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Review

ORGAN-PROTECTIVE EFFECTS OF *ARONIA MELANOCARPA*: AN UPDATED REVIEW

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ABSTRACT

PURPOSE: To collect current information about the organ-protective effects of Aronia melanocarpa obtained in experimental and human studies. METHODS: An advanced search using the keyword "Aronia melanocarpa" was conducted in Medline (via PubMed) and Scopus databases. The following inclusion criteria were applied: original articles published in the time frame between January 2014 and December 2024. A total of 940 reports were identified in both databases, 250 duplicates were eliminated and 597 were excluded due to lack of relevance to the topic. Finally, 93 articles were determined for review. RESULTS: Aronia melanocarpa showed protective effects on the cardiovascular system, central nervous system, liver, stomach, intestines, kidneys, reproductive organs, skin, skeletal muscles, bones, eyes, immunocompetent organs, exocrine and endocrine glands. Most of studies were conducted in animals and in cell cultures. Human studies were scarce and not sufficient to draw final conclusions. CONCLUSIONS: Aronia melanocarpa showed numerous organ-protective effects proven predominantly in animal studies. Therefore, more information is required to obtain objective information related to its protective effects in humans.

Keywords: Aronia melanocarpa, organ-protective, review

INTRODUCTION

Health-promoting benefits of foods gather remarkable scientific and public interest in recent years. In this regard, the concept of functional foods was introduced for the first time in 1980s in Japan. Functional foods, also known as nutraceuticals, are defined as foods which contain bioactive compounds with health-promoting or disease-preventing effects achieved after regular consumption. Their health impact could be studied in *in vitro* and *in vivo* settings (animal studies, clinical trials). Common functional foods include probiotics-rich foods (yogurt, kefir), fish (rich in omega fatty acids), nuts (rich in vitamins, minerals, polyunsaturated fatty acids), certain vegetables (broccoli, tomato, curcuma, soy bean) and berry fruits (rich in polyphenols) (1).

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In the last three decades, the number of studies investigating the health effects of polyphenol (PP)-rich foods is progressively growing. These phytochemicals possess one or more phenolic rings. They are categorized as non-flavonoids (phenolic acids, lignans, stilbenes) and flavonoids (flavonols, flavanols, flavanones, flavones, isoflavones, anthocyanins). Additional functional groups attached to the phenolic ring(s) contribute to their diverse biological activities. PPs are ubiquitously distributed in different plant parts (trunk, leaves, roots, fruits, blossoms). After consumption, very small amounts of PPs are absorbed. Gut microbiota plays a significant role for their bioavailability. Understanding these peculiarities is crucial in order to improve their absorption rate (for instance, through gut microbiota regulation) and therefore, their effects (2).

Aronia species are shrubs belonging to the *Rosaceae* family, subfamily *Maloideae*, originating in North America and introduced later to European and Asian countries. Among

them, *Aronia melanocarpa* (Michx.) Ell. (black chokeberry) has attracted considerable interest in science since it is resistant to low temperatures, damage during transportation and mechanized harvesting. This plant is harvested in the late summer (August, September) and its mature berry-type fruits are used for production of food colorants, juice, jam, wine, nectar and tea. Chokeberry fruits are purplish black, characterized by astringent taste due to the presence of a large amount of PPs, especially proanthocyanidins (3). The effects of *Aronia* fruit consumption (or fruit extract administration) on health have been extensively studied but better understanding of the validity of the data in real practice requires objective interpretation of the information. Thus, the purpose of this paper was to collect the available updated information about the organ-protective effects of *Aronia melanocarpa* obtained in experimental and human studies.

MATERIALS AND METHODS

An advanced search was conducted by independent reviewer (MR) in scientific databases Medline (via PubMed) and Scopus for identification of articles using the keyword "*Aronia melanocarpa*". The following inclusion criteria were applied: original articles; records published in the time frame between January 2014 and December 2024. The exclusion criteria were: irrelevant topic; review articles.

In Medline database, 451 reports were identified, of which 245 were excluded in accordance to the inclusion and exclusion criteria utilized, and 206 reports were used for retrieval of information by an independent reviewer (MR). Additionally, 127 reports were excluded due to lack of organ effects specified, utilization of chemical and/or phytochemical analysis only, non-relevant topic or identification of review articles. Finally, 79 articles were determined for review.

In Scopus database, 489 papers were identified, of which 250 duplicates from Medline database were eliminated. Thus, 239 reports were used for retrieval of information. Before the final review, 225 papers were excluded in compliance with the selected inclusion and exclusion criteria due to lack of organ effects specified, utilization of chemical and/or phytochemical analysis only or identification of review articles. Finally, 14 articles were selected for review.

Thus, a total of 93 papers were approved for final objective review and analysis by two independent reviewers (MR and SVK).

RESULTS

Cardiovascular protection

A total of 16 papers emphasizing on the cardiovascular protection of *Aronia melanocarpa* were identified in the current research from which 1 study was conducted *ex vivo* (on isolated organ), 2 studies were conducted *in vitro*, 13 – *in vivo* (7 animal studies and 6 clinical trials, respectively). Careful analysis of the reports included in this review has found that the cardiovascular benefits could be ascribed to the following main effects: antihypertensive, antithrombotic, improvement of endothelial function and protection of the myocardium and/or blood vessels.

Antihypertensive effect of *Aronia melanocarpa* was reported in one animal study and 4 clinical trials. Cujic et al. tested the effect of 4-week dry chokeberry fruit extract administration on the blood pressure (BP) in spontaneously hypertensive rats. It was found that the extract reduced the systolic (SBP) as well as the pulse pressure which were associated with the established diuretic effect (4). In *Aronia*-supplemented humans compared to placebo, significant SBP reduction was documented in 3 studies (5-7), DBP reduction was reported in 2 studies (5, 8) while both SBP and diastolic (DBP) were reduced remarkably only in 1 study (5). The BP-lowering effect was more pronounced in hypertensive than normotensive subjects and this effect was dose-dependent. These findings were in accordance with the studies focusing on the effects of *Aronia* on the BP-regulating enzymes and mediators. Although Sikora et al. indicated a weak *in vitro* angiontesin-converting enzyme (ACE)-inhibitory activity of chokeberry extract, the same research group found a significant positive correlation between the reduction in ACE activity and SBP/DBP after prolonged (in the course of 2 months) *Aronia melanocarpa* extract supplementation in clinical settings in metabolic syndrome (MS) individuals who are considered a high cardiovascular risk group (5). Moreover, one study found that the intake of chokeberry-containing PP-rich juice was able to reduce the BP variability, especially in hypertensive patients (7). BP variability is a predictive marker for cardiovascular diseases and acute events (9). Thus, its reduction is indicative for cardioprotection. The

phosphorylated form of the endothelial NOS (eNOS), responsible for production of the vasodilatory and antithrombotic nitric oxide (NO), was found to be up-regulated after *Aronia* treatment, leading to NO increase in a time-dependent and dose-dependent manner – this effect peaked at the 10th minute and persisted as long as 48 hours after acute administration (10). This finding was confirmed by Cebova et al. in a model of N-nitro-L-arginine methylester (L-NAME)-induced arterial hypertension in Wistar Kyoto rats. The administration of *Aronia* extract for three weeks resulted in an increase of the total NOS activity in the aorta and the left ventricle (LV). However, eNOS activity was elevated only in the LV. The authors have also reported a reduction in the conjugated dienes, as an index of lipid peroxidation, in both investigated tissues indicating the antioxidant effect of the treatment. The authors hypothesized that the antioxidant activity of chokeberry contributed to the improvement of NOS synthesis since oxidative stress triggered dissociation of NOS from its dimer to the inactive monomer form (10). A clinical study from 2020 involving 20 healthy participants (10 males and 10 females) showed an increase in nitrite/nitrate (NOx) without sex differences registered after 4-day supplementation with two *Aronia*-containing nitrate-rich supplements (11). In addition, these findings were supported by the improvement in the arterial stiffness markers (flow-mediated dilation, pulse wave velocity, augmentation index) in two studies in humans supplemented with *Aronia* berry (poly)phenols (12, 13).

