Chapter

Cardioprotective Effects of Cultivated Black Chokeberries (*Aronia* spp.): Traditional Uses, Phytochemistry and Therapeutic Effects

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Abstract

Cardiovascular diseases represent the main cause of morbidity and mortality worldwide. Obesity, sedentary life style, diet, smoking and stress are the principal inducers of hypertension, endothelium dysfunction and insulin resistance in the developed countries. The latest *in vitro* and *in vivo* studies on different type of extracts obtained from black-fruited Aronia highlight its excellent cardioprotective actions for the prevention and treatment of cardiovascular and metabolic disorders. So, this chapter aims to bring an up-to-date regarding the antioxidant, anti-inflammatory, anti-atherosclerotic, antiplatelet, blood pressure, glucose and lipid reduction properties of black-fruited Aronia, as a possible new therapeutic strategy for the primary and secondary prevention of cardiovascular pathologies.

Keywords: black chokeberries, cardioprotective, black-fruited Aronia, antioxidant, anti-atherosclerotic, antithrombotic

1. Black chokeberries: historical background, taxonomy and botanical aspects

The use of medicinal plants in the prevention and treatment of diseases is an old practice that has been maintained over time and is currently being given special attention by scientists, as well as by people aiming to preserve their health or treat a disease. In the past, the therapeutic properties of the plants were discovered by observing their effects on the animals that ate them [1–3]. People gathered knowledge about the therapeutic effects of plants and several traditional medicines were born [4]. At the beginning of the nineteenth century, the first plant compounds were isolated and later some of them served as a model for the development of synthetic drugs [4, 5]. Plant-derived products are intensively studied and they are

important sources in drug discovery [1]. Studies are directed toward the identification of mechanisms of action of plant compounds [6], exploiting their capacity of reducing the side effects of synthetic drugs [7] and development of new delivery systems that can increase the efficacy of phytochemicals [8].

One of the plants whose health benefits led to numerous studies is black chokeberry, also known as black-fruited Aronia. Its fruits enjoy presently a high recognition as a health food, despite lacking the historical advantage of other well-known rosaceous berry crops (strawberries, raspberries and blackberries). The highly regarded berries (botanically: pomes), cultivated in many European countries and North America, have an unusual history, which reflects on the taxonomic confusions surrounding the plant.

Unlike other edible plants, black Aronia spread from America to Europe due to its decorative interest. The ancestor of today's cultivated black-fruited Aronia is *Aronia melanocarpa* (Michx.) Elliott, a species that grows wildly in North America [9] along with two other *Aronia* species. The small genus *Aronia* Medik. (chokeberry) includes multistemmed, deciduous shrubs that differ in the color and size of fruits (pomes), as well as in the pubescence of leaves, stems and inflorescences. Three North American and one European species are recognized [10]. Red chokeberry, *A. arbutifolia* (L.) Pers., grows up to 3 m tall and has obovate to elliptical leaves, which are shiny green on the upper side and tomentose, slightly gray on the lower side. The margins are serrated and the tip is short, acuminate. Flowers are white, in compound corymbs. In late September to early October, red fruits are produced. Black chokeberry, *A. melanocarpa* (Michx.) Elliott, has a smaller habit (up to 1.5 m) and is not pubescent [11]. Its fruits are black, shiny, with a diameter of 0.8–1.3 cm; they typically ripen in August. A third species native to North America, *A. prunifolia* (Marshall) Rehder, or purple chokeberry, is considered by certain authors to be a hybrid between the former two species, a distinct species or a variety of black or red chokeberry. It has dark purple to black fruits, and the leaf pubescence is intermediate between *A. arbutifolia* and *A. melanocarpa* [12].

A. melanocarpa is cold-hardy member of the Rosaceae family, where it is assigned to the Amygdaloideae subfamily and Maleae tribe [13]. Wild-growing black chokeberry plants were introduced to Europe in the nineteenth century as ornamental shrubs. The first record of the species in Russia (1816) mentions black Aronia under the name *Mespilus melanocarpa* in the catalog of plants of the Kremenets Botanical Garden [14]. Soon, it was grown as a cold-resistant ornament in other botanical gardens; before the twentieth century, it was however not grown for its fruits, which, though edible, are poorly palatable and astringent. The black-fruited Aronia is cultivated nowadays as a distinct morphology in comparison to its wild-growing North American counterparts: its leaves are wider, flowers are larger and more numerous, fruits have a larger diameter, and corymbs bear a higher number of fruits [15]. It has been proposed that this species is the result of breeding and selection experiments performed in the early twentieth century by Ivan Michurin, and that all *Aronia* plants cultivated in the former Soviet Union originate from the Russian pomologist's nursery [14]. Then, it spread to other European countries such as Poland, Norway, Finland, Sweden or Germany [16]. In 1976, chokeberry was introduced to Japan [17]. The distinct differences from its wild-growing progenitors and the constancy in characteristics supported the assignment of the large-fruited chokeberry to a new species, *Aronia mitschurinii* A.K.Skvortsov & Maitul [18]. Its origin is however not completely elucidated. One of the hypotheses is that the species is a hybrid obtained by backcrossing *A. melanocarpa* with an F1 x*Sorbaronia* hybrid (*Sorbus aucuparia* × *A. melanocarpa*). Leonard and co-workers using amplified fragment length polymorphism (AFLP) were able to show similarities of *A. mitschurinii* to x*Sorbaronia* hybrids [19]. Further studies with more sensitive and

complex tools (like multilocus nuclear data) are proposed in order to firmly establish the origin of *A. mitschurinii* [20].

An important feature of the cultivated black-fruited Aronia is its extremely low variability due to the apomictic formation of seeds, a process occurring without fertilization of the egg. Plants resulting from apomixis are clones of the mother plant. Cultivated varieties of *A. mitschurinii*, including 'Nero,' 'Viking' and 'Galicjanka,' have in fact undistinguishable phenotypes [21] and are tetraploid (2n = 68) [14]. On the contrary, 'Hugin' and 'Elata' cultivars are considered true *A. melanocarpa* genotypes [21].

