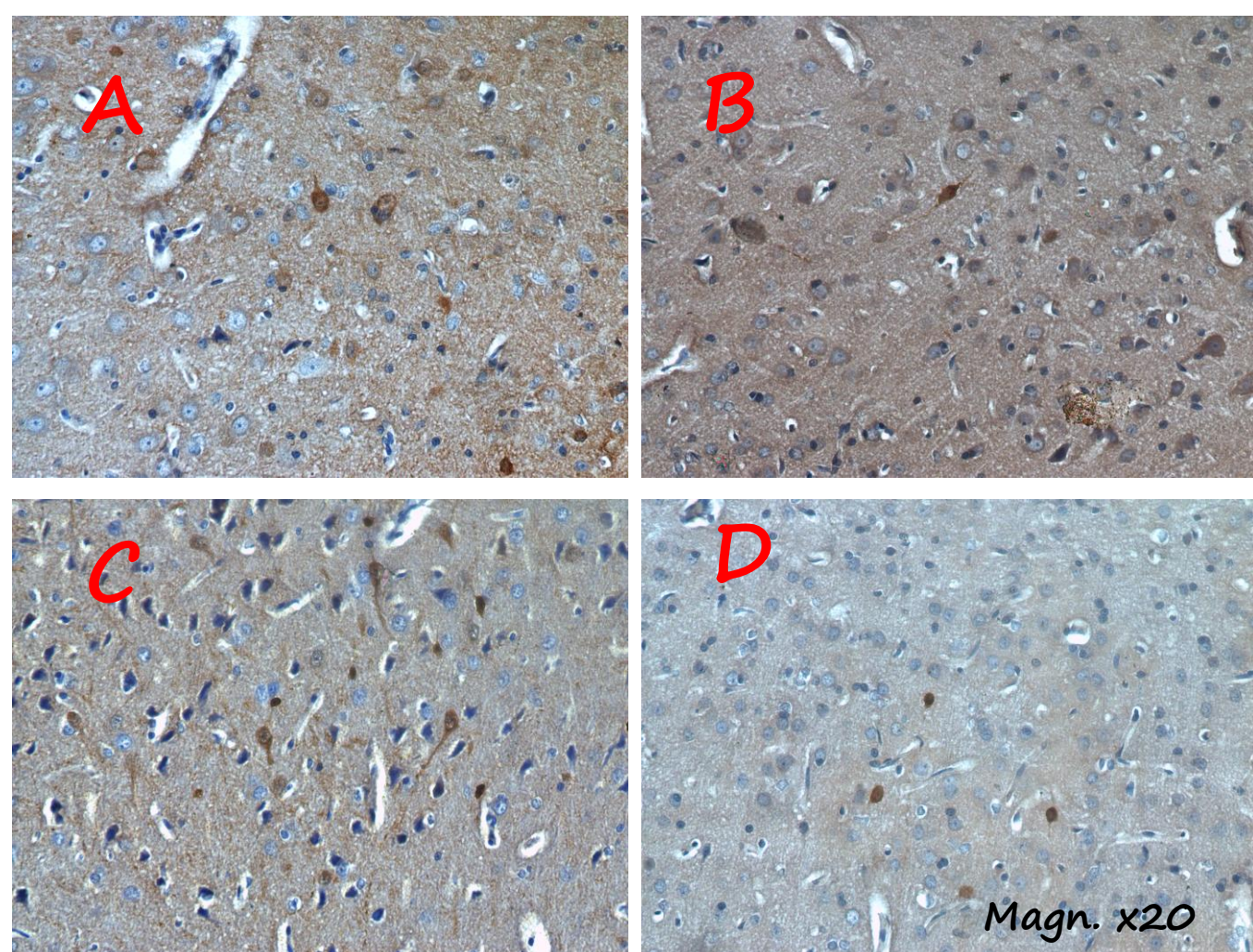


Fig. 2. Calretinin expression in the claustrum of the rat after consumption the beetroot/carrot juice

Materials

- ✓ 8-week-old Wistar rats (n=32)
- ✓ four equal groups A-D (n=8)
- ✓ group A - control
- ✓ group B - Cd²⁺ dissolved in 100 ml tap water/day
- ✓ group C - beetroot/carrot juice
- ✓ group D - Cd²⁺ dissolved in 100 ml juice/day

- ✓ Cd²⁺ 5mg/kg bw
- ✓ time : 12 weeks

Method

- ✓ the brains - 12 hours 10% formalin (pH=7.0; +4°C)
- ✓ the 10 µm thick frontal slices
- ✓ immunohistochemical PAP Method

PAP Method

- ✓ 2.5% normal horse serum (S-2012; Vector, USA)
- ✓ Mouse primary antisera raised against calretinin (dilution 1:2000; C7479, Sigma, Germany)
- ✓ anti-mouse/rabbit Ig (ImPRESS™; MP-7500 Vector, USA), for 1 hour (RT)
- ✓ 3,3'-diaminobenzidine (DAB, Vector, USA) chromogen.
- ✓ Calretinin IR neurons - Axiolab (Zeiss) light microscope

Table 2. Percentage number of neurons immunoreactive to CR to total number neurons with standard deviation in CL. Different letters in superscript indicate statistically significant differences (Kruskal-Wallis p<0.05).

CLAUSTRUM	
Group A. CONTROL GROUP	5.07±0.83 ^a
Group B Cd ²⁺ dissolved in 100 ml tap water/day	1.33±0.48 ^b
Group C beetroot/carrot juice	4.9±0.66 ^{ac}
Group D Cd ²⁺ dissolved in 100 ml juice/day	1.87±0.78 ^c

Main bioactive components (polyphenols and betalains):

- ✓ may be an efficient factor influencing the calcium metabolism of CNS neurons, and may have a neuroprotective effect on claustral neurons

Conclusions

- ✓ may reduce the toxic effects of cadmium on CNS neurons