Antithrombotic effect of chokeberry was documented by Sikora et al. (14) and Stevanovic et al. (15). The first study group tested the effect *Aronia melanocarpa* extract on the spontaneous and ADP-activated platelet (PLT) adhesion as well as on the activity of thrombin and plasmin *in vitro*, while the second group assessed in runners prior to race the acute effect of *Aronia* juice consumption on PLT activation markers P-selectin and GP IIb/IIIa. Both studies found a decrease in PLT activation and in addition, the first experiment indicated an anticoagulant as well as fibrinolytic activity of the nutraceutical.

Improvement in the endothelial function was confirmed in studies assessing the effect of *Aronia* on the surrogate endothelial markers eNOS (expression and activity) and NO as well as on endothelial cell proliferation. As mentioned before, chokeberry extract was able to increase the activity of eNOS and elevate NO production (10, 11). Interestingly, in one investigation, the extract was able to increase the proliferation of angiotensin II-exposed endothelial progenitor cells as well as their telomerase activity. These outcomes were linked to the antioxidant activity of the extract since it activated the transcription of nuclear factor erythroid-2 related factor 2 (Nrf2), triggering the expression of the endothelium-protective antioxidant enzyme hemeoxigenase-1 (HO-1) (16).

Protective effects on the cardiac and/or vascular structure were documented by Daskalova et al. (17), Yuste et al. (18), Catalan et al. (19), Shvets et al. (20), Reyzov et al. (21) and Buda et al. (22). The first study found that *Aronia* juice administrated for 90 days preserved the endothelium, the elastic membrane in the tunica media and prevented the subendothelial lipid accumulation as well as decreased the aorta thickness in aging rats (17). The second study described similar outcomes of chokeberry administration on aorta thickness and confirmed its vasoprotective effect in hypercholesterolemic rats (18). The third study evaluated the expression of cardiac and vascular proteins in hypercholesterolemic rats receiving *Aronia* fruit infusion. In the aorta, the infusion was found to down-regulate the protein kinase cAMP-dependent catalytic alpha and as a result could prevent the thrombus formation. The infusion downregulated the vascular smooth muscle proliferative proteins IQ motif containing GTPase activating protein 1 and heat shock protein HSP 90-beta as well as the PCSK9-regulatory adenylyl cyclase-associated protein 1 in the aorta. Moreover, it was established that the infusion could increase the gene expression of the platelet-aggregating fibromodulin and cell contractile proteins transgelin 1 and 2 in the aorta (19). The fourth study aimed to examine the effect of intraperitoneal *Aronia melanocarpa* extract administration on the phospholipid composition of the myocardium of rats at a different age during immobilization stress. In aged rats, the composition of the myocardium changed as the amount of phosphatidylcholine decreased but the total amount of lysophosphatidylcholine, phosphatidylserine and phosphatidylinositol increased significantly. These changes were less pronounced in adult rats where only an increase in the phosphatidylcholine hydrolysis was detected. *Aronia melanocarpa* extract

administration 60 minutes before the stress test was able to reduce the amount of phospholipids in the myocardium of aged rats without similar effect observed on adult rats and this effect was not antioxidant-dependent (20). The fifth study aimed to assess the effect of 10-week *Aronia melanocarpa* juice treatment on the structure of the myocardium and coronary arteries in MS rats. MS induced cardiomyocyte degeneration and necrosis as well as endothelial cell necrosis while the juice was able to protect against these histopathological changes in the tested tissues (21). The sixth study evaluated the effects of chokeberry extracts obtained from dry or frozen berries in six increasing concentrations (10, 50, 75, 100, 500 μg/mL), on the isolated mice aorta rings exposed to angiotensin II, lipopolysaccharide and glucose to simulate the renin-angiotensin system in *ex vivo* settings. Both extracts decreased H_2O_2 and superoxide production in a concentration-dependent fashion and promoted vascular relaxation in the stimulated aorta rings at the concentration of 100 μg/mL (22).

Neuroprotection

Neuroprotective effects of *Aronia melanocarpa* were documented in a total of 16 *in vitro* studies or *in vivo* experiments. Based on the available data, neuroprotective properties of chokeberry were associated with the following effects: antioxidant and anti-inflammatory; preservation of brain tissue morphology; cognition-enhancing; anti-aging; anxiolytic-like; antidepressant-like; anti-ischemic; anti-nociceptive.

Antioxidant and anti-inflammatory activities in the central nervous system are the main mechanisms of *Aronia*-mediated neuroprotection. Several studies documented the plant could potentiate the endogenous antioxidant defence. In one study, rats fed either high-fructose or high-fat diet receiving *Aronia* juice for 5 weeks had higher catalase (CAT) and paraoxonase activity as well as decreased pro-oxidant marker protein carbonyl groups (PCGs) in the brain (only in high-fat diet group) (23). These findings were confirmed later by Case et al., who described that low dose *Aronia melanocarpa* concentrate could reduce the production of superoxide, hydroxide and oxidized glutathione (GSH) in a model of paraquat-induced neurotoxicity. Interestingly, the authors documented opposite effects of the higher doses – superoxide production increased independent of paraquat contributing to increased neuronal cell death (24). A recent study on animals found chokeberry was able to shift the pro-oxidant/antioxidant status in the brain and exert neuroprotection in rats with a model of human exposure to cadmium (Cd) (25). Anti-inflammation was associated with the ability of *Aronia* to suppress AMPK/SIRT1/NF-κB signalling as described by Zhao et al., who detected *Aronia*-induced reduction in the expression of p-NF-κB and its downstream protein IκB-α, as well as in the NLRP3 inflammasome in the brains of D-galactose-induced aging mice (26).

Preservation of brain tissue morphology were documented in 5 studies. Using selective staining for neurofibrillary networks, axons and dendrites, Daskalova et al. examined the effect of continuous (for 105 days) daily administration of *Aronia melanocarpa* juice on the hippocampal structure in aged rats. Significant increase in the nerve fibre density in the hippocampal perforant pathway was documented compared to the untreated controls (27). *Aronia*-mediated stimulation of the hippocampal neurogenesis was described in Aβ-induced memory damaged rats, in which a higher number of preserved neurons and increase in the number of pyramidal cells in CA1 and CA3 regions from chokeberry anthocyanins were observed (28). Zhao et al. found that intraperitoneal injection of *Aronia melanocarpa* polysaccharide to D-galactose-induced aging rats was protective against the brain senescence as detected by reduced microscopic and macroscopic features of aging in the dentate gyrus (26). Two studies indicated that cyanidin 3-O-galactoside from *Aronia* berries could protect the brain neurons and reduce the accumulation of amyloid in experimental models of aging in rats/mice (29,30).

