TITLE:

Exposure to the natural alkaloid Berberine affects cardiovascular system morphogenesis and functionality during zebrafish development

DAVIDE MARTINI[1#], CECILIA PUCCI[1,2 #,§], CHIARA GABELLINI[1], MARIO PELLEGRINO [3] and MASSIMILIANO ANDREAZZOLI[*,1,4]

[1]*Cell and Developmental Biology Unit, Department of Biology, University of Pisa, Pisa, Italy*

[2]*Sant'Anna School of Advanced Studies, Pisa, Italy*

[3]*National Institute of Optics, National Research Council, Pisa, Italy*

[4]*Interdepartmental Research Center Nutrafood "Nutraceuticals and Food for Health", University of Pisa, Pisa, Italy*

[#] These authors equally contributed to this work.

[§]Present address: Institute of Genomic Medicine, Catholic University, 00168 Rome, Italy.

[*] Corresponding author:

massimiliano.andreazzoli@unipi.it

Cell and Developmental Biology Unit, Department of Biology, University of Pisa SS12 Abetone e Brennero, 56127 Pisa, Italy

Tel. +39-050-2211485; FAX + 39-050-2211495

Supplementary Table S1

Percentage of mortality at different timepoints and BRB concentrations

	24 h	48 h	72 h	96 h
control	0.5 ± 0.5	1.3 ± 0.83	2.28 ± 0.64	2.75 ± 0.81
50 mg/L	0.98 ± 0.6	2.25 ± 0.99	2.75 ± 0.81	2.75 ± 0.81
100 mg/L	0.98 ± 0.6	3.08 ± 1.32	8.33 ± 1.21	(***)(###) 25.16 ± 5.06
200 mg/L	3.08 ± 1.54	5.65 ± 2.74	(***)(###)($$$) 42.13 ± 10.12	(***)(###)($$$) 87.24 ± 4.95
400 mg/L	4.88 ± 1.85	(***)(###)($$$) 36.74 ± 3.67	(***)(###)($$$)(ϕϕϕ) 95.27 ± 2.36	(***)(###)($$$) 100 ± 0.0

Data are cumulative and expressed as mean ± standard error of the mean (SEM) from 5 independent experiments, each with 40 larvae per experimental condition. One-way ANOVA followed by Tukey post hoc test. Statistical significance refers to the same exposure time, in the various experimental conditions. ***$p<0.001$ vs control, $^{###}p<0.001$ vs 50 mg/L, $^{\$\$\$}p<0.001$ vs 100 mg/L, $^{\phi\phi\phi}p<0.001$ vs 200 mg/L.

Supplementary Table S2

Percentage of embryos with pericardial edema at different timepoints and BRB concentrations

	24 h	48 h	72 h	96 h
control	0.00	0.192 ±0.192	0.5448 ± 0.29	0.5448 ± 0.29
50 mg/L	0.00	1.34 ± 0.73	3.947 ± 0.90	6.38 ± 1.57
100 mg/L	0.00	(*)(#) 12.14 ± 2.64	(***)(###) 26.67 ± 2.93	(***)(###) 54.88 ± 5.01
200 mg/L	0.00	(***)(###) 25.17 ± 8.65	(***)(###)($) 40.81 ± 6.74	(***)(###) 40.81 ± 6.74
400 mg/L	1.82±1.82	(***)(###)($) 27.69 ± 7.27	(***)(###) 27.69 ± 7.27	(***)(#)($$$) 27.69 ± 7.27

Data are cumulative and expressed as mean ± SEM from 5 independent experiments, each with 40 larvae per experimental condition. One-way ANOVA followed by Tukey post hoc test. Statistical significance refers to the same exposure time, in the various experimental conditions:
$^{*}p<0.05$ vs control, $^{***}p<0.001$ vs control, $^{\#}p<0.05$ vs 50 mg/L, $^{\#\#\#}p<0.001$ vs 50 mg/L, $^{\$}p<0.05$ vs 100 mg/L, $^{\$\$\$}p<0.001$ vs 100 mg/L.

Supplementary Table S3

Sequences of primers used in qRT-PCR

Gene name		Sequences
cdh5	**F**	5' - TGGAATGAGTGTCAGTGCCC - 3'
	R	5' - CAGCTGCTCATGGATTTCGC - 3'
vegfaa	**F**	5' - AAAAGAGTGCGTGCAAGACC - 3'
	R	5' - GACGTTTCGTGTCTCTGTCG - 3'
vegfab	**F**	5' - TGTTGGTGGAAATTCAGCAG - 3'
	R	5' - CACCCTGATGACGAAGAGGT - 3'
phd3	**F**	5’ - CCTGGAAATGGAGCTGGATA - 3’
	R	5’ – CCGGTCAAATAAAGGCTCAA - 3’
β-actin	**F**	5' - CGAGCAGGAGATGGGAACC - 3'
	R	5' - CAACGGAAACGCTCATTGC - 3'