PHYTOTHERAPY RESEARCH
Phytother. Res. **24**: 1107–1114 (2010)
Published online 22 June 2010 in Wiley InterScience
(www.interscience.wiley.com) **DOI**: 10.1002/ptr.3226

REVIEW ARTICLE

The Clinical Effectiveness of Chokeberry: A Systematic Review

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Products derived from the black chokeberry, *Aronia melanocarpa,* are claimed to be beneficial in disorders or diseases associated with oxidative stress. The claims are based on evidence from *in vitro* studies and animal experiments. The active principle – a mixture of procyanidins, anthocyanins and phenolic acids – constitutes one of the most potent natural antioxidants. A systematic review was carried out of the quality of the clinical trials on chokeberry products that had been published up to December 2009, and conventionally established criteria were used to assess the strength of the evidence for their clinical effectiveness. Thirteen studies were identified. The quality of most of the trials and, correspondingly, the evidence of effectiveness for *Aronia* products is poor. Though laboratory and clinical data indicate that chokeberry products may well be useful as 'functional food' for disorders or diseases related to oxidative stress, these promising indications need to be confirmed in more rigorous studies before putative therapeutic uses can be confidently recommended for chokeberry products. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: chokeberry; *Aronia melanocarpa*; systematic review; clinical trials; antioxidative effect.

INTRODUCTION

The black chokeberry, *Aronia melanocarpa* (Family Rosaceae), is native to North America and Canada and was introduced into Europe about a century ago. Because of the astringent taste of the raw berries and their smell of bitter-almonds, pure *Aronia* products are not particularly popular, although they have been documented as a 'functional food' in Russia since the 1940s. Chokeberry juice has been used primarily for blending with other juices or in the preparation of syrups, jellies, teas, liqueurs, spirits and fruit wine. The interest in *Aronia* products has recently increased, not only because of the putative health-promoting effects of *Aronia*, but also because the plant is pest-resistant and easy to cultivate (http://opus.haw-hamburg.de/volltexte/2008/513/pdf/med_y_210.pdf). Laboratory reports of potentially useful activities began to appear in the mid 1990s, and these have been accompanied since 2000 by a modest number of reports of its use in a variety of clinical settings.

Chokeberries have a greater content of phenolic constituents than most of the other black berries (Kahkönen *et al.*, 2001, Wu *et al.*, 2004). These comprise procyanidins, anthocyanins and phenolic acids, and the amounts reflect antioxidative potency in laboratory testing (Zheng and Wang, 2003). Procyanidins and some anthocyanins may also form complexes with free iron and copper, which catalyse free-radical reactions and contribute to the reduction in pro-oxidative activity seen with *Aronia* products (Hider *et al.*, 2001). Popular health claims for *Aronia* products are that they: (i) combat heart disease and other cardiovascular problems, (ii) mitigate hypertension, (iii) maintain a healthy urinary tract, (iv) fight against bacteria and viruses, (v) strengthen memory, (vi) aid digestion and (vii) help to decrease the amount of cholesterol carried as low density lipoproteins (LDL) (www.associatedcontent.com/article/870111/10_benefits_of_aronia_berry.html?cat=51). Additional claims by association have been made in diabetes and arthritis (www.doverpost.com/lifestyle/x124604982/Studies-show-aronia-berries-may-be-full-of-health-benefits), as well as in cancer (http://hubpages.com/hub/aronia). These claims are primarily based on research with other black berry products or isolated anthocyanins (Zafra-Stone *et al.*, 2007) or are transferred from *in vitro* and experimental studies with chokeberry preparations (Kulling and Rawel, 2008).

The aim of this systematic review is to evaluate the current *clinical* evidence for effectiveness of chokeberry preparations.