Cognition-enhancing effects were documented in experimental models of neurotoxicity/memory impairment/aging in animals. *In vitro* study by Lee et al. described the protective effect of *Aronia* berry extract on the hippocampal HT22 cells in glutamate-induced neurotoxicity due to its antioxidant activity demonstrated by stimulation of glutathione reductase (GR) and glutathione peroxidase (GPx), and increase of GSH (31). Anti-dementia activity was detected in another *in vitro* experiment where *Aronia* leaf extract was able to suppress the acetylcholinesterase (AchE) activity by 60-70%, thus promoting

acetylcholine-mediated cognitive improvement (32). Moreover, *Aronia melanocarpa* anthocyanins were able to reduce Aβ-induced neuronal apoptosis by regulating the intracellular Ca^{2+} concentration, elevating ATP, suppressing apoptotic cytochrome c, caspase-9, cleaved caspase-3 and Bax, and stimulating the anti-apoptotic Bcl-2 (33). The experiment of Young et al. revealed that ethanol extract obtained from lactic acid fermented *A. melanocarpa* was able to produce stronger anticholinesterase activity and showed higher memory-enhancing effect compared to the non-fermented extract, as confirmed by the decreased latency time in Morris water maze (MWM) test. Prevention of the Aβ-induced memory impairment in rats was later confirmed in the study of Wen et al. who documented an improvement of the spatial memory in MWM test in rats after one-month oral supplementation with *Aronia* anthocyanins (29). In scopolamine-induced memory impairment model, *Aronia melanocarpa* extract administration was able to reduce the escape latency and swimming distance of mice in MWT test, as well as the latency time in passive avoidance test. This effect was ascribed to the reduced AchE activity and up-regulation of brain-derived neurotrophic factor as well as p-CREB in the hippocampus (34).

Anti-aging activities of chokeberry in the brain were documented in five animal studies. Three studies incorporated D-galactose to induce brain senescence. In one of them, chokeberry anthocyanins were found to prevent D-galactose-induced cognitive decline in mice and this effect was associated with reduced neuronal DNA damage, increased brain monoamines (norepinephrine, dopamine, serotonin), reduced oxidative stress and neuroinflammation (35). In another study, *Aronia* fruit juice administration to aged rats for 105 days also improved the cognitive performance as it increased the numbers of vertical movements in the activity cage and the number of avoidances on the 3rd, 4th and 5th days of the learning session in the active avoidance test (27). In the third study, Zhao et al. found that *Aronia* polysaccharide was able to improve the moving distance and latency in 8-arm maze test in mice. These results correlated with decreased AchE activity and reduction of oxidative stress (decreased malondialdehyde (MDA), increased Nrf2 and superoxide dismutase (SOD)/CAT/HO-1 levels, respectively), as well as inflammation (suppressed NF-κB pathway), pyroptosis (reduced Gasdermin D, caspase-1, interleukin (IL)-1β) and apoptosis (increased phosphorylated PI3K/AKT/mTOR/Bcl-2 and reduced Bax/caspase-3) in the brain and improved energy metabolism (via AMPK). An interesting finding of this experiment was the association between the improved composition of the intestinal microbiota and the retarded brain senescence (26). Two studies implemented senescence accelerated mouse prone 8 aging mice to test the effects of *Aronia melanocarpa*-derived cyanidin 3-O-galactoside (C3G) on the brain senescence. Wen et al. found that C3G co-administered with the antidiabetic drug metformin improved the spatial learning by preventing amyloid accumulation and neuronal damage in the brain (29). In the study of Fan et al., C3G improved spatial memory and reduced amyloid load and improved energy metabolism in the brain, as measured by the improved glucose uptake (30).

Anxiolytic-like and antidepressant-like activities were documented in two studies. Tomic et al. tested the effect of one-month *Aronia melanocarpa* juice administration on the behavior of rats and documented anxiolytic-like effects in elevated plus maze (EPM) test and a decreased state of despair in forced swimming test (FST) which indicated an anti-depressant-like effect. These results were supported by *in vitro* assays which showed monoamine oxidase (MAO)-A/MAO-B-inhibitory activity of the juice ingredients (36). Georgieva et al. used ovariectomy-induced oestrogen deficit model in female Wistar rats to assess the behavioural effects of 3-month *Aronia melanocarpa* fruit juice treatment. Ovariectomy induced anxiety assessed in the EPM and social interaction test as well as depression-like behaviour evaluated in the FST, and the juice effectively antagonized these negative outcomes (37).

Anti-ischemic effect in the brain was documented by Liu et al. Network pharmacological analyses revealed that anthocyanins in *Aronia melanocarpa* could be the main protective components against cerebral ischaemia, and the tumour necrosis factor (TNF)-signalling pathway – their main target. Molecular docking revealed a strong binding between anthocyanins and matrix metalloproteinase (MMP)-2. An *in vivo* model of middle cerebral artery occlusion in rats was implemented to explore the effects of 7-day anthocyanin treatment. It was found that

anthocyanins antagonized the blood-brain barrier damage, down-regulated the extracellular MMP-2 and the pro-inflammatory TNF-α, IL-6, iNOS 24 hours after cerebral ischemia. The results were confirmed in an *in vitro* assay, where anthocyanin therapy reduced the nitrite content and the expression of TNF-α, IL-1β, IL-6 and iNOS in oxygen-glucose deprived cultured microglia (38).

Anti-nociceptive effect was documented in one study. *Aronia melanocarpa* juice, administered in the course of 3 months, reduced the nociceptive perception in oestrogen-deprived female rats subjected to ovariectomy as assessed by the hot plate test (37).

Hepatoprotection

Protective effects on the liver were documented in 14 studies (3 *in vitro* studies, 10 animal studies, 1 human study).

In vitro studies documented that *Aronia* could antagonize hepatocyte damage or fibrotic changes. Kondeva-Burdina et al. reported that *Aronia* juice, applied on isolated hepatocytes, exposed to carbon tetrachloride or tert-butyl hydroperoxide, was able to prevent the depletion of hepatic GSH and thus, it could alleviate hepatocyte oxidative stress and cell death. Interestingly, the highest dose used was superior to the effect of the hepatoprotective agent sylimarin (39). In another investigation, *Aronia melanocarpa* extract mixed with *Ligularia fischeri* extract was found to increase the activities of alcohol dehydrogenase and aldehyde dehydrogenase in HepG2 cells (40). This enzyme-inducing activity was one of the proposed mechanisms for the liver protection established after the oral ingestion of the mixture of extracts in animal models alcohol-induced injury (40). The most abundant flavonoid fraction in *Aronia* berries – anthocyanins, applied on HSC-T6 cell, exerted an anti-inflammatory effect by suppressing the production of IL-1, Il-6, TNF-α and cyclooxygenase (COX)-2, as well as an anti-fibrotic effect by blocking the expression of the pro-fibrotic factors transforming growth factor (TGF)-β1, phosphorylated-Smad2 protein, α-smooth muscle actin and collagen I (41). Likewise, an anti-fibrotic effect was documented in the study of Zhao et al. who reported that *Aronia* polysaccharide could preserve the hepatocytes and could prevent collagen accumulation in the liver by suppressing the pro-fibrotic TGF-β/Smad pathway and PI3K/Akt pathway, respectively, and by improving the gut microbiota diversity (42).