Taking into account the better understanding of *Aronia* taxonomy and genetics, in recent years, it has become accepted that the cultivated black-fruited Aronia berries, which were the subject of most biomedical and phytochemical studies, are not *A. melanocarpa* fruits as reported. Research results should rather be assigned to *A. mitschurinii*, which is the only species used for commercial fruit production [21].

Further developments on the subject of black-fruited Aronia taxonomy should certainly lead to a more stable and correct nomenclature of the chokeberry species investigated by biomedical research. A unified, proper nomenclature is essential to enable researchers to assign correctly a therapeutic activity to *A. melanocarpa*, *A. mitschurinii*, or even *X Sorbaronia mitschurinii,* all of them used in various publications to assign cultivated black-fruited chokeberry.

2. Traditional use of black chokeberries

Although wild-growing black chokeberries (*Aronia melanocarpa*) were known to the North American settlers, the taste of the fruits was dissuading for a use as foodstuff. They employed fruits and bark as an astringent. The Forest Potawatomi Native Americans used the fruits of this plant in cold treatment and the preparation of traditional pemmican [17].

In Russia and Lithuania, the cultivated black chokeberry fruits were used as adjuvant treatment for high blood pressure and as anti-atherosclerotic agent [22]. Other uses include treatment of hemorrhoids, achlorhydria, avitaminosis and convalescence [17].

Because of their astringent taste, black chokeberry fruits are not usually consumed as such, but they are used for the production of juices, wines, teas, jellies or syrups, especially in fruit blends [23, 24]. Chokeberry powders are used as a natural dye in the food industry [25].

The high content of phenolic compounds, mainly anthocyanins, which are responsible for the black color of the fruits, determined an increased interest in the medicinal properties of this plant. *Aronia* ssp. fruits have a higher antioxidant activity than other berries. Various studies emphasized antioxidant, anticancer, anti-diabetic, anti-inflammatory and hepatoprotective properties for the juice or fruit extract, which made cultivated black-fruited Aronia an important health food and dietary supplement [26].

3. Phytochemistry

Several analyses have been performed in order to evaluate the organic as well as inorganic constituents of black chokeberries. They include the research of the differences in the chemical composition of wild vs. cultivated chokeberry [21, 27], the influence of the cultivar type [28–30], the degree of fruit maturity [31], fertilization [32], the application of biosynthesis regulators [33] and geographic location [34].

Wild *Aronia* genotypes contain less water and more phenolics and have a higher antioxidant activity than cultivated black chokeberries [21]. Typical values for the dry weight of cultivated black chokeberry fruits are 17.9–26%; fresh chokeberries afford 11.1–17.4% juice and 44.6–50% pomace [35]. The majority (72%) of the dry weight is constituted by dietary fiber, higher than other fruits like bilberries and currants. The study of dietary fiber with solid-state NMR could show that its components are water-insoluble fibers (cellulose, hemicellulose, lignin, cutin and pectins), soluble fibers and other constituents, which are valuable antioxidants, anthocyanins and procyanidins [36].

The sour taste of *Aronia* ssp. berries is due to the presence of organic acids, mainly malic and citric acids. Their total content is however lower than in other berries [37]. Cultivated black chokeberries contain between 5.71 and 19.36% reducing sugars [23] and are characterized by a relatively high sorbitol amount (median content of 70 g/kg) [34] when compared to other berries. The high sorbitol content, shared as well by rowan berries (*Sorbus aucuparia*), may be related to the possible hybrid nature of cultivated chokeberry. Large-fruited Aronia cultivars have been hypothesized to contain the genomes of *Aronia melanocarpa* and *Sorbus aucuparia* in a ratio of 3:1 [19, 21]. The high sorbitol content has been proposed to serve as an analytical tool in the control of juice blends [38]. Another feature of the sugar profile in cultivated black chokeberry is the absence of sucrose; its presence in black Aronia-based products suggests the addition of sugar or other fruits [34].

The lipid content of black cultivated chokeberries is reduced (below 0.2%) [23] and is mainly owed to hydrophobic constituents of the skin and seeds. The content in proteins is also low, with values of 10.7% in the pomace. The main amino acid was glutamic acid (19.8%), followed by aspartic acid (8.9%) and arginine (7.9%) [39].

Regarding the content in minerals, chokeberries may be valuable to complement dietary potassium and zinc intake. Among vitamins, Aronia fruits contain vitamins B1, B2, B6 and C, pantothenic acid and niacin; the detailed content has been extensively reviewed [23, 38].

The analysis of the volatile constituents in Aronia berry juice afforded the detection of 74 constituents, of which the most abundant were 3-penten-2-one, 3,9-epoxy-p-menth-1-ene and benzaldehyde. Among the aroma-active compounds, ethyl-2-methyl butanoate, ethyl-3-methyl butanoate and ethyl decanoate could account for the "fruity" aroma notes, while nonanal is responsible for the "green" notes [40]. The bitter-almond scent of the fruits is due to the presence of amygdalin [34]. This compound is a cyanogenic glycoside present in many representatives of the Rosaceae family [35]. Interestingly, a recent research aimed at identifying compounds, which inhibit adipocyte differentiation, was able to isolate from the butanol fraction of a chokeberry extract amygdalin and prunasin as active compounds [41]. These two cyanogenic glycosides suppress the expressions of peroxisome proliferator-activated receptor γ, CCAAT/enhancer-binding protein α (C/EBPα), sterol regulatory element-binding protein 1c, fatty acid synthase (FAS), and adipocyte fatty-acid–binding protein (aP2) [42].