METHODS

Several electronic databases were searched: Medline/OVID, Silverplatter, and CENTRAL from 1950 up to December 2009, using some or all of the search terms, chokeberry, *Aronia*, *Aronia melanocarpa*, and searched by hand for publications not stored electronically (e.g.: theses, webpages). Two authors (CC and SC) extracted the data independently and evaluated the quality of the

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Received 31 March 2010
Accepted 21 April 2010

studies and the strength of the evidence of clinical effectiveness using the same criteria as in previous reviews (Chrubasik *et al.*, 2004; Gagnier *et al.*, 2004; Chrubasik *et al.*, 2006, 2007a, 2007b, 2008; Vlachojannis *et al.*, 2009, 2010a, 2010b).

Briefly, the assessment of quality was based on 'yes' or 'no' answers to the following questions (to the extent that they were applicable in the context of each study):

Was or were:

(i) patients included on the basis of specified eligibility criteria;
(ii) randomization appropriate;
(iii) treatment allocation concealed;
(iv) baseline values of the groups similar;
(v) outcome measures and control interventions explicitly described;
(vi) co-interventions comparable;
(vii) outcome measures relevant;
(viii) adverse events fully described;
(ix) attrition of patients from the study (the 'drop-outs') fully described;
(x) sample size based on *a priori* power calculation;
(xi) in the event of attrition of patients during the study, analysis by intention-to-treat;
(xii) point estimates and measures of variability presented for the primary outcome measure;
(xiii) studies undertaken over an appropriate time-course to demonstrate the putative effect.

Clearly, for some studies, particularly simple observational studies, some of the questions are not applicable but the inability to supply a 'yes' answer is, in itself, a marker of 'absence of quality' in systematic reviews of this sort.

Disagreements between the assessors over these deliberately simplified questions were few (arising mostly from lack of transparency in describing methods) and were resolved after discussion. Adding up the 'yes' answers applicable to each study gave it a Total Score (TS) out of a maximum of 13. The distribution of Total Scores for the studies in the review is one way to indicate the strength of evidence of effectiveness from a group of individual studies. Early in the history of the clinical investigation of a particular product, most of the studies will be 'exploratory' in that they determine the conditions, study design and sample size needed in planning to maximize the chances of demonstrating useful clinical effects in later 'confirmatory' studies.

A conventional way of assessing evidence of clinical effectiveness from a more mature body of work is to define the evidence as 'strong', 'medium' or 'weak', according to the following criteria: (i) 'strong' – pooling of data from at least two confirmatory studies demonstrating a clinically relevant effect; (ii) 'moderate' – consistent findings from one confirmatory study with a clinically relevant effect, multiple exploratory studies of high internal validity, or both; (iii) poor – multiple exploratory studies of low internal validity or a single study of high internal validity.

RESULTS

Thirteen clinical trials were identified. They have been assigned a number from 1 to 13 in chronological order, to facilitate reference between the tables and the text. Table 1 gives some characteristics of the publications in

Table 1. The published clinical trials, each with the reference number (No) used in this paper's text and other Tables, some publication characteristics and the setting for the study

No	Year	Authors	Journal	Volume & Pages	Language	Full or Abstract	Setting
1	2000	Pawłowicz *et al.*	*Ginekol Pol*	**71:** 848–853	Polish	Full	Intrauterine growth retardation
2	2001	Pawłowicz *et al.*	*Ginekol Pol*	**72:** 983–988	Polish	Full	Oligospermia
3	2002	Yaneva *et al.*	*Folia Med (Plovdiv)*	**44(1–2):** 22–25	English	Full	After radiation for breast cancer
4	2002	Simeonov *et al.*	*Folia Med (Plovdiv)*	**44(3):** 20–23	English	Full	Non insulin-dependent diabetes
5	2005	Kowalczyk *et al.*	*Pol Merkur Lekarski*	**19:** 651–653	Polish	Abstract	Hypercholesteraemia
6	2005	Pilaczynska-Szczesniak *et al.*	Int J Sport Nutr Exerc Metab	**15(1):** 48–58	English	Full	Healthy volunteers
7	2007	Sikora *et al.*	www.science24.com/paper/10824[a]	paper/10824	English	Abstract	Metabolic syndrome
8	2007	Naruszewicz *et al.*	*Atherosclerosis*, also[b]	**194:** e179–e184	English	Full	After myocardial infarction
9	2007	Broncel *et al.*	*Pol Merkur Lekarski*	**23:** 116–119	Polish	Full	Metabolic syndrome
10	2007	Skoczynska *et al.*	Pharmacol Rep	**59** (Suppl 1): 177–182	English	Full	Metabolic syndrome
11	2007	Koziróg-Kołacińska *et al.*	*Atherosclerosis*[c]	**8** (Suppl): 166–167	English	Abstract	Hypercholesteraemia
12	2008	Jensen *et al.*	*J Agric Food Chem*	**56:** 8326–8333	English	Full	Healthy volunteers
13	2009	Poreba *et al.*	*Ann Agric Environ Med*	**16:** 305–308	English	Full	Hypercholesteraemia