One clinical study involving 80 pre-diabetic subjects was detected in the reviewed literature regarding the liver-protective properties of *Aronia*. The participants were given a mixture containing *Aronia melanocarpa*, red ginseng, shiitake mushroom and nattokinase for 12 weeks. At the end of the experimentation, serum levels of aspartate aminotransferase, alanine transaminase and gamma-glutamyl transferase were significantly lower in the treated group. These improved liver parameters were accompanied by improved insulin resistance markers such as homeostatic model assessment of insulin resistance (HOMA-IR) and fasting serum insulin level as well as the inflammatory marker high-sensitivity C-reactive protein (43). Three animal studies tested the effects of *Aronia* in models of liver damage associated with metabolic dysfunction. Park et al. used high-fat high-fructose diet to induce non-alcoholic fatty liver disease in mice. Co-administration of *Aronia* powder (0.5% and 1%) with the diet for 8 weeks led to a reduction of the expression mRNA of lipogenic sterol regulatory element-binding protein, acetyl-CoA carboxylase and fatty acid synthase accompanied by a decrease of liver triglyceride (TG) levels. These results were supported by the histological examination of the liver which showed reduced hepatocyte fat accumulation (44). Chen et al. used high-fat diet and streptozotocin to induce type 2 diabetes mellitus and to evaluate the effects of *Aronia melanocarpa* anthocyanin extracts supplementation for 5 weeks. The extracts were shown to down-regulate the NF-κB, JAK/STAT and iNOS pro-inflammatory signalling cascades as well as TNF-α, IL-6, monocyte chemoattractant protein-1 (MCP-1), intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1. The expression of suppressor of hepatocyte signalling 3 (SOCS3) was reduced and thus, hyperinsulinemia was antagonized. Moreover, the treatment facilitated hepatic glucose uptake and prevented the hepatocyte enlargement and inflammatory cell infiltration in the liver (45). *Aronia melanocarpa* treatment was also tested in the settings of alcohol-induced liver damage. C57BL/6 mice were administered alcohol in the course of 6 months and *Aronia melanocarpa* was administered in the last 6 weeks in a study of Wang et al. The treatment protected the liver by alleviating liver inflammation through

reduction of IL-2/IL-4/IL-6, thioesterase 4, fibrinogen-like protein 1, fibrinogen γ by preventing liver fat accumulation and as well as by exerting antioxidant effect resulting from the activation the Nrf2 pathway, suppression of Keap1 and increase of the activities of the antioxidant factors CAT, SOD, GPx, coenzyme Q3 and microsomal glutathione S-transferase 3 (46). In another report, *Aronia melanocarpa* Elliot anthocyanins mitigated alcohol-induced liver injury by alleviating liver inflammation: the proliferative index Ki67, the inflammatory molecules IL-6 and TNF-α were reduced, PI3K/Akt and Keap1/HO-1 cascades were suppressed and the expressions of the nicotinic receptor α7nAChR and collagen I were restored (47). Furthermore, in another study it was documented that *Aronia*, administered together with *Ligularia fischeri* in a form of an extract, induced the activity of alcohol-metabolizing enzymes and reduced the blood alcohol concentration as well as its area under the curve (40).

The effects of *Aronia melanocarpa* in Cd-exposed experimental animals were assessed by Mężyńska et al. and Kozlowska et al in a total of three studies. The animals were exposed to Cd in the diet for 3-24 months and *Aronia* extracts were administered orally for up to 24 months. These reports indicated that the extracts, used in low to moderate doses, were able to restore the liver antioxidants (SOD, CAT, GPx, GSH), to alleviate the oxidative hepatic damage (as evidenced by a decrease in lipid peroxidation markers lipid peroxides and 8-isoprostane, protein oxidation markers protein carbonyl groups and 3-nitrotyrosine, as well as the DNA oxidative damage marker 8-hydroxy-2′-deoxyguanosine), and to antagonize Cd-induced disturbance in hepatic collagen homeostasis (48-50). Interestingly, in the livers of rats exposed to cisplatin, *Aronia* extract was also able to prevent cisplatin-induced reduction of trace elements (Cu, Fe and Mn), important for stress oxidative enzymes (51). These studies suggest that the hepatoprotective effect of *Aronia* was complex and multifactorial.

Intestinal protection and effects on gut microbiota

The effects of *Aronia melanocarpa* on the intestines were investigated in a total of 16 studies.-The research of databases identified ten reports focused on the intestinal microbiota outcomes, three – on the effects in experimental models of colitis, two – on the effects on intestinal wall integrity, and one – on the anti-cytotoxic effect.

The first study documenting **improvement of gut microbiota** was conducted by Wu et. al who examined the effect of *Aronia* juice applied to human intestinal microbial ecosystem with a co-culture of intestinal and endothelial cells. The results revealed an increase in the relative abundance of *Firmicutes* in the ascending, transverse and descending colon, *Proteobacteria* in the ascending colon and *Akkermansia* in transverse and descending colon as well as reduction in the relative abundance of *Bifidobacterium*. These changes were associated with an increased production of metabolically beneficial short-chain fatty acids (SCFAs) propionate and butyrate (52). An increased growth of SCFA-producing *Anaerostipes* and *Bacteroides* was documented in a clinical trial among 66 healthy participants who were administered PP-rich chokeberry extract or chokeberry fruit powder, respectively. Interestingly, a significant positive correlation was noted between the flow-mediated dilation of extract-treated subjects and SCFA-producing *Dialister*, *Phascularctobacterium* and *Roseburia* (12). Similar results were found in another study showing a decrease in the *Firmicutes*/Bacteroidetes ratio in the gut flora and an increased relative abundance of anti-obesity *Bacteroides*, *Preotella* and *Akkermansia* after *Aronia* extract-treated high-fat diet-fed mice. Moreover, a decreased bile acid content with a reduction of cholic and deoxycholic acids and an increase in chenodeoxycholic acid was noted, and these findings correlated positively with *Bacteroides* and *Prevotella* and negatively with *Clostridium*, *Eubacterium* and *Ruminococcaceae*. In addition, these gut microbiota changes were associated with improvement of the lipid profile and anti-obesity effect (53). The anthocyanins in *Aronia* fruits might be responsible for the improvement of the microbiota as *in vitro* gastrointestinal digestion and colonic fermentation model highlighted the increased abundance of *Bacteroides*, *Lactobacillus*, the increased growth of *Bifidobacterium*, *Blautia*, *Faecalibacterium* and the decreased growth of *Prevotella*, *Megamonas*, *Escherichia*, *Shigella*, *Fusobacteria*, *Klebsiella*, *Enterococcus* and *Streptococcus* after anthocyanin treatment (54). Likewise, Zhu et al. (55) and Zhao et al. (26) confirmed the effects of *Aronia* ingredients on *Bacteroides*. On the contrary, one report

showed an increase in *Firmicutes* in *Aronia* pomace-fed pigs (56). New studies showed stimulatory effects of *Aronia* products on *Intestinomonas butyriciproducens*, *Lawsonibacter assacharolyticus*, *Butyricimonas faecihominis*, *Bacteroides xylanisolvens* (13), *Parasuterella* (57) and *Eggerthellaceae* (58) and inhibitory effects on *Senegalimassilia anaerobia* and *Haemophilus* parainfluenzae (13).

Effects in experimental models of colitis was documented in the reports of Kang et al. (59), Valcheva-Kuzmanova et al. (60), Pei et al. (61) and Li et al (62). Kang et al. used dextran sulfate sodium (DSS) to induce ulcerative colitis in BALB/c mice and *Aronia* berry extracts as therapeutic substances. The extracts reduced the histological signs of colitis and prevented the weight loss, the reduction of the colon length and the increase of the disease activity index. The results were accompanied by inhibition of prostaglandin E_2 synthesis and reduction of the inflammatory markers (59). Valcheva-Kuzmanova et al. investigated the effects of *Aronia melanocarpa* fruit juice in a model of trinitrobenzensulfonic acid-induced colitis in Wistar rats. Due to its antioxidant and anti-inflammatory activities, the juice ameliorated the macroscopic signs of colitis (area of ulceration, adhesions to adjacent organs, increased colon weight/length ratio), reduced the histopathological changes (epithelial destruction and inflammation) and lipid peroxidation in the colon (60). Interesting outcomes were described in a model of T-cell transfer-induced colitis in mice. Five-week *Aronia* diet reduced the inflammation in the colon as evidenced by the lower weight/length ratio, reduced 2′-deoxy-2′-[18F]fluoro-d-glucose uptake in the diseased colon, down-regulation of the pro-inflammatory IL-6 mRNA in the colon and up-regulation of the anti-inflammatory IL-17A and IL-10. Furthermore, it decreased the production of mitochondrial peroxide production in the spleen and antagonized the increase of MDA by maintaining the colonic GSH and GPx activity and preventing the down-regulation of the colonic mRNA expression of the antioxidants SOD2, GPx, GR, gamma-glutamylcysteine synthetase and peroxiredoxin (61). Li et al. documented that 3-week administration of *Aronia* extract to DSS-induced inflammatory bowel-diseased mice could amend the clinical deterioration and could suppress the expression and levels of apoptosis-related Bax, Bcl-2, caspase-3 and caspase-9 through NF-κB and MAPK/ERK inhibition and Keap1/Nrf2 stimulation (62).