The most intensively studied compounds in cultivated black chokeberries are phenolic compounds, mainly anthocyanins, procyanidins and phenolic acids. The total phenolic content ranges from 3440 mg/100 g dry weight to 7849 mg/100 g dry weight, depending on cultivar, ripening stage at harvest, cultivation conditions or analytical methods used for the quantification [38]. Analysis of several chokeberry cultivars identified a higher polyphenolic content for the fruits of 'Hugin' cultivar compared to 'Viking,' 'Galicjanka' and 'Nero' cultivars [29]. There are differences in the polyphenols content in chokeberry products; for example, the pomace has a five-fold higher content compared to juice [16].

Polymeric proanthocyanidins represent 66% of chokeberry polyphenols, while anthocyanins represent about 25%. The fruits contain up to 5181.60 mg/100 g dried weight polymeric procyanidins [43]. The constitutive unit is mainly (−)-epicatechin and the units are connected by C4—C6 and C4—C8 bonds (B-type bonds). The degree of polymerization ranges from 2 to 23 units. The main proanthocyanidins found in the fruits and bark of chokeberry are dimeric procyanidin B2 and procyanidin B5 and trimeric procyanidin C1 (**Figure 1**) [44].

Anthocyanins are a class of flavonoids that give blue, dark red or purple color of the fruits and they are important compounds for the biological activity of chokeberries [38]. Black chokeberries have a higher content of anthocyanins than other berries, such as blackberries, strawberries or red raspberries [45]. Anthocyanins are found mainly in fruit skin [46]. They are represented by cyanidin glycosides such as

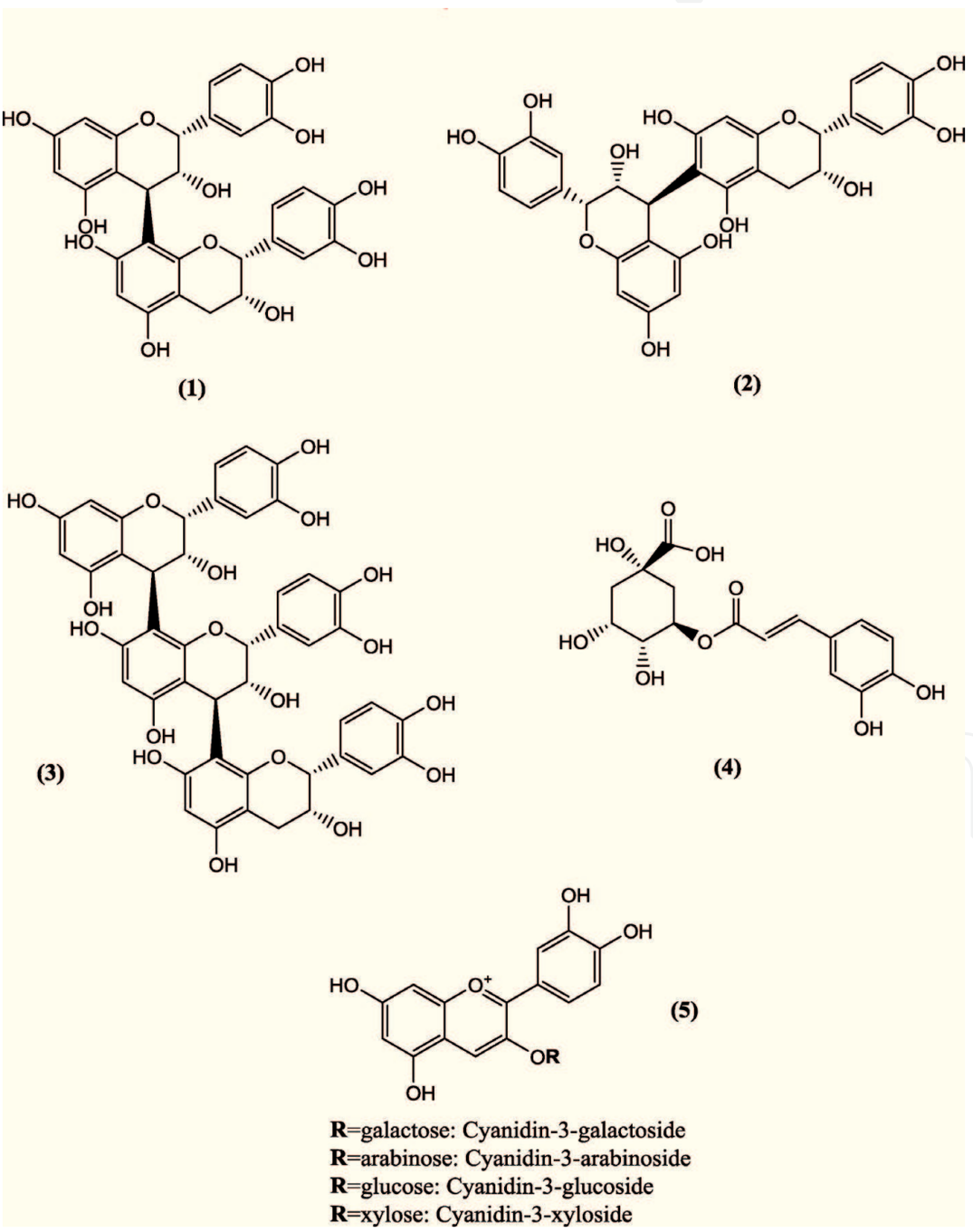

Figure 1.
Chemical structures of polyphenolic compounds from cultivated black-fruited chokeberries. (1) Procyanidin B2; (2) procyanidin B5; (3) procyanidin C1; (4) chlorogenic acid; (5) anthocyanins.

cyanidin-3-glucoside, cyanidin-3-galactoside, cyanidin-3-xyloside and cyanidin-3-arabinoside [43]. The study of the fruits of black chokeberry, 'Viking', 'Nero' and 'Galicjanka' cultivars revealed that 'Nero' and 'Viking' cultivars had higher anthocyanin content. Cyanidin-3-galactoside and cyanidin-3-arabinoside are the major anthocyanins in chokeberries, while cyanidin-3-xyloside and cyanidin-3-glucoside are found in lower amounts [47]. The fruits also contain pelargonidin-3-arabinoside and pelargonidin-3-galactoside in trace amounts [17, 38]. Other anthocyanins such as cyanidin-3-pentoside-(epi)catechin, cyanidin-3,5-hexoside-(epi)catechin, and cyanidin-3-hexoside-(epi)cat-(epi)cat were identified in black chokeberry juice and powder [46].