[a] 42nd meeting of the Polish Biochemical Society.
[b] *Atherosclerosis* (Suppl) 2003;**4:** 243 (2P-0473).
[c] IV Congress of Polish Soc of Clinical Pharmacology and Therapy, 'Safety and rational pharmacotherapy', 24–25.05.2007, Poznan.

which the studies appeared, and the general setting. Nine papers/abstracts were in English and the remaining four in Polish. In eight papers (numbers 4, 5, 7–11, 13), the setting was either the 'metabolic syndrome (papers 7, 9, 11) – a constellation of features under the general headings: abdominal obesity, atherogenic dyslipidaemia, hypertension, insulin resistance, and prothrombotic and pro-inflammatory state – or conditions presenting with a single or limited selection of those features (papers 4, 5, 8, 10, 13). Three of the remaining five studies (papers 1–3) investigated effects of *Aronia* in other conditions with increased oxidative stress, and the other two (papers 6 and 12) were of the effects of *Aronia* on the antioxidant capacity in healthy volunteers (Table 1).

Table 2 gives some indication of how the studies were carried out. Five studies (papers 5, 7, 10, 12, 13) had only one group, two being simple observational studies of the effects of giving an *Aronia* product (paper 5 and 7), and the rest incorporating a formal, single or double crossover, either to a placebo or no treatment (10, 12, 13). The remaining studies were comparisons between an '*Aronia'* group (receiving an *Aronia* product) and either a 'Placebo' group (receiving a placebo) or a 'Control' group of patients receiving nothing (papers 3, 4, 6, 8, 9, 11) or healthy subjects receiving nothing (1, 2).

In seven studies (papers 1, 2, 5, 7–9, 11) the *Aronia* product was Aronox^{R}, prepared from the berries, not by a solvent extraction, but by purification on a chromatographic bed, drying and vaporization (annaskoc@ak.am.wroc.pl), and standardized in 100 mg tablets each containing 15 mg of anthocyanins. In the remaining papers, the *Aronia* product consisted of juices with varying anthocyanin contents (Table 1). We tried unsuccessfully to contact Dr Naruszewicz by phone and email (marek.naruszewicz@wum.edu.pl), in an attempt to clarify how and why he appears to have used three 85 mg tablets of Aronox^{R} per day as his study medication – although Aronox tablets contain a 100 mg dose of dried juice.

The duration of the studies ranged from 2 h to 3 months, with most lasting between 4 and 8 weeks. In almost all of the studies, there was a striking multiplicity in the measurements undertaken as potential outcome measures – indicating a strong 'exploratory' trust to those studies. The use of the *Aronia* product was associated, in every study, with a favourable change in at least one or more of the measurements that had been undertaken (Table 3).

Table 4 shows the responses to the questions by which the quality of each study was assessed. All scored at least four 'yeses', for questions (i), (v), (vii) and (xiii) but, although all outcomes were explicitly described and

Table 2. Aronia product studied, and dose, study groups, duration of study and measurements undertaken. The abbreviations for the many measurements undertaken are given, as far as possible, at first use in the cell at first use, or at the top of the column

No	Aronia product Dose/day	Anthocyanins (mg/day)	Groups (Number in group) A Aronia, P placebo, CP control patients, CH control healthy subjects	Duration of study	Measurements undertaken CD, T lymphocyte subsets; (H/LDL), high/low density lipoprotein; TC, total cholesterol; (ery), erythrocyte; PG, prostaglandin; hs, high sensitivity; (S-ICAM, S-VCAM), soluble cellular adhesion molecules
1	Aronox 300 mg	45	A(50), P(55), CH(60)	8 weeks	oxidized LDL (oLDL)
2	Aronox 300 mg	45	A(22), P(16), CH(25)	8 weeks	oLDL, semen fructose
3	Concentrate 40 mL	not stated	A(42), CP (25)	not stated	CD3 total T lymphocytes, CD4 helper + inducer T cells, CD8 suppressor and cytotoxic T cells, natural killer cells
4	Juice 400 mL with 15 mL apple pectin	not stated	A(21), CP(23)	3 months	Fasting blood glucose (fG), HbA1c, cholesterol (chol), lipids, blood pressure (BP)
5	Aronox 200 mg	30	A(16) observational	30 days	ery Pb, Al, Cu, Zn; superoxide dismutase (SOD), glutathione peroxidase (GP), catalase (CAT), thiobarbiturate-reactive substances (TRS)
6	Juice 150 mL/day	35	A, CP(N not stated)	24 hours	TRS, superoxide dismutase (SOD), GP
7	Aronox 300 mg	45	A(25) observational	4 weeks	TC, H/LDL-chol, triglycerides (TG), platelet function
8	Aronox 255 mg (see text)	38	A(22), CP(22)	6 weeks	BP, 8-iso-PGF2α, oLDL, hs-IL-6, hs-C-reactive protein (CRP), S-ICAM, S-VCAM, monocyte chemotactic protein-1 (MCP-1), adiponectin
9	Aronox 300 mg	45	A(25), CP(22)	8 weeks	BP, TC, LDL- & HDL-chol, TG, Endothelin-1, FbG uric acid (UA), ery membrane cholesterol (emChol)
10	Juice 250 mL	29	A(58) double crossover	6 weeks	BP, TC, LDL- & HDL,TG, lipid peroxides (LPO) hs-CRP, homocysteine, fibrinogen, fG, vitamins A&E
11	Aronox 300 mg	45	A(25), CP(22)	8 weeks	body mass, waist circumference, blood lipids, fG, GP, CAT, SOD
12	Juice 120 mL	27	A(12), crossover to/from placebo	2 hours	ery-based antioxidative protection, TRS
13	Juice 250 mL	29	A(35) double crossover	6 weeks	TC, H/LDL-chol, TG, nitric oxide, flow-mediated dilatation, brachial artery diameter

Table 3. The measures that improved in the patients or healthy volunteers while they were taking the assigned *Aronia* product

No	Benefits claimed for *Aronia* product
1	Women with IUGR had higher oLDL levels than women with normal pregnancy; during *Aronia* intake oLDL levels were in the normal range
2	Men with oligospermia had higher oLDL levels than normal men; during *Aronia* intake oLDL levels were in the normal range
3	Significantly higher CD4 and CD8 T cell counts in the *Aronia* group only
4	All measures had improved in the *Aronia* group only
5	Increase of glutathione peroxidase and catalase activities, and erythrocyte Zn; decrease of erythrocyte Pb, Al, Cu
6	All measures were significantly lower in the *Aronia* group only
7	Platelet aggregation and all blood lipids except HDL cholesterol changed beneficially during intake of *Aronia*
8	Blood pressure, all measures of oxidative stress, and the adhesion molecules all reduced in the *Aronia* group only
9	Decrease of all measures except HDL-chol, fG and UA in the *Aronia* group only
10	Decrease of all measures except UA, HDL-chol, fibrinogen, hs-CRP and vitamin E; increase in HDL subfraction HDL-2 during intake of *Aronia*
11	Significant increase in SOD and GP activities in the *Aronia* group only
12	Increase of antioxidant capacity and inhibition of lipid peroxidation during intake of *Aronia*
13	Beneficial effect on endothelial function and lipid metabolism during intake of *Aronia*

Table 4. Assessment of 13 quality criteria in the 13 clinical trials identified. nk, not known

Paper number (from Table 1)	1	2	3	4	5	6	7	8	9	10	11	12	13
(i) patients included on the basis of specified eligibility criteria	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
(ii) randomization appropriate	nk	nk	nk	no	no	yes	no	semi	no	no	no	yes	no
(iii) treatment allocation concealed	nk	nk	no	no	no	nk	no	nk	no	no	no	no	no
(iv) baseline values of the groups similar	nk	nk	nk	nk	nk	nk	nk	yes	no	yes	no	yes	yes
(v) outcome measures and control interventions explicitly described	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
(vi) co-interventions comparable	nk	nk	nk	nk	n/a	yes	nk	yes	no	yes	nk	yes	yes
(vii) outcome measures relevant	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
(viii) adverse events fully described	nk	nk	nk	nk	nk	nk	nk	nk	nk	nk	nk	yes	nk
(ix) attrition of patients from the study fully described	nk	nk	nk	nk	nk	nk	nk	yes	nk	nk	nk	nk	nk
(x) sample size based on *a priori* power calculation	no	no	no	no	no	no	no	no	no	no	no	no	no
(xi) in the event of attrition, analysis by intention-to-treat	nk	nk	nk	nk	nk	nk	nk	yes	nk	nk	nk	nk	nk
(xii) point estimates and measures of variability for the primary outcome measure	no	no	no	no	no	no	no	no	no	yes	no	yes	yes
(xiii) timing appropriate	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Total score (sum of 'yes' responses)	4	4	4	4	4	6	4	8	4	7	4	9	7

relevant to the condition under study, there is no transparency as to whether any single outcome had been pre-specified for the purposes of hypothesis testing and *a priori* planning of sample size as would be required for a study to be considered 'confirmatory' rather than 'exploratory' as defined in the Methods. Thus, although the collection of ten studies centring on the metabolic syndrome and aspects thereof produced generally consistent favourable results for the chosen *Aronia* product, the collection does not include even a single 'confirmatory' study, so that the overall strength of evidence, in even this reasonably coherent body of work, must be classified as 'poor' by the criteria outlined in the Methods section. Besides the lack of transparency over advance stipulation of a critical outcome measure, only one study (paper 12) gave any adequate account of adverse events and only one gave adequate account of attrition (paper 8, in which the attrition was zero!). Attrition needs to be considered in relation to whether the results should be analysed *per protocol* or by intention-to-treat). Nonetheless, an encouraging feature of Table 4 is the trend towards improved study quality over the 9 years for which the *Aronia* products have been studied.

DISCUSSION

Although the limited body of work up to December 2009 presents evidence of clinical effectiveness which must be classified as 'poor' by conventional criteria, it is by no means unpromising. However, for the products to gain the acceptance that they may well deserve, a new generation of 'confirmatory' studies needs to be planned and undertaken. The evidence from laboratory investigations (reviewed recently, for example, by (Kulling and Rawel, 2008), as well as the clinical studies covered in this review provide potentially valuable pointers for the planning process. The laboratory investigations consist of product stability studies and studies in animal models and cell lines of various areas of potential benefit and toxicity, and dose finding.

Product stability studies

The rate of degradation of anthocyanins is affected by the type of anthocyanidin, the amount of glycosylated

sugar and the care taken to reduce contact with air (specifically oxygen) during preparation before packaging in bottles or cartons (Kaack and Austed, 1998), and during storage (the degradation of total anthocyanins has been shown to be 22% faster in cartons, for example, than in glass bottles (Trost *et al.*, 2008).

Vitamin C can reduce the oxidative degradation of the anthocyanins (Kaack and Austed, 1998) and the near black anthrocyanidin-containing juices are often presented as dilutions other fruit juice, e.g. lemon juice. The *in vitro* antioxidant activity of a lemon juice mixture was twice as high when pure lemon juice was enriched with 5% of *Aronia* concentrate (González-Molina *et al.*, 2008). Dilutions of *Aronia* juice in lemon juice have an attractive red colour that depends on a mixture of glycosylated and non-glycosylated anthocyanins (aglycones). The ranking order of individual non-glycosylated anthocyanins in a blueberry-*Aronia* nectar (from the most to least stable) was as follows: cyanindin > peonidin > petunidin > malvidin = delphinidin. For the glycosylated anthocyanins, the sugar with which the anthocyanidin is glycosylated makes a difference, degradation rate being least with glucose as the glycosylating sugar, intermediate with galactose and most with arabinose (Trost *et al.*, 2008). In dilutions of *Aronia* concentrate 2.5% and 5% in a lemon juice, the red colour was retained over 60 days despite a degradation of more than 90% in the antioxidative capacity supplied by the anthocyanidins – so colour is no guide to potential effectiveness (González-Molina *et al.*, 2008).

Temperature is important. Keeping *Aronia* juice concentrate for 8 h at 60°C resulted in a decrease of the polyphenol and anthocyanidin content by 22% and 35%, respectively, with an associated decrease in antioxidant activity of 56%. Thus the prolonged drying processes used in the manufacture of some *Aronia* berry products may be better avoided (Kasparaviciene and Briedis, 2003; Kähkönen *et al.*, 2001). Freeze-drying may be a better option – or, depending on the cost of the raw materials, it may be cheaper simply to increase the dose of a product such as Aronox^{R}. Whatever may be the case, there seems to be some scope for increasing the content of the putative active principle in future *Aronia* products.

Laboratory studies indicating potential benefits of *Aronia* or anthocyanins

The earliest documented laboratory demonstration of a potentially useful action of *Aronia* was an antiinflammatory effect shown in 1994 by Borissova and co-workers. *Aronia* anthocyanins were more effective than rutin in suppressing inflammation in the rat paw oedema test. More recently, a potent antiinflammatory effect of a proprietary extract of *Aronia* was demonstrated in endotoxin-induced uveitis in rats (Ohgami *et al.*, 2005). After 24 h, the number of inflammatory cells, the protein concentrations, and the levels of NO, PGE2, and TNF-alpha in aqueous humour from both eyes were dose-dependently decreased. The antiinflammatory effect of 100 mg of *Aronia* extract was as strong as that of 10 mg of prednisolone and stronger than that of either anthocyanin or quercetin, another component of the extract, when given alone. The same extract also dose-dependently suppressed LPS-induced nitric oxide synthase and COX-2 protein expressions in a mouse macrophage cell line, indicating that it acts in pathways that involve several inflammatory mediators (Ohgami *et al.*, 2005).

In 1995, Niedworok *et al.* described 'protection against damage from cyclophosphamide' and radiation disease (1995 theses quoted in Niedworok's review; bdb.trzcianka.com.pl/oferujemy/Aronox badania/ANG/Table I-25_eng.pdf).

Another early demonstration of potential benefit was by Atanasova-Goranova *et al.* in 1997. Their study in rats showed a hepatoprotective effect: *Aronia* nectar (fruit juice and pulp from the black chokeberry) inhibited nitrosamine production induced by aminopyrin and sodium nitrite in rats with a concomitant reduction in organic liver damage. *Aronia* juice also showed a hepatoprotective effect when given as a pre-treatment in a rat model of hepatotoxicity (Valcheva-Kuzmanova *et al.*, 2004, 2006). The juice prevented the carbon tetrachloride-induced increase in transaminase activities, and also the elevation of plasma and liver malondialdehyde.

Two studies revealed some antimutagenic potential of *Aronia* extract or its anthocyanin ingredients (Gasiorowski *et al.*, 1997; Gasiorowski and Brokos, 2001). Rats treated with the colonic carcinogen 'azoxymethane' and fed a diet with *Aronia* extract (385 mg of anthocyanins and 1645 mg of total phenolics per g of diet) had fewer foci of aberrant colonic crypts and less proliferation of colonic cells, suggesting that anthocyanin-rich *Aronia* extracts may offer some protection against colonic cancer (Lala *et al.*, 2006). However, although force feeding of rats with 8 mL/kg of chokeberry juice for 28 days was apparently harmless, doing so in combination with the chemical carcinogen, *N*-nitrosodiethylamine, caused more toxicity and DNA damage than the carcinogen alone (Krajka-Kuźniak *et al.*, 2009).

Isolated anthocyanins from *Aronia* were also shown to reduce pancreatic swelling in rats with PAF- or ceruleine-induced pancreatitis, with a concomitant decrease in lipid peroxidation and adenosine deaminase activity (Jankowski *et al.*, 2000). Generally 'antidiabetic' effects have been shown in various models of diabetes and hyperlipidemia (mimicking the metabolic syndrome) in rats. Large doses of *Aronia* juice (10 or 20 mL/kg) given for 6 weeks significantly reduced elevated plasma glucose and triglycerides in rats with streptozotocin-induced diabetes and diet-induced hyperlipidemia (Valcheva-Kuzmanova *et al.*, 2007b). Given for only 30 days they reduced plasma total cholesterol, LDL-cholesterol and triglycerides (Valcheva-Kuzmanova *et al.*, 2007a). In a similar rat model of pancreatic dysfunction, benefits have been demonstrated from feeding for 4 weeks with 0.2% chokeberry extract (714 mg/g total polyphenols, 57% anthocyanin glycosides; Agropharm) (Jurgoński *et al.*, 2008).

Matsumoto *et al.* (2004) demonstrated a gastro-protective effect of a methanol extract of *Aronia* (DER 1:10) that was as effective as that of quercetin and was caused by the red pigment fraction of the extract. In a rat model in which gastric ulcers were induced by indomethacin, a significant reduction of the depth and severity of mucosal lesions was found when *Aronia* juice was given concomitantly with the indomethacin (Valcheva-Kuzmanova *et al.*, 2005). Isolated *Aronia* anthocyanins have also been shown in rats to mitigate the effects of

poisoning by lead (Kowalczyk *et al.*, 2002) and cadmium (Kowalczyk *et al.*, 2003).

Several *in vitro* studies, one open study in chimpanzees and two exploratory studies in humans have suggested that an aqueous elderberry extract may have a place in the treatment of viral infections including the pandemic ones (Vlachojannis *et al.*, 2010b). Black chokeberries contain even more anthocyanins than do elderberries (Wu *et al.* 2004), and an *in vitro* study has shown that *Aronia* juice inhibited the reproduction of Influenza virus in its initial stages. Thus, proprietary standardized *Aronia* products may possibly have some use as antiinfluenza preparations. (http://www.antibiotikamonitor.at/34_03/34_03_1_04.htm).

Such an extraordinary multiplicity of effects claimed for *Aronia* has suggested to many researchers and practitioners that the *Aronia* products affect very basic cellular processes that are common to its many putative effects – namely effects that reduce the pathological generation of oxygen free radicals and/or promote scavenging of oxygen free radicals (Kulling and Rawel, 2008).

Safety

The Polish company Agropharm in Lodz has some unpublished data showing that intragastric administration of up to 5 g/kg of *Aronia* anthocyanins was not associated with any toxic symptoms in mice. Likewise, giving 10 mg/kg of anthocyanins for 6 weeks was well tolerated and the internal organs did not show any histological changes (http://bdb.trzcianka.com.pl/oferujemy/Aronox%20badania/ANG/Table%20I-25_eng.pdf). Nonetheless, the observations of (Krajka-Kuźniak *et al.*, 2009) indicate that some attention needs to be given to the possibility that *Aronia* products and related substances may act synergistically with other foodstuffs, medicines, poisons, chemotherapeutic agents, etc., that may be relevant in patients for whom in future *Aronia* products might otherwise be indicated.

Pointers to planning 'confirmatory' clinical studies

Setting. The metabolic syndrome and related disorders seem to be a promising target population to be studied, in that the conditions are prevalent, and increasingly so. Finding suitable subjects to study will be relatively easy and any demonstrated benefits are likely to reduce, not only the morbidity and mortality of the conditions, but also their enormous financial burden. The same might be said of endemic viral diseases such as influenza.

Dose. Doses do, of course, need to be tailored to the target populations to be investigated, but current empirical experience suggests that the daily dose of an *Aronia* product should contain 300–600 mg of anthocyanins – considerably more than in the clinical studies reviewed here (http://bdb.trzcianka.com.pl/oferujemy/Aronox%20badania/ANG/Table%20I-25_eng.pdf). If the doses used in animal studies are extrapolated to humans, the daily dose would correspond to an anthocyanin content of 1600–3000 mg contained in about 700–1400 mL juice per day (see below). Considerations of dose need to take into account considerations of how different *Aronia* products are manufactured and stored (see above – Product stability).

Adverse reactions. Considerations of dose also need to be tempered by considerations of possible adverse interactions with co-medications (see above), and for effects that may conceivably not have been studied fully in animals. Although no adverse events were reported in any of the clinical studies reviewed, squeezed *Aronia* juice may have mild diuretic and laxative effects from its potassium content and sorbitol-related laxative effects. Long-term use of *Aronia* preparations may conceivably cause anaemia because of the iron-chelating effect of anthocyanins. Like other polyphenol-containing products, *Aronia* juice or concentrate can also cause superficial staining of the teeth (Shellis *et al.*, 2005). As with almost any product containing molecules of sufficient size, hypersensitivity may occur, albeit rarely.

It is, of course mandatory that, for any adverse reactions that occur during any studies, a full description is provided, and a formal consideration of the likelihood that it is causally related to the product being studied. If subjects fail to complete the study because of adverse effects or any other reason, consideration of the possible effects of that attrition must be allowed for by comparing intention-to-treat and *per protocol* analyses of the results.

Outcome measures. Those used in the clinical trials reviewed to be reliable and relevant to improved well-being, and primary outcome measures need to be prespecified as the basis of hypothesis testing and sample size calculations (see above).

Duration. Short and medium term studies of biochemical and cytological measurements are needed as well as simple clinical measurements such as body weight and arterial blood pressure (as in Table 2) which are indications of potential benefit on terms of risk reduction. Longer term studies are also required to determine whether the potential risk reduction is translatable into better clinical outcomes from disease, improved life expectancies and greater confidence that the potential benefits are not offset by toxicity in the longer term (www.fda.gov/cder/guidance/index.htm, www.emea.europa.eu/pdfs/human/ich/030095en.pdf).

CONCLUSION

In conclusion, there is appreciable experimental evidence of the effectiveness of *Aronia* products in an extraordinary range of pathological conditions in which the underlying damage is probably mediated by uncontrolled oxidative processes. The current evidence of effectiveness in several clinical contexts is promising, but does not yet meet the accepted standards that would secure them an indisputable place in therapy. Fortunately, there are several promising avenues of investigation that may be pursued to remedy the present shortcomings.

We wish to announce the tragic and untimely death of Dr med Cosima Chrubasik on 26th March 2010, which deprived not only her family of a much-loved daughter, but also denied medical science the talents of a gifted young researcher. Her intelligent and tenacious curiosity in her chosen field means that she will be greatly missed by those who knew Cosima and her work.

Acknowledgement

The authors sincerely thank Andrew Black, Bristol, for his valuable suggestions during the preparation of the manuscript.

Conflict of Interest

The authors have declared that there is no conflict of interest.

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