Effect of novel plant antioxidant from *Aronia melanocarpa* on cardiovascular risk factors in patients after MI

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Proposed mechanism by which flavonoids may reduce risk for cardiovascular diseases

- **Oxidative stress**
  - Scavenge reactive oxygen and nitrogen species
  - Chelate redox-active transition metal ions
  - Spare and interact with other antioxidants
  - Inhibition of the redox-sensitive transcription factors
  - Inhibition of pro-oxidant enzymes
  - Induction of antioxidant enzymes
Proposed mechanism by which flavonoids may reduce risk for cardiovascular diseases

- Growth of atherosclerotic plaque
  - Reduce adhesion molecule expression
  - Anti-inflammatory
  - Reduce the capacity of macrophages to oxidatively modified LDL
Proposed mechanism by which flavonoids may reduce risk for cardiovascular diseases

- Platelet function and haemostasis
  - Inhibit platelet aggregation
- Blood pressure and vascular reactivity
  - Promote nitric oxide-induced endothelial relaxation
The role of CD36 and LOX-1 receptors in foam cell development

Endothelial cells

Macrophage

NO

H₂O

MPO

CD36

OX-LDL

LDL

s-ICAM

s-VCAM

PPARγ

Transcription

Cholesterol esters

IL-6, MCP-1

MPO

OX-LDL

LOX-1
Aronia (Photinia melanocaarpa, also known as Aronia melanocarpa and Aronia nigra), is a native American bush that has been successfully exported to Eastern Europe and is commercially grown in Denmark, Poland, Russia and elsewhere.

Known commonly as “chokeberry” or “blak chokeberry”, (Photinia melanocarpa) has at least two cousins worth mentioning. These are Photinia floribunda, also known as Aronia atropurpurea, the “purple chokeberry”, and Photinia pyrofilia, also known as Aronia arbutifolia, the “red chokeberry”. The former ranges from Mississippi to Wisconsin, Georgia into Canada, and all areas in between. The latter ranges from Texas-Oklahoma eastward through Tennessee to the Atlantic and from Florida northward into Canada. The Aronia berry has all of the healthy attributes of the cranberry, but also contains five to ten times the amount of anthocyanins and polyphenols of a cranberry.
Antioxidant activity (ORAC), Anthocyanin Content and Phenolic Content in Fruit of Blueberry, Cranberry, Lingonberry and Chokeberry

<table>
<thead>
<tr>
<th>species</th>
<th>ORAC (µmol of TE/g)</th>
<th>Anthocyanin (mg/g)</th>
<th>Total phenolic (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blueberry</td>
<td>28.9</td>
<td>1.20</td>
<td>4.12</td>
</tr>
<tr>
<td>Cranberry</td>
<td>18.5</td>
<td>0.32</td>
<td>3.15</td>
</tr>
<tr>
<td>Lingonberry</td>
<td>38.1</td>
<td>0.45</td>
<td>6.52</td>
</tr>
<tr>
<td>Chokeberry</td>
<td>160.2</td>
<td>4.28</td>
<td>25.56</td>
</tr>
</tbody>
</table>

Effect of *A. melanocarpa* extract on superoxide production by human arterial wall cells from patients with cardiovascular risk factors

Data are shown as means +/-SEM, * - p<0.05 vs. native using appropriate tests depending on data distribution.
Effect of *A. melanocarpa* extract on vasoconstruction

Increasing concentration of *A. melanocarpa* extract

Increasing concentration of *A. melanocarpa* extract

![Graph showing the effect of increasing concentration of *A. melanocarpa* extract on vasoconstruction.](image)

- **Polifenole ekstraktu *A. Melanocarpa* (µg/ml)**
  - 0,001
  - 0,01
  - 0,1
  - 1
  - 5
  - 10
  - 100

- **Rozkurcz (% przykurczu wstępnego)**
  - 0
  - 10
  - 20
  - 30
  - 40
  - 50
  - 60
  - 70
  - 80

**Graph Key**
- IMA
- HSV
Correlation between endothelium function and vasorelaxation activity of *A. melanocarpa* extract

Highest relaxation after administration of *A. melanocarpa* polyphenols extract [%] vs. ACh maks. [%]

R = 0.65, p = 0.001; n = 25
Mechanism of vasorelaxation activity of *A. melanocarpa* polyphenols

**Endothelium function**

**Role of eNOS** (incubation with L-NAME)
Superoxide production in platelets and effect of *A. melanocarpa* polyphenols

Production of $O_2^-$ in platelets
- correlation with risk factors
Superoxide production in platelets and effect of *A. melanocarpa* polyphenols

Risk factors

[Graph showing platelet superoxide production (RLU/sek/4x10^5 płytek) in response to different concentrations of A. melanocarpa polyphenols.]

Control

[Graph showing production of O_2^-^ (RLU/sek/4x10^5 płytek) for different concentrations of A. melanocarpa extract.]

* * *
Effect of increasing *A. melanocarpa* polyphenols concentrations on platelet aggregation

**Control**

**Risk factors**

- A. melanocarpa polyphenols extract conc. (ug/ml)
- Aggregation (RU) thrombin induced (20mU/ml)

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*Note: The graphs show the effect of increasing polyphenols concentrations on platelet aggregation for control and risk factors. The asterisk (*) indicates statistical significance.*
The effect of increasing concentrations of Aronia flavonoids from chokeberry on eNOS expression in HUVEC cells
The effect of increasing concentrations of Aronia flavonoids from chokeberry on LOX-1 expression in HUVEC cells.
Product: Aronia fruits extract /ARONOX/
Scientific name: Aronia meelanocarpa (chokeberry)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Powder</td>
</tr>
<tr>
<td>Colour</td>
<td>Red-brown</td>
</tr>
<tr>
<td>Humidity</td>
<td>max 8%</td>
</tr>
<tr>
<td>Anthocyanins Cy-3-O-Ga</td>
<td>20-25%</td>
</tr>
<tr>
<td>Polimeric procyanidins (+)(-) epicatechin</td>
<td>&gt;55%</td>
</tr>
<tr>
<td>Phenolic acids</td>
<td>9%</td>
</tr>
<tr>
<td>Antioxidant activity</td>
<td></td>
</tr>
<tr>
<td>(μ M Trolox/g dried weight) DPPH radicals</td>
<td>118.7</td>
</tr>
<tr>
<td>ABTS radicals</td>
<td>106.1</td>
</tr>
</tbody>
</table>
Combination therapy of statin with flavonoids rich extract from chokeberry fruits enhanced reduction in cardiovascular risk markers in patients after myocardial infarction (MI)

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Methods

- This was a double-blind, placebo-controlled, parallel trial. Forty-four patients (11 women and 33 men, mean age 66 years) who survived myocardial infarction and have received statin therapy for at least 6 months (80% dose of 40 mg/day simvastatin) were included in the study. The subjects were randomised to receive either 3 x 85 mg/day of chokeberry flavonoid extract or placebo for a period of 6 weeks.
<table>
<thead>
<tr>
<th>Clinical and Demographic Characteristics of MI Patients</th>
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<tbody>
<tr>
<td><strong>Aronia flavonoids (n=22)</strong></td>
</tr>
<tr>
<td><strong>Age</strong></td>
</tr>
<tr>
<td><strong>Sex (women, men)</strong></td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
</tr>
<tr>
<td><strong>MI (years after)</strong></td>
</tr>
<tr>
<td><strong>Diabetes (%)</strong></td>
</tr>
<tr>
<td><strong>Hypertension (%)</strong></td>
</tr>
<tr>
<td><strong>Current smokers (%)</strong></td>
</tr>
<tr>
<td><strong>Medication</strong></td>
</tr>
<tr>
<td><strong>Statins (%)</strong></td>
</tr>
<tr>
<td>simvastatin 40 mg</td>
</tr>
<tr>
<td>atorvastatin 20 mg</td>
</tr>
<tr>
<td><strong>Aspirin (%)</strong></td>
</tr>
<tr>
<td><strong>ACE inhibitors (%)</strong></td>
</tr>
</tbody>
</table>
Effect of Aronia flavonoids or placebo on lipids, lipoproteins and homocysteine levels in patients with a history of MI

<table>
<thead>
<tr>
<th></th>
<th>Anthocyanins (n=22)</th>
<th>Placebo (n=22)</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before</td>
<td>after</td>
<td>before</td>
</tr>
<tr>
<td>TG [mg/dl]</td>
<td>162.7 ± 66.2</td>
<td>152.7 ± 55.5</td>
<td>131.8 ±</td>
</tr>
<tr>
<td>Chol [mg/dl]</td>
<td>198.0 ± 25.6</td>
<td>199.8 ± 28.2</td>
<td>202.2 ±</td>
</tr>
<tr>
<td>LDL [mg/dl]</td>
<td>117.3 ± 25.9</td>
<td>116.9 ± 27.9</td>
<td>116.8 ±</td>
</tr>
<tr>
<td>HDL [mg/dl]</td>
<td>42.2 ± 9.56</td>
<td>43.4 ± 9.7</td>
<td>45.6 ± 9.2</td>
</tr>
<tr>
<td>Lp(a) [mg/dl]</td>
<td>27.3 ± 33.6</td>
<td>28.2 ± 37.0</td>
<td>25.2 ± 36.4</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>17.2 ± 7.3</td>
<td>17.1 ± 8.2</td>
<td>16.0 ± 7.0</td>
</tr>
<tr>
<td>[µmol/l]</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The effect of Aronia flavonoids on plasma total F2-isoprostanes level

[pg/ml]

before      after

↓ 38%

p < .005

Aronia
Placebo
The effect of Aronia flavonoids on plasma OX-LDL level

- **Aronia**
  - p < .0001
  - ↓ 29%
The effect of Aronia flavonoids on systolic blood pressure

![Graph showing the effect of Aronia flavonoids on systolic blood pressure. The graph compares the blood pressure before and after treatment with Aronia or placebo. The graph indicates a significant decrease in blood pressure (\(p < .0001\)) with Aronia treatment, resulting in a 11 mmHg reduction.]
The effect of Aronia flavonoids on diastolic blood pressure

Before: [mmHg] 88
After: [mmHg] 78

↓ x = 7.2 mmHg

P < .0000

Aronia
Placebo
The effect of Aronia flavonoids or placebo on plasma ACE activity
The effect of Aronia flavonoids on plasma IL-6 level

[Diagram showing a decrease in IL-6 levels from before to after treatment with Aronia flavonoids. The graph indicates a significant decrease with p<.003.]
The effect of Aronia flavonoids on plasma hs-CRP level

The graph shows the change in plasma hs-CRP level before and after treatment with Aronia flavonoids. The level decreases by 23% compared to the placebo group, with a statistically significant difference (p<.001).
The effect of Aronia flavonoids on plasma adiponectin level

[p<.03] 23.6% ↑

Aronia
Placebo

[μg/ml]
The effect of Aronia flavonoids on plasma MCP-1 level

Before: 340 pg/ml
After: 220 pg/ml

↓ 29%

p < .001

Aronia vs Placebo
Role of EPC in reendothelisation and neovascularisation
Regulation of EPCs following the natural history of atherosclerosis

Endothelial progenitor cells and atherosclerosis

- EPCs are a circulating bone marrow-derived cell population that express surface CD34, CD133 and vascular endothelial growth factor receptor 2 (VEGFR-2).
- EPCs are incorporated into the sites of endothelial denudation and provide an endogenous repair mechanism to endothelial injury.
- EPCs from subject with CVD showed in vitro accelerate senescence and reduction in telomerase activity. This effect was partially through increase of oxidative stress and inhibiting eNOS.
Effect of *Aronia melanocarpa* polyphenols on telomerase activity in EPC
Effect of chokeberry extract on telomerase activity in vivo in patients with CVD
Cells incubated with 50μg of Aronia extract
Cells incubated with oxLDL
Cells incubated with Aronia extract and oxLDL
Conclusions

- These results confirm the potential usefulness if natural antioxidants in the treatment of atherosclerosis, also through their effect on EPC senescence inhibition.
- Funding. This work was supported by non-restricted grants from Polish Society for Atherosclerosis Research and from Agropharm.
Conclusion

- In view of the fact that chokeberry flavonoids reduce the severity of inflammation, regardless of statins, they can be used clinically for secondary prevention of ischaemic heart disease.

- Funding. This work was supported by non-restricted grants from Polish Society for Athrosclerosis Research and from Agropharm.