The main phenolic acids in black chokeberries are chlorogenic and neochlorogenic acids. They represent about 7.5% of fruit polyphenols [43]. In addition to chlorogenic and neochlorogenic acids, other phenolic acids such as cryptochlorogenic acid, 3-*O*-p-coumaroylquinic acid and di-caffeic quinic acid have been reported in chokeberries [46]. Chokeberry juice contains a greater amount of these compounds than pomace [43]. Fruits of 'Viking' cultivar and wild chokeberry have a higher content of phenolic acids compared to fruits of 'Nero' and 'Galicjanka' cultivars [47].

Chokeberry also contains flavonol glycosides such as quercetin 3-*O*-galactoside, quercetin 3-*O*-rutinoside, quercetin 3-*O*-glucoside, quercetin 3-*O*-arabinoside, isorhamnetin 3-*O*-rutinoside or kaempferol 3-*O*-glucoside [48]. Other phenolic compounds identified in chokeberry fruits and flowers are the flavanone eriodictyol-7-*O*-β-glucuronide and the flavonols quercetin-3-robinobioside and quercetin-3-vicianoside [49]. Even though the fruits were the most investigated for the polyphenols in their composition, black chokeberry leaves also contain these compounds, with a higher content in the young leaves compared to the old ones (**Figure 1**) [50].

4. Cardioprotective actions: *in vivo* and *in vitro* studies: clinical trials

Diseases of the circulatory system were reported to be the main cause of death in Europe and America [51, 52]. The affections of the circulatory system are related to high blood pressure, smoking, cholesterol and diabetes (the main pathologies of modern times), resulting in stroke or ischemic heart disease as the major pathologies causing invalidity and death in the developed countries [53].

Obesity, sedentary lifestyle, diet, smoking and stress are the principal inducers of endothelial dysfunction and insulin resistance, which will later induce hypertension and diabetes [54]. Thus, targeting endothelial dysfunction, insulin resistance and the altered metabolic state represents important strategies for decreasing the incidence and complications of cardiovascular diseases, as well as the medical costs [53, 55].

As well known, the vascular endothelium is the single layer of cells that lines the internal lumen of blood vessels [54, 56]. A healthy endothelium is essential for the cardiovascular system and is considered nowadays an organ [53, 57]. It possesses several important functions such as relaxation of vascular smooth muscle cells, inhibition of platelet aggregation, limitation of leukocyte adhesion, regulation of the vascular tone, inhibition of vascular smooth proliferation, and specialized autocrine and paracrine secretion (producing and secreting vasoactive, vasoprotective, angiogenic, inflammatory and thrombotic/antithrombotic molecules) [53, 55, 57]. It also produces growth factors and responds to physical and chemical signals. The term "endothelial dysfunction" is used not only to describe the impaired metabolism of nitric oxide (NO) or the imbalance of

endothelium-derived relaxing and constrictor factors, but also to describe an aberrant endothelium activation and abnormalities between endothelium and leukocytes, platelets and other regulatory molecules [53, 58, 59]. Thus, it plays an essential role in the pathogenesis of several cardiovascular (hypertension, atherosclerosis, systemic and pulmonary hypertension, etc.), as well as other diseases (inflammatory diseases or cancer) (**Figure 2**) [60–62].

Elevated oxidative stress is another major risk factor of cardiovascular pathologies as an altered metabolic state will increase the reactive oxygen species (ROS) production, which will determine an increase in lipid peroxidation, inflammation, endothelial dysfunction, platelet aggregation and activation [63]. On endothelial cells, oxidative stress has been shown to immediately increase vessel damage. Moreover, the lesion caused by oxidative stress can affect the entire body's organs and systems (**Figure 2**) [64].

Thus, it is important to find new therapeutic strategies to improve endothelial function and decrease ROS as strategies for the primary prevention of cardiovascular diseases in the ever-aging Western societies.

In the last decade, several plant bioactive substances (called nutraceuticals) have proven to have outstanding properties, which suggest that they could play an important role in preventing the onset and development of chronic diseases. The term nutraceutical refers to a natural bioactive compound with several properties, such as health promoting, disease preventing or medicinal properties [65].

Cultivated black chokeberry fruits have been discovered to contain an enormous source of bioactive compounds such as antioxidants-especially polyphenols, flavonoids (anthocyanins, flavonols and flavanols), vitamins (C and E), minerals (potassium, magnesium and calcium), pectins, carotenoids and carbohydrates [16]. Moreover, the *in vitro* and *in vivo* studies have highlighted its antioxidant (one of the richest plants in antioxidants), anti-inflammatory, anti-proliferative,