Improvement of the intestinal wall integrity was reported by Valdez et al. (63) and Ren et al. (56). In the first study, Caco-2 cells were cultured in order to form a model intestinal barrier. Barrier function was damaged by an inflammatory cocktail (TNF-α, IL-1β, and IFN-γ in the basolateral media and lipopolysaccharide in the apical media). *Aronia* berry powder or individual PPs applied to the basolateral media prevented the inflammation-induced permeability by increasing zonula ocludens-1 expression and occludin in tight junctions. Interesting to note, gut microbiota-derived PP metabolites were not able to improve Caco-2 barrier function (63). In the second study, *Aronia* pomace, administered to pigs in concentrations of 4% and 8% in the basic diet, led to the following outcomes: 4% *Aronia* pomace increased the jejunal expression of zonula occludens-1, occludin and claudin-1 genes and 8% pomace increased the jejunal expression of zonula occludens-1 and claudin-1. These data suggested that the lower concentration was more beneficial to overall health of pigs (56).

Anti-cytotoxic effect was documented in an *in vitro* study of Kšonžeková et al., who used anthocyanin-rich *Aronia melanocarpa*, *Sambucus nigra*, *Vaccinium myrtillus* and *Vaccinium corymbosum* extracts, respectively, to treat porcine intestinal epithelial cell line IPEC-1. The results revealed that cyanidin glycoside-rich *Aronia* and *Sambucus* extracts exhibited better antioxidant and anti-cytotoxic effects compared to the other two extracts with complex anthocyanin compositions. Furthermore, extracts that were rich in cyanidin glycosides promoted the growth of IPEC-1 cells without showing cytotoxic effects at equivalent *in vivo* concentration (64).

Gastroprotection

Three studies (one *in vitro* and two animal studies) evaluating the effects of *Aronia* and *Aronia*-derived phytochemicals on the stomach were identified.

Paulrayer et al. used ethanol-induced gastric ulceration model to test the effects of *Aronia melanocarpa* hydro-alcoholic extract (AMHAE) pre-treatment. AMHAE reduced dose-dependently the size of the ethanol-

induced ulcers, as assessed by the reduction of the ulcer index and microscopic damage score (comparable to the proton-pump inhibitor omeprazole). The ulcer-preventing effect was ascribed to the antioxidant and anti-inflammatory effects of the extract since it reduced myeloperoxidase, MDA, NF-kB p65, TNF-α and MCP-1 and increased SOD, CAT and GPx levels as well as the anti-inflammatory IL-4 and the protective HSP-70. Pre-treatment with L-NAME, indomethacin, naloxone and capsazepine prevented the gastroprotective activity of AMHAE indicating the involvement of NO, PGs, opioids and TRPV (vanilloid receptor-related transient receptor potential) in the protection (65). Valcheva-Kuzmanova et. al investigated the effects of four different *Aronia*-based juices (AM1, AM2 – obtained from *Aronia* fruits at 20 °C and 60 °C, respectively), AMRC (a mixture of AM2 with *Rosa canina* extract) and AMAV (*Aronia* juice with *Alchemilla vulgaris*). The juices were given as pre-treatment in a model of indomethacin-induced gastric ulceration in rats. The four juices abolished indomethacin-induced gastric ulceration manifested by superficial erosions, focal desquamation and zonal destruction of gastric glands. They also reduced apoptosis (assessed by increase in Bcl-2 and decrease in Bax) as well as lipid peroxidation (measured by the decrease of TBARS). The most notable effects were achieved with AMAV pre-treatment (66). The third study investigated the *in vitro* effects of *Aronia* anthocyanin pre-treatment on gastric epithelial cell line NCI-N87. The authors documented a decrease in the IL-1β-mediated secretion of the pro-inflammatory IL-8 as well as lack of cytotoxic effects (67).

Pulmonary protection

Pulmonary protective effects were reported in three studies.

In the study of Valcheva-Kuzmanova et al., the effect of *Aronia melanocarpa* fruit juice was assessed in amiodarone-induced pneumotoxicity model in rats. The juice effectively counteracted the amiodarone-induced increase of the lung weight coefficient, reduced the fibrosis as measured by the decrease in the level of hydroxyproline and mitigated the oxidative stress as measured by the decreased level of MDA. In bronchoalveolar lavage, the juice treatment reduced the protein content, total cell count, polymophonuclear cells, lymphocytes as well as the activities of the enzymes lactate dehydrogenase, acid phosphatase and alkaline phosphatase (ALP) (68). Jang et al. tested the effect of *Aronia* bioactive fraction pre-treatment on lipopolysaccharide-exposed BEAS-2B cells. The pre-treatment significantly decreased the expression of the inflammatory factors IL-1β/-6/-8, TNF-α, COX-2, iNOS and arrested the cell cycle at G0/G1 and S phases (69). Another study was designed to evaluate the effect of *Aronia*-derived C3G, administered for 56 days to mice exposed to silica particles-containing solution. The authors detected a reduction in the pulmonary fibrosis in a dose-dependent manner and relief of the silica particles-induced epithelial-mesenchymal transition as shown by the up-regulation of the epithelial factor E-cadherin mRNA and down-regulation of the mesenchymal cell protein α- α-smooth muscle chain mRNA. Moreover, inflammatory cell infiltration was suppressed. These effects were mediated by C3G-stimulated Nrf2/HO-2 signaling which inhibited the proliferative TGF-β/mTOR pathway (70).

Kidney protection and effects on urinary tract infections

A total of 7 studies describing the effect of *Aronia melanocarpa* on the urinary tract were identified.

Protective effects for the kidneys were documented in 6 studies (5 animal studies and 1 human study).

One of the animal studies was conducted by Song et al., who investigated the effects of 8-week walnut and chokeberry mixture on oxidative and antioxidant markers in rat kidney samples in a model of d-galactose-induced aging. The results showed amelioration of the d-galactose-induced MDA increase in the kidneys which was accompanied by the up-regulation of the hepatic SOD and GPx (71). Similar results were documented in another animal study, where cisplatin-induced oxidative stress and reduction in the total antioxidant capacity (TAC) of the kidneys were antagonized by 4-week chokeberry *Aronia melanocarpa* extract treatment. In addition, the treatment prevented cisplatin-induced decrease of trace elements (Cu, Zn, Fe and Mn) important for the stress oxidative enzymes (51). Comprehensive investigation of the effects of *Aronia melanocarpa* on the kidneys were pursued by Li et al. and Smereczanski et al., who tested the effects of chokeberry-derived anthocyanins and

chokeberry extract in renal ischemia-reperfusion injury and Cd-induced kidney damage models, respectively. The first model was accomplished using renal pedicle clamping and subsequent 24-hour reperfusion in mice. The animals were pretreated with cyanidin-3-glucoside (C3GL), C3G, cyanidin-3-arabinoside (C3A) or with the mix of these anthocyanins (AC) for 2 weeks. Serum creatinine, blood urea nitrogen (BUN), IL-1β, TNF-α and MCP-1 were significantly reduced in all anthocyanin-treated groups compared to the untreated group and this effect was most pronounced in C3A-treated animals. In the kidneys, reduction in the levels of IL-1β, Il-6, TNF-α and MCP-1 were most demonstrative in AC- and C3A-treated groups. The activity of GSH, SOD and CAT increased most notably again in AC and C3A groups (72). In Cd-induced kidney damage model, Cd was administered in two different doses for 24 months – 1 mg/kg and 5 mg/kg. The experiment investigated and the effect of *Aronia* extract on kidney Cd concentration, on markers of kidney function (urea, uric acid, total protein presented as protein/creatinine ratio (PCR) as well as on markers of glomerular injury (creatinine clearance, serum albumin presented as albumin/creatinine ratio (ACR), and tubular injury (N-acetyl-β-D-glucosaminidase, ALP, β2-microglobulin). Histological examination of kidney samples as well as determination of pro-inflammatory (chemerin, macrophage inflammatory protein 1 alpha) and apoptosis (Bax) markers in the kidneys were also carried out. A positive correlation was established between blood Cd levels and markers of tubular and glomerular damage as well as between blood Cd concentration and chemerin. The extract prevented Cd-mediated increase in N-acetyl-β-D-glucosaminidase, ALP, β2-microglobulin, kidney injury molecule-1, chemerin and macrophage inflammatory protein 1 alpha, and preserved ACR, PCR, uric acid, urea, creatinine and creatinine clearance within control values. Chokeberry extract also prevented Cd-induced tubular damage (vacuolization, hyalinization, hypertrophy and hyperplasia of the convoluted tubule epithelium, interstitial proliferation and tubular lumen extension) and glomerular damage (congestion of the cortex/medulla interface, perivascular oedema) (73). In another experiment, gentamicin was used to induce nephropathy in rats, manifested by hypoalbuminemia, increased serum levels of globulins, urea and creatinine, haemolysis, echinocytosis and platelet aggregation. Chokeberry extract, administered as pre-treatment, was associated with hypochromic anaemia. However, it prevented the rest of the haematological disturbances. When administered as post-treatment, *Aronia* extract reduced the severity of the anaemia and normalized the urea and creatinine values (74).
One clinical study testing the effects of standardized *Aronia* extract on the redox status in haemodialysis patients was identified in the literature. The study involved 30 patients, receiving the extract for 30 days. The results showed reduction in the levels of nitrites and superoxide anions as well as elevation in the CAT and GSH which subsequently led to increased haemoglobin and haptoglobin levels as well as reduced ferritin and lactate dehydrogenase levels (75).

One human study in the reviewed databases evaluated the effects of *Aronia melanocarpa* juice the incidence of **urinary tract infections** (UTIs). In a cross-over study, 236 nursing home residents were included, consisting of 2 subject groups – A and B. Group A received a placebo drink for 3 months followed by chokeberry juice for the next 3 months while it was vice versa for group B. It was found that the number of patients treated with antimicrobial drugs for UTIs in the periods of juice consumption was reduced. However, this effect was transient as it disappeared after the treatment (76).

Effects on reproductive organs and gonadal functions

A total of six animal studies were identified in the search process.

Protective effects on male reproductive organs were reported in two studies. Kim et al. investigated the effect of *Aronia melanocarpa* on the prostate in rats with testosterone propionate-induced benign prostate hyperplasia. Four *Aronia* extracts (T1, T2, T3, T4) obtained under different extract conditions, were orally administered in the course of 6 weeks. It was found that T1 antagonized testosterone-induced prostate weight increase, reduced the level of dihydrotestosterone and 5α-reductase in the prostate and in the serum as well as the mRNA expression of the prostate-specific protein proliferating cell nuclear antigen (77). Daskalova et al. aimed to examine the effect of 3-month *Aronia melanocarpa* juice treatment on the rat testicular histology and

oxidative stress in aged rats. The following age-related changes were detected: thinning and vacuolization of the spermatogenic epithelium, reduced spermatozoa count, decreased number of tubules with active spermatogenesis, expanded extracellular space and reduced vascularity around the Leydig cells. Moreover, the expression of the spermatogenesis-stimulating factors neurotrophin-2 and TRK-C was markedly reduced in aged animals compared to young rats. In the testicular tissue of aged *Aronia*-treated rats, the authors documented decrease in the oxidative stress, manifested by increased expression of nNOS, eNOS and MAS1 in the seminiferous tubules and in the Leydig cells. In addition, enhanced peritubular vascularization and improved expressions of neurotrophin-3 and tyrosine protein kinase receptor C in Leydig cells were noted (78).

Four studies documented **beneficial effects on female reproductive organs**. Rudic et al. conducted a detailed study to test the effect of standardized *Aronia melanocarpa* extract, administered alone or combined with metformin for 28 days, in dehydroepiandrosterone-induced polycystic ovary syndrome (PCOS) in female rats. Upon induction of PCOS, a marked deterioration was observed in several key parameters, such as body weight, menstrual cyclicity, ovarian histomorphology and ovarian volume. Furthermore, serum levels of testosterone and progesterone were significantly altered, along with an increase in lipid profile markers, specifically LDL, TG, and total cholesterol levels. Both extract-receiving groups had improvement in the ovarian cyclicity after 4-5 days, reduced occurrence of follicular cysts, increased number of the corpora lutea, antral and tertiary follicles, decreased number of atretic follicles and reduced final body weight. Moreover, in rats treated with the extracts, there was a decrease in the transverse and longitudinal ovarian diameter and absence of hyperthecosis or fluid-filled formations. *Aronia* extract supplementation reduced testosterone and estradiol and increased progesterone levels. Improvement in the glucose tolerance was also achieved, as registered by the fasting glucose values as well as glucose values and glucose area under the curve during GTT. *Aronia* treatment mitigated the oxidative stress, as seen by the decrease in the O_2^- (serum), H_2O_2 (serum) and TBARS (serum and ovaries), increase in CAT and SOD activities, and improvement of the lipid profile by decreasing TG and LDL-cholesterol levels (79). Another comprehensive investigation conducted by Çelik et al. studied the effects of *Aronia melanocarpa* extract on cyclophosphamide-induced premature ovarian failure (POF). The model impaired the oestrus cycle (assessed by cytological examination of vaginal smears), increased the inflammatory cell infiltration, haemorrhages and cystic structures with fibrosis in the ovaries. Additionally, the experimental model induced decrease in the number of functional follicles and increase in the number of atretic follicles in the ovaries. Moreover, decreased immunoreactivity to the angiogenetic factor VEGF and increased immunoreactivity for apoptosis-related caspase-3 and caspase-9 were detected. *Aronia melanocarpa* extract administration restored the oestrus cycle, increased the numbers of the primordial, secondary and tertiary follicles and decreased the numbers of the atretic follicles. Compared to the cyclophosphamide-induced POF rats, the expression of VEGF increased and the caspase-3/-9 expression decreased to control values (80). In another study, 8-week administration of *Aronia melanocarpa* powder solutions were able to alleviate oxidative stress (as evidenced by reduced Keap1 and increased Nrf2, HO-1 and SOD expression) and improve the oestrogen status (increased oestradiol in the serum) during ovulation of laying hens (81). Kang et al. investigated the effects of *Aronia melanocarpa* hot water extract on the menopausal symptoms in ovariectomized rats. Operated animals showed dyslipidaemia characterized by increased serum levels of total cholesterol, LDL-cholesterol and TG as well as impaired activity of bone-specific ALP. Moreover, ovariectomy reduced the overall collagen content in the connective tissue. Administration of the extract improved the collagen content in the connective tissue, significantly increased the ALP activity and decreased the TG levels (82).

Effects on skeletal muscle and bone

A total of six studies (2 – *in vitro*, 4 – animal studies) were found focusing on the effects of *Aronia melanocarpa* on skeletal muscle and bone.

Effects of *Aronia melanocarpa* on skeletal muscles were investigated by Makanae et al., Yun et al. and Liu et al. In the study of Makanae et al., *Aronia melanocarpa* extract was administered orally to rats and its effects on

muscles were tested alone or in response to isometric contraction of selected skeletal muscles on the 1st and 6th hour. The phosphorylation of ERK1/2 was increased on the 1st hour, while on the 6th hour increased phosphorylation of Akt at Ser^{473}, p70S6K, rpS6 and AMPK was documented. It was found that the extract was able to stimulate the mTOR1 anabolic pathway components (Akt, p70S6K, rpS6, AMPK) especially after isometric muscle contraction. However, muscle protein synthesis was not stimulated. Moreover, increase in muscle degradation mediator MAFbx was detected (83). The study of Yun et al. confirmed these findings. It investigated the effect of *Aronia melanocarpa* extract on muscle cell differentiation and proliferation in *in vitro* and *in vivo* settings. A myoblast cell culture C2C12 was used for the *in vitro* model in which *Aronia* treatment increased the expression of MHC, myogenin and phosphorylated Akt, stimulated the phosphorylation of mTOR and increased the number of MHC (+) multinucleated myotubes which also became larger in size. In addition, no cytotoxic effect was registered. These findings highlight the pro-myogenic effect of the extract via Akt activation. The same culture was used to investigate the effect of the extract on dexamethasone-induced myotube atrophy. The results showed that *Aronia melanocarpa* extract could prevent dexamethasone-induced reduction in myotube diameter and elevation of muscle-specific ubiquitin ligases MuRK1 and Atrogin-1. Wild type C57BL/6 mice were included in the *in vivo* experiment. They received *Aronia* extract in the course of 8 weeks. *Aronia*-treated animals showed an increase in the mass of the muscles soleus and extensor digitorum longus, as well as enlarged myofibers, which were linked to enhanced grip strength test outcomes compared to the control group. Additionally, increase in the number of MyhIIa and MyhIIb (+) fibres were documented via immunofluorescence method. Moreover, increased activity of enzymes of the oxidative and glycolytic metabolic pathways were detected. These changes were accompanied by elevation of the myoglobin content and the phosphorylated S6K protein, key mediator in the Akt/mTOR signalling (84). A recent study exploring the effects of *Aronia* powder, *Aronia* anthocyanins and their metabolites on TNF-α-treated muscle satellite cells confirmed the protective effect of *Aronia*. Cell differentiation was impaired by TNF-α while *Aronia*, and especially its metabolites, prevented this negative impact. Moreover, dexamethasone-mediated myotube atrophy was counteracted by *Aronia* and myotube formation was enhanced. The authors detected decreased expression of toll-like receptor 4 and NF-kB genes indicating that the protection was mediated by the anti-inflammatory effect of *Aronia* berries (85).

Protective effects in bones were documented in the studies of Brzoska et al. and Ghosh et al. The first study group tested the effects of *Aronia melanocarpa* extract in rats chronically exposed to Cd for up to 24 months. Cd produced pro-oxidative status as indicated by the elevated oxidant markers and suppressed antioxidant markers in the bones. *Aronia* treatment stimulated the antioxidant defence as evidenced by improved SOD, CAT and GPx levels and mitigated the elevation of oxidative stress parameters both in the bone and in the serum. Additionally, a positive correlation between bone markers of antioxidant defence and markers of bone formation was detected, while a negative correlation was established between bone antioxidant defence markers and bone resorption markers (86). Another report of the same study group established that *Aronia* extract was able to prevent the suppression of bone procollagen I synthesis induced by low and moderate Cd exposure, and improved femoral biomechanical characteristics (87). Ghosh et al. reported that *Aronia* extract dose-dependently prevented the nuclear factor kappa-B ligand-induced activation of osteoclast-specific extracellular signal-regulated kinase, c-Jun-N-terminal kinase, p38, c-Fos and NFATc1 by reducing ROS generation. Therefore, the osteoclastic differentiation of RAW 264.7 cells was inhibited and the number of tartrate-resistant acid phosphatase (+) multinucleated osteoclasts was reduced (88).

Ocular protection

Two studies by Xing et al. used sodium iodate ($NaIO_3$)-induced dry age-related macular degeneration model in rats (57, 89). *Aronia* extract alone (89) or combined with Lactobacillus fermentum NS9 (57) was administered orally for 28 days. Improvement in the amplitude of b-waves (89) or both a-waves and b-waves (57) as well as reduced retinal damage were detected. The retinal thickness was preserved, retinal and serum MDA levels were lowered, crystallin proteins were up-regulated (57, 89) and caspase-3 was down-regulated (57). Interestingly, the second study also found higher prevalence of

Parasutterella species in the rat's gut microflora which could contribute to the protective effects (57).

A recent study explored the effect of combined *Aronia melanocarpa, Lonicera caerulea*, and Vacc*inium myrtillus* extract among 23 presbyopia patients in a double-blind, randomized, cross-over study conducted in 2 phases – stage 1 (42 days) and stage 2 (42 days). The extract improved the results of the Schirmer test (used to assess dry eye syndrome) during stage 1 and the near visual acuity during stage 2 (90).

Skin protection

A total of 6 studies were found in literature.

Goh et al. investigated the anti-inflammatory effects of *Aronia* concentrate on the skin, using TNF-α-treated human keratinocyte cell line HaCaT for *in vitro* evaluation and 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced ear oedema in mice. It was found that the concentrate counteracted TNF-α-induced ICAM-I expression, monocyte adhesiveness, ROS generation and MAPK activation *in vitro*. Moreover, the treatment antagonized the increase of IKK, IκB inactivation, p65 phosphorylation and nuclear translocation, thus, suppressing NF-κB cascade. These results were confirmed in the *in vivo* model of TPA-induced ear oedema, where the concentrate prevented the increase in the ear thickness and weight (91). In another study, 7-day topical *Aronia melanocarpa* extract administration after UV-B-induced photodamage in ICR mice reduced the skin injury, as shown by the decrease in the epidermal thickness and restored fibroblast count in the dermis. The extract counteracted the UV-B induced fibrosis, increased the immunoreactivity to collagen type I and III fibres and decreased the immunoreactivity to MMP-1 and MMP-3 (92). Lee et al. confirmed these results in two model systems: the first one was a monolayer of cells – either human epidermal keratinocytes (HaCaT cells) or neonatal human dermal fibroblasts (HDFn cells) and the second – a bioprinted 3D dermal equivalent. *Aronia* extract showed no cytotoxic effect and increased the proliferation of HaCaT cells in a dose-dependent manner. Likewise, the expression and the levels of COL1A1 were elevated and the MMP-1 and MMP-3 proteins were down-regulated (93). Youn has documented that *Aronia* melanocarpa extract, administered to HaCaT cell line in a form of liposome, was able to stimulate hyaluronic acid synthesis through up-regulation of the hyaluronan synthase 2 gene and this effect was superior to the effect of the comparator all-trans retinoic acid. Moreover, the liposome did not induce local reaction in the skin irritation test in healthy humans (94). In a study of Lee et al., a combined *Triticum aestivum* and *Aronia melanocarpa* extract was administered orally for 11 days to elucidate its effect on or 2,4-dinitrochlorobenzene-induced atopic dermatitis-like signs and symptoms in mice. The extract reduced the skin levels of the pro-inflammatory markers IL-1β/-4/-6, TNF-α and chemokines CCL17, CCL22, CX29, CXCL10, CXCL11, decreased mast cell and macrophage infiltration in the skin and potentiated the antioxidant Nrf2/HO-1 pathway. Likewise, the extract dose-dependently reduced the epidermal and dermal thickness, skin IgE and IL-4 levels as well as increased the skin moisture content (95). Another study demonstrated that fermented *Aronia melanocarpa* extract suppressed melanogenesis *in vitro* through inhibition of melanin synthesis factors (tyrosinase, TRP-1/-2 and MITF) and stimulation of melanogenesis-inhibitory factors (Akt, GSK-3β). The authors reported that the extract was rich in gallic acid, which was achieved by fermentation. Gallic acid was the probable contributor for the described benefits since it exerted similar effects on the aforementioned melanin-regulating factors in the same study (96).

Advantages and disadvantages of the research

The research revealed multiple and diverse organ-protective effects of *Aronia melanocarpa* in almost all systems of the human body (**Figure 1**). The number of papers published in the selected time interval between January 2014 and December 2024 was enormous and impressive.

However, for objective interpretation, the drawbacks of the current paper should be noted. First, articles published only in time interval between January 2014 and December 2024 were selected for review. Our research showed that there are numerous studies related to the beneficial effects of *Aronia melanocarpa* on human health before 2014, however, for topicality, conciseness and clarity reasons, the last ten years were selected as appropriate time period. Second, two databases (Medline (via PubMed) and Scopus) were utilized in the search process. However, we believe papers indexed in these databases possess high scientific quality. Another shortcoming of the

current review is the inclusion of *in vitro* studies. Although cell cultures could promote valuable information about cell behavior under certain pathologic or therapeutic influences, they do not reproduce and reflect the pathological or therapeutic outcomes entirely as they occur in the human body. Moreover, variability of the cell culture assays is a main drawback which, on the other hand, is associated with cell density alterations, medium changes and contamination issues (97). Likewise, results gathered from animal models cannot be translated directly to humans. However, compared to *in vitro* assays, animal studies possess higher informative value as the response in a real biological system is investigated. Another critical issue of the paper is that many studies use chokeberry in different forms – fruit juice, fruit powder, fruit extract, leaf extract or separate phytoconstituents (e.g. PPs, polysaccharide). In addition, some investigations utilized combination of *Aronia* product with another test substance making the results difficult to interpret. Last but not least, the overall number of human trials is inconclusive and the trial parameters (number of participants, trial duration, study design) diversity additionally complicates the interpretation of the outcomes.

Therefore, taken together, *Aronia melanocarpa* possesses various benefits in terms of organ protection, nonetheless, more human studies conducted under unified standards should be prioritized in order to support its role in real clinical settings.

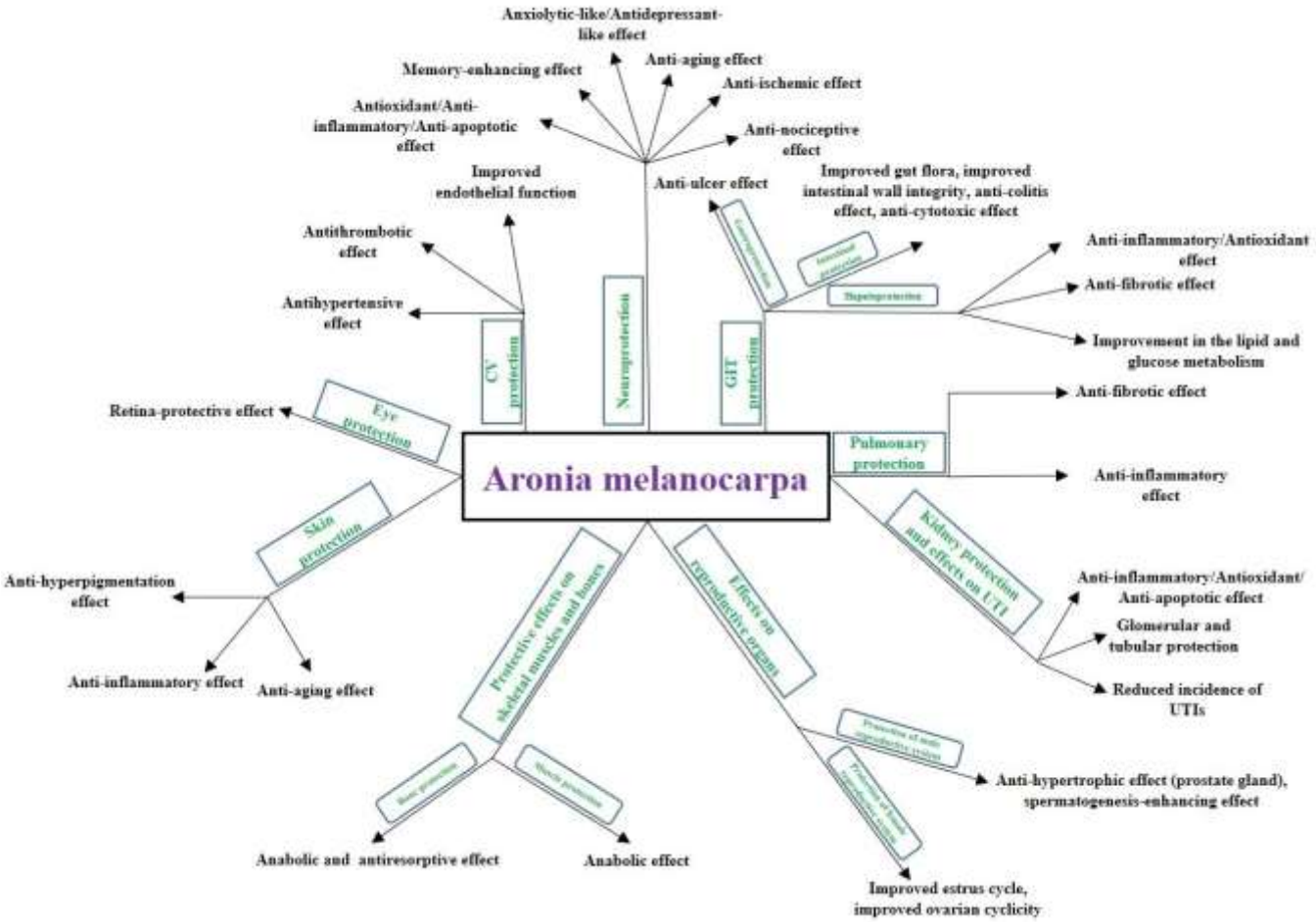

Figure 1. Organ-protective effects of *Aronia melanocarpa* and related mechanisms of action; CV: cardiovascular, GIT: gastrointestinal, UTI: urinary tract infections

CONCLUSION

In conclusion, *Aronia melanocarpa* and its constituents have been shown to exert protective effects on the cardiovascular system, central nervous system, liver, stomach, intestines, kidneys, gonads, skin, skeletal muscles, bones, eyes. However, *in vitro* assay and animal studies involving different experimental models do not necessary reflect results in humans. Moreover, human studies are scarce and insufficient. Therefore, in order to draw a final conclusion, more information is required related to the organ-protective effects of *Aronia melanocarpa* in humans.

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