Effect of *Ribes nigrum* leaf extracts on endothelium-dependent vasorelaxation

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Introduction:

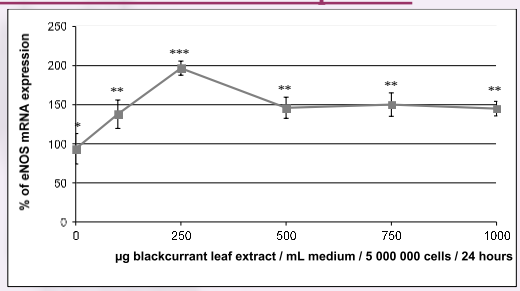
Endothelial cells produce various vasodilating substances such as endothelium-derived factors or nitric oxide (NO), endothelium-derived hyperpolarizing factors (EDHF) and vasodilator prostaglandins. NO produced by endothelial-type nitric oxide synthase (*eNOS*) is a key factor in vascular protection. One of the mechanisms involved in the endothelium dysfunction, implied in the development of various vascular pathologies, is a reduced NO synthesis and/or an increase of vascular NO degradation.
Blackcurrants are among the berries these with the higher amount of phenolic compounds, especially anthocyanins. This study aimed at investigating the effect of blackcurrant leaf extract on the endothelium relaxation by examining *in vitro* the expression of *eNOS* and the NO production, by human endothelial cells. Its effect was also observed on the relaxation of isolated vessels.

Results:

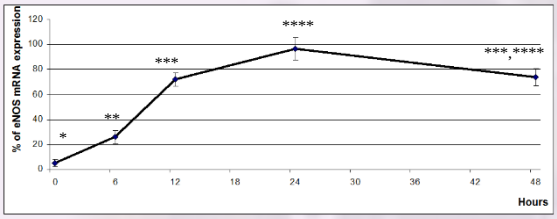
I. Blackcurrant leaf extract stimulates the endothelial NO formation and *eNOS* mRNA expression

	Conc.		Formation of NO (µM)				
			5 min	6 h	12 h	24 h	48 h
Control	Med.		0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Leaf extr.	100	µg/ml	1.5 ± 0.6	1.0 ± 0.4	2.2 ± 0.7	1.4 ± 0.9	4.0 ± 0.8
	200	µg/ml	5.6 ± 0.7	9.7 ± 0.4	8.3 ± 2.5	6.3 ± 1.2	7.3 ± 0.9
	300	µg/ml	2.1 ± 1.8*	12.8 ± 0.8**	11.4 ± 0.9**	4.2 ± 1.2*	6.8 ± 1.5*
	400	µg/ml	9.8 ± 1.3*	11.5 ± 1.1*	16.0 ± 1.6**	7.6 ± 2.1*	6.5 ± 1.8*
	500	µg/ml	9.4 ± 3.7*	10.0 ± 1.7*	23.5 ± 2.2**	7.6 ± 1.7*	6.3 ± 1.4*
	750	µg/ml	0 ± 0	1.9 ± 4.0*	22.1 ± 2.8**	5.0 ± 1.4*	12.2 ± 2.9***
	1000	µg/ml	0 ± 0	9.6 ± 7.1*	3.0 ±7.9*	7.2 ± 1.4*	31.9 ± 6.5**

Effect of leaf extracts on NO formation in EAHy926 cells. 5x10^5 cells were treated with various concentrations of extract (0 to 1 mg/mL). After 5 min, 6h, 12h, 24h and 48h, NO content was assessed by the Griess method. Data are shown as means ± SD of six independent experiments. Significantly differences at p<0.05 are indicated by a different number of * symbols during the kinetic, for each concentration tested (n=6).

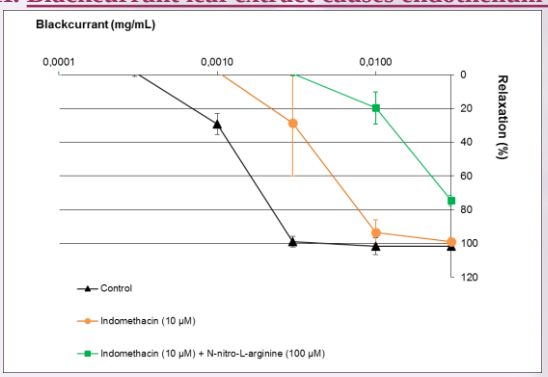


Effect of extracts on eNOS mRNA expression in EAHy926 cells (5x10^6 cells) after a 24h of incubation period with various concentrations of the extract. The results are expressed in % of expression and normalized to β-actin mRNA expression taken at 100%. Data are shown as means ± SD of three independent experiments. Significantly differences at p<0.05 are indicated by a different number of * symbols (n=3). Representative agarose gel showing the effect of 0, 250, 500 and 1000 µg/mL of leaf extract on the expression of eNOS and β-actin.

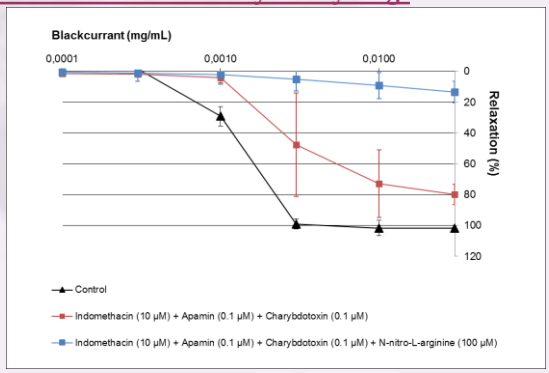


Time course of eNOS mRNA expressionafter incubation of 5x10^6 cells (EAHy926) with 250 µg/mL of the extract in the medium up to 48h. The results are expressed in % of expression and normalized to β-actin mRNA expression taken at 100%. Data are shown as means ± SD of three independent experiments. Significantly differences at p<0.05 are indicated by a different number of * symbols (n=3).

II. Blackcurrant leaf extract causes endothelium-dependent relaxations in coronary artery rings



Characterization of the relaxation induced by increasing concentrations of blackcurrant extract (0,0001 to 0,03 mg/mL) in porcine coronary artery rings, precontracted with U46619. Rings with an intact endothelim were incubated with N^G-nitro-L-arginine (100µM). All experiments were performed in the presence of Indomethacin (10µM), excepted control. Data are shown as mean ± SD of two independent experiments.



Characterization of the relaxation induced by increasing concentrations of blackcurrant extract (0,0001 to 0,03 mg/mL) in porcine coronary artery rings, precontracted with U46619. Rings with an intact endothelim were incubated with N^G-nitro-L-arginine (100µM) and/or apamin + charybdotoxin (0,1 µM each). All experiments were performed in the presence of Indomethacin (10µM), excepted control. Data are shown as mean ± SD of two independent experiments.

Conclusion:

Blackcurrant leaf extract can moderately up-regulate the production of NO and the expression of eNOS mRNA. The mechanism by which the leaf extract induced endothelium-dependent relaxation was investigated using several inhibitors. The data indicate that the extract induced a pronounced endothelium-dependent relaxation involving NO and also to some extent vasorelaxant prostanoids. These effects could contribute to the cardiovascular protection from atherosclerosis.