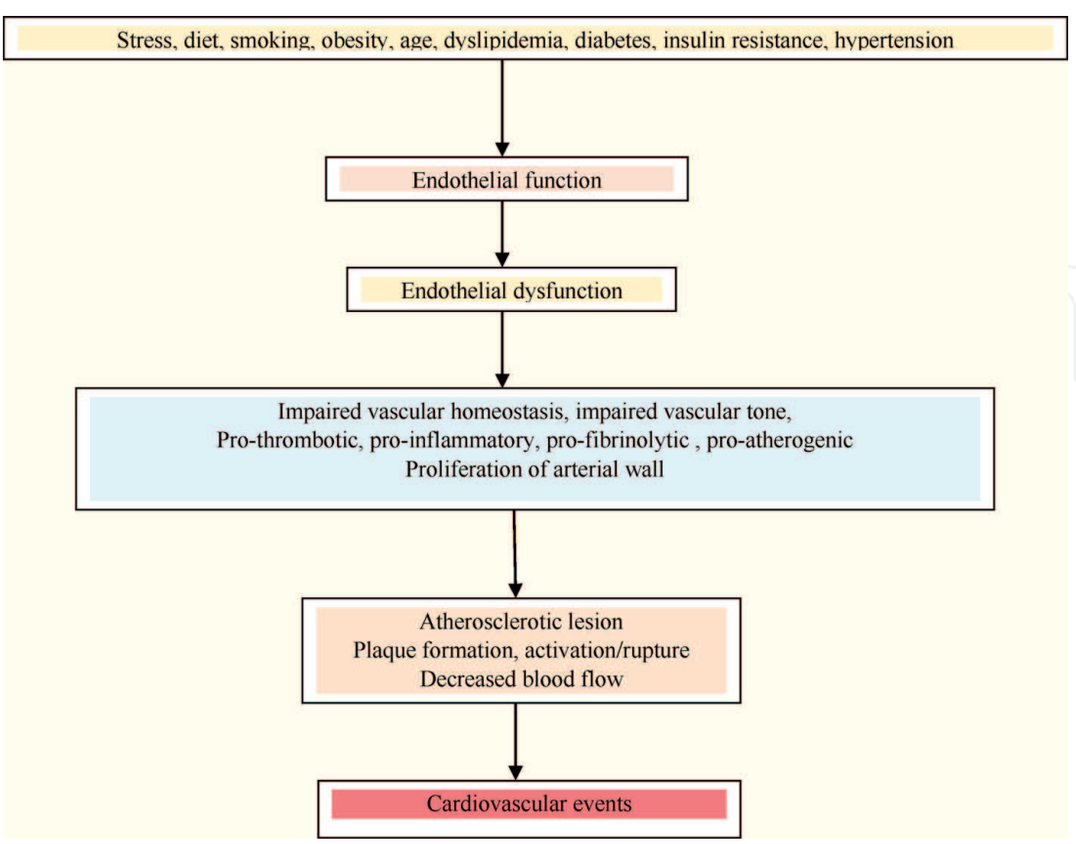

Figure 2.
Main aspects regarding the development of cardiovascular disease events.

anti-atherosclerotic, hypotensive, antiplatelet, gastroprotective, antimicrobial (antibacterial and antiviral), immunomodulatory and anti-tumor profile, making it an excellent candidate for the prevention of cardiovascular and metabolic disorders [16, 66].

Herein, the main and the latest findings regarding the cardioprotective properties of cultivated black chokeberry will be presented and discussed (**Figure 3**).

4.1 Antioxidant properties

There are currently a large number of studies that presents the antioxidant activity of black chokeberry both *in vivo* (animal studies and clinical trials) and *in vitro* (isolated cells and cell lines) explaining the capability of different types of extracts obtained from this vegetal product to protect against free radicals. Black chokeberry fruits are considered to contain a huge level of antioxidants-more precisely vitamin C and polyphenols, such as anthocyanins, phenolic acids, tannins, flavanols, flavonols and flavonoids [16, 45, 67]. The antioxidant mechanism includes radical scavenging, inhibition of reactive oxygen and nitrogen species formation, of prooxidant enzymes, stimulation of antioxidant ones and most probably cellular signaling to manage the enzymes and antioxidant substance levels [68]. The berries highlighted the highest antioxidant capacity among other berries and fruits investigated via the oxygen radical absorbing capacity (ORAC) assay [28, 38, 69, 70]. Moreover, it was noted that the pomace compared with the berries or the juice has a higher antioxidant capacity (being five times more concentrated than the juice) and the highest content of phenolic compounds (mainly polymeric proanthocyanidins and (−)-epicatechin) [43]. On the other hand, Rop et al. [71], showed the same high antioxidant property for the fruit product, pomace, fresh berry fruits and juice [16, 71]. An important amendment is the fact that the antioxidant activity levels and the polyphenols concentration are species dependent [16].

A recent *in vivo* study, published in 2019, showed the additive effect of chokeberry and walnut in increasing the expression of antioxidant enzyme genes in the

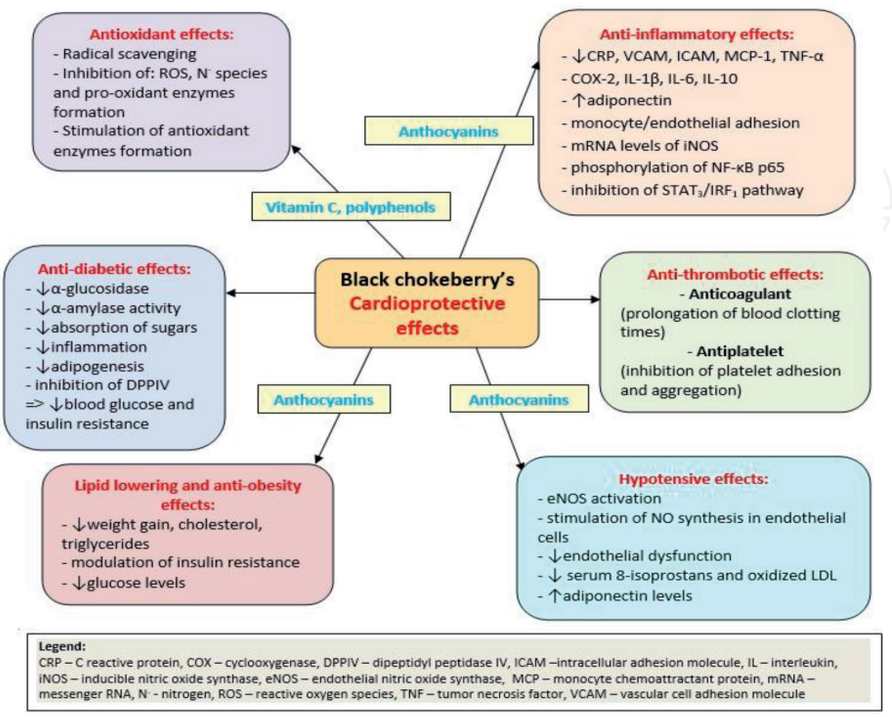

Figure 3.
The main cardioprotective effects of cultivated black chokeberry and its mechanism of action.

liver and decreasing lipid peroxidation in the liver, serum and kidneys in an aging mouse model [72]. The study performed by Jo et al. [73] described that dietary supplementation with Aronia extract increased the lifespan and improved the oxidative injuries related to the age in *Drosophila melanogaster*. Other *in vitro* studies outlined an increased antioxidant profile by decreasing the levels of ROS after incubation with *Aronia melanocarpa* bioactive compounds [74, 75]. Moreover, a decreased level of plasma lipid peroxidation was observed *in vitro*, after treatment with ziprasidone [76]. The antioxidant effect was also evidentiated in humans, as the black chokeberry juice supplementation decreased the exercise-induced oxidative damage to red blood cells in rowers [77].

The antioxidant activity is in general attributed to the polyphenolic compounds [3, 78]. It was observed that proanthocyanidin and anthocyanin content in berries, as well as neochlorogenic acid, cyaniding 3-arabinoside and (−)-epicatechin, are the main substances responsible for this action [71, 79].

4.2 Anti-inflammatoSry effect

Vascular inflammation is a primary key step process underlying endothelial dysfunction and later atherosclerosis [59]. It is responsible for endothelial activation, which will further stimulate the leukocytes' recruitment [56]. Moreover, it is a self-maintaining process, which is mediated through the expression of several molecules such as cell adhesion molecules (ICAM and VCAM), cytokines, neutrophils, fibrinogen, C-reactive protein, etc. [68]. Apart from endothelial dysfunction, it will induce smooth muscle cell migration, vascular calcification, oxidative stress, degradation of extracellular matrix and collagen, elastolysis and increased activity of metalloproteinases. Vascular inflammation was observed in patients with hypertension, metabolic syndrome, diabetes, infections, preeclampsia, coronary heart disease or peripheral arterial disease [58, 62]. Thus, the anti-inflammatory effect exhibited by several bioactive natural, synthetic or semi-synthetic compounds is important for the prevention of chronic diseases (especially the cardiovascular, metabolic and immune system disorders) and also for decreasing their complications [16]. Supplementation with black chokeberry extract for patients who suffered myocardial infarction and are under treatment with statins decreased the plasmatic concentration of several inflammatory biomarkers such as C-reactive protein (CRP), monocyte chemoattractant protein-1 (MCP-1), intercellular adhesion molecule (ICAM), vascular cell adhesion molecule (VCAM) and interleukin 6 (IL-6). Moreover, adiponectin concentration (an anti-inflammatory cytokine) was found to be increased [80]. The study performed by Zapolska-Downar et al., in 2012, on TNF-α–treated human aortic endothelial cells (HAECs) highlighted the fact that various concentrations of Aronia extract decreased the expression of ICAM-1, VCAM-1, the phosphorylation of NF-κB p65 and intracellular ROS production [26].

Other research groups reported that black chokeberry extract induces a vasorelaxation of coronary arterial rings, which is dose-dependent and endothelium-dependent, compared with other extracts (bilberry and elderberry) [81].

Recently, Iwashima et al. [82], showed that Aronia extract decreased TNF-α–induced monocyte/endothelial adhesion and decreased VCAM-1 expression, although it did not affect ICAM-1 expression on a vascular endothelial inflammation model of human umbilical vein endothelial cells (HUVECs). Moreover, it lowered the phosphorylation of signal transducer and activator of transcription 3 (STAT3), its nuclear levels and the interferon regulatory transcription factor-1 (IRF1) nuclear level. Thus, the researchers concluded that Aronia extract could exert an anti-atherosclerotic effect through the inhibition of STAT3/IRF1 pathway in vascular endothelial cells [82].

Lee et al. [83] highlighted the anti-neuroinflammatory effect of an ethanolic extract of Aronia in a mouse model of Alzheimer's disease. Aronia extract significantly reduced the generation of NO, as well as mRNA levels of iNOS (inducible nitric oxide synthase), COX-2 (cyclooxygenase 2), IL-1β (interleukin-1 beta) and TNF-α (tumor necrosis factor alpha), suggesting its neuroprotective effect against the development of Alzheimer's disease [83].

Wei et al. [84] showed that the anthocyanins from black chokeberry delayed the degenerative changes of brain, related to aging, in an aged mouse model, possibly by reducing inflammation, regulating the balance of redox system and inhibiting DNA damage [84].

In another study, it was observed that the administration of Aronia cold-pressed juice and oven-dried black chokeberry powder by patients with mildly elevated blood pressure levels reduced only the levels of TNF-α and IL-10 and did not influence other inflammatory markers [85].

The anti-adhesion effect of Aronia extract can be due to the presence of anthocyanins (alone or by a synergistic effect), more precisely cyaniding-3-glucoside, cyaniding-3-galactoside, cyaniding-3-arabinoside, peonidin-3-glucoside and delphinidin-3-glucoside [86].

Moreover, the anti-apoptotic effect of Aronia extract was also showed in an animal model of cardiomyoblasts [87]. In diabetic patients, the reversing of atherosclerosis development by Aronia polyphenols was also showed through augmenting the immune defenses and reducing inflammation, as the extract decreased the monocyte and granulocyte levels responsible for inflammation and also the number of lymphocytes, thus blocking the formation of atherosclerotic lesions [23, 88].

4.3 Hypotensive properties

Chronic inflammation can lead to cardiovascular diseases characterized by high blood pressure levels, altered metabolism of lipids, endothelial dysfunction, oxidative stress, etc. Endothelial dysfunction can be a cause, as well as a consequence of hypertension [59]. Thus, due to multiple mechanisms of action, black chokeberry can induce a cardioprotection by having positive effects on multiple risk factors for cardiovascular diseases (e.g., antioxidant effect, anti-inflammatory, hypolipidemic, antithrombotic, etc.) [16, 26, 89].

Over the years, the blood pressure lowering effects of black chokeberry were highlighted in several studies and nowadays black chokeberry preparations are recommended as a nutritional supplement in the management of essential arterial hypertension [16]. In one study, spontaneous hypertensive rats that were treated with commercial Aronia extract had decreased blood pressure levels compared with controls [90], although the effect was term-limited and maximal 3 h after intake. Other studies suggested that Aronia polyphenols could induce a positive effect on blood vessels by stimulating NO synthesis in endothelial cells (via activation of eNOS), through a mechanism related to ACE inhibitors (angiotensin I-converting enzyme inhibitors) and endothelium-dependent [68, 91]. Sihora et al. studied the effects of 2 months administration of black chokeberry preparation in patients with metabolic syndrome on the activity of ACE. They observed a 25% decrease of ACE after 1 month of administration and 30% decrease after the second month. Moreover, systolic blood pressure, diastolic blood pressure and CRP levels correlated positively with the activity of ACE [92]. The studies performed by Bell et al. [81] highlighted the vasoactive and vasoprotective potential of black chokeberry extract on the coronary arteries, due to the high concentrations of anthocyanins. The following bioactive substances were found to be responsible for the vasorelaxation and the endothelial protective effects: coumaric acid (the most potent), ferulic

acid, caffeic acid and chlorogenic acid [92]. Moreover, Aronia induced a protective action in aorta and coronary arteries against atherogenic changes [93, 94].

A double-blind, placebo-controlled study performed by Naruszewicz et al. [80] showed that black chokeberry polyphenols decreased the severity of inflammation in patients after myocardial infarction and thus, Aronia can be used as a strategy treatment for the secondary prevention of ischemic heart disease [80]. Black chokeberry flavonoids significantly decreased serum 8-isoprostanes and oxidated LDL (formed as a consequence of the action of oxidative stress in the vascular wall that are contributing to the formation of foam cells, the basis of atherosclerotic lesions) [95]. Moreover, it decreased the levels of adhesion molecules (ICAM, VCAM and MCP-1) compared with the control group and increased the level of adiponectin, an anti-inflammatory molecule [80]. Anthocyanins (mostly conjugated cyanidins and chlorogenic acid) from black chokeberry juice were found to be potent stimulators of endothelial NO formation via Sirc/PI3-kinase Akt pathway, in a study performed by Kim et al. [96].

The study performed by Loo et al. [85] showed that consumption of chokeberry products can modestly lower blood pressure levels and decrease low-grade inflammation in hypertensive patients (under no regular use of anti-hypertensive drugs) with mildly elevated blood pressure levels.

4.4 Lipid-lowering and anti-obesity effects

Several studies, including large clinical trials, have showed over the time the relationship between LDL cholesterol, triglycerides and the development and progression of atherosclerosis. Moreover, it is well known that hyperlipidemia is one of the major risk factors for developing undesirable cardiovascular events [68]. Black chokeberry products had been reported to manifest lipid-lowering properties and anti-obesity effect in culture cells, in animal as well as in human studies. Qin et al. [97] showed that a dose of 10 or 20 mg/kg/body weight of anthocyanin attenuates weight gain, decreases triglycerides and cholesterol and modulates insulin resistance in Wistar rats under a fructose-rich diet of 6 weeks. Moreover, it decreased TNF-α and IL-6 [97]. The black chokeberry pomace administered in Polish Merino lambs decreased glucose and total cholesterol levels and increased HDL [98]. The juice administered *ad libitum* for 28 days decreased glucose, insulin and body weight in C57BL/6JmsSIc and KK-Ay mice [99]. Another study performed by Daskalova et al. [94] on male Wistar rats who received a daily dose of 25 ml commercial juice for 90 days showed a decrease in LDL and also retarded age-related changes in the aortic wall. Ciocoiu et al. [100] showed a decrease of total cholesterol, systolic and diastolic blood pressure levels and an increased HDL value after 8 weeks administration of an ethanol extract of black chokeberry.

Results from clinical trials have shown that administration of 100 ml juice before meal decreased the blood glucose levels of 37 healthy subjects [101]. A 12-week randomized, placebo-controlled trial showed that consummation of 500 mg Aronia extract although did not changed blood pressure levels or inflammatory and oxidative stress biomarkers, it decreased total and LDL cholesterol in healthy adults former smokers. The cholesterol-lowering capacity was closely linked with cyanidin-3-*O*-galactoside and peonidin-3-*O*-galactoside urinary levels [102]. Kardum et al. [103] showed that 100 ml of glucomannan-enriched Aronia juice-based supplement decreased the body mass index, waist circumference and systolic blood pressure, concluding that the juice had a positive effect on cellular oxidative damage, anthropometric indices of obesity and blood pressure levels.

In 2016, Shin et al. observed that adipogenesis in 3 T3-L1 preadipocytes was blocked after administration of a black chokeberry extract. Moreover, the expression of mRNA levels of some adipogenesis key genes was impacted and that the

levels of PPARϒ (peroxisome proliferator-activated receptor ϒ), FABP4 (fatty acid–binding protein 4), adiponectin, MCP-1 (monocyte chemoattractant protein-1) and leptin were decreased [104].

Other studies performed concluded that anthocyanins may contribute to the prevention of obesity by lowering the sugar and lipid absorption in the digestive system [16, 105]. Although the mechanism underlying the lipid-lowering effect is not completely elucidated, it seems that the blocking of cholesterol absorption (by flavonoids), increased lipoprotein catabolism (by cyanidin), inhibition of 3-hydroxy-3-methylglutaryl-CoA reductase and thus decreased synthesis of cholesterol (by flavonoids) could explain this effect [68].

4.5 Anti-diabetic effect

Several studies describe the hypoglycemic effect of black chokeberry [16, 98, 106–108]. Moreover, its property of decreasing the insulin resistance was also highlighted. It seems that Aronia extracts can lower the risk factors associated with insulin resistance, by decreasing inflammation and adipogenesis and by modulating several pathways linked to insulin signaling [92, 97]. As a general view, metabolic disorders, more specifically plasma glucose and the lipid profile, were improved after long-term juice consumption [106–108]. It was observed that polyphenolic compounds can decrease blood glucose levels by inhibiting α-glucosidase and α-amylase activity and, thus, controlling the postprandial hyperglycemia [16]. Anthocyanins (more specifically cyaniding 3-rutinoside) might inhibit intestinal α-glucosidase and might slow down the absorption of sugars [106, 109, 110]. Moreover, anthocyanins were found to exert a positive action on normalizing the carbohydrate metabolism in diabetic patients and rats, throughout several mechanisms such as reversing the beta cells' integrity and physiology and stimulating the release of insulin [111]. Chlorogenic acid was found to be the most potent inhibitor of pancreatic α-amylase [16]. Other studies postulated that the antidiabetic effect of black chokeberry juice may be due to the cyaniding 3,5-diglucoside inhibition of DPP IV (dipeptidyl peptidase IV) [112]. It seems that in humans, black chokeberry juice can be an excellent natural alternative therapeutic strategy for the treatment of metabolic syndrome disorders, the dose varying from 100 ml to 300 ml per day, for at least 3 months [16].

A recent study, published in 2019, showed the anti-adipogenic effect of cyaniding-3-O-galactoside–enriched black chokeberry extract in C57BL/6 obese mice. The extract reduced the serum levels of insulin, leptin, triglyceride, LDL and total cholesterol and suppressed adipogenesis by decreasing the expression of several key proteins [113].

The study performed by Jakovljevic et al. [114] on a rat model with metabolic syndrome highlighted the fact that 4-week administration of Aronia extract managed to reduce blood pressure levels and to induce benefits on the heart function. It also improved glucose tolerance and oxidative stress levels and attenuated the pathological alterations of the liver, thus conferring an excellent cardioprotection, alone or in combination with other dietary regimens [114]. A fermented chokeberry extract administered for 8 weeks in obese mice decreased weight gain and increased glucose tolerance and insulin sensitivity. These results also led to the conclusion that the anti-obesity effect was not closely correlated with the cyaniding content [115].

4.6 Effects on erythrocytes

Studies showed that the anti-oxidative effect of black chokeberry may impact erythrocyte's proper functioning [23]. The juice increased the protection against

oxidation in erythrocyte membranes of 25 healthy women who drank 100 ml daily for 3 months [103]. The same effect (anti-oxidative effect on erythrocytes and increased PUFA concentration) was observed in obese women who drank 100 ml juice with glucomannan for 4 weeks [103]. Moreover, in patients with hypercholesterolemia, Aronia extract decreased cholesterol level and lipid peroxidation in erythrocytes, improved the rheological properties of red cells and increased their membrane fluidity [116].

4.7 Effects on neutrophils

As neutrophils produce high concentrations of ROS, this impacts the tissue-damaging effects of inflammatory reactions. The study performed by Zielinska-Przyjemska et al. [117] showed that the oxidative metabolism of neutrophils decreased after treatment with Aronia juice, both in non-obese and in obese patients.

4.8 Antithrombotic properties

Black chokeberry exhibited *in vitro* strong anticoagulant properties by prolonging blood clotting times (APTT, prolonged PT and TT) and by decreasing the maximal velocity of fibrin polymerization in human plasma [16, 118]. Malinowska et al. showed its action on clot formation and fibrin lysis in patients with hyperhomocysteinemia [74]. Black chokeberry proved to possess properties in inhibiting also the platelet aggregation [119], as the extract decreased *in vitro* several steps of platelet activation, such as adhesion of platelets to collagen and platelet aggregation. Moreover, it decreased the production of ROS in resting blood platelets and in those activated by thrombin [119]. Sikora et al. [92] noted that 1 month of administration of Aronia extract in men did not influence the number of platelets in the blood; the extract led to prolonging the time required to reach the maximal aggregation. The study performed by Ryszawa et al. [120] assessed the effects of Aronia extract on ROS production and aggregation in the thrombocytes of smoker patients with high cardiovascular risk factors, such as hypertension, diabetes and hypercholesterolemia, who had an increased production of ROS, compared with the control group. The Aronia supplementation managed to neutralize the difference in ROS production between the studied group and the control group and induced significant anti-aggregation effects dependent on the concentration, in both groups, concluding that these effects might be independent of its capacity of modulating ROS production [120].

5. Conclusions and perspectives

From all the information presented above, one can conclude that black chokeberry is an extremely rich source of bioactive molecules that are offering an excellent cardioprotection among other fruits and that can be used in both primary and secondary prevention of cardiovascular events. Despite the low bioavailability of polyphenols and their variability, they are exceptionally important for their health-promoting properties. Based on the present findings, it can be definitely included in a healthy daily diet. However, much more research is needed to completely understand the exact mechanisms as well as the full-length actions of black chokeberry, especially in humans. Complete bioavailability studies are required concerning Aronia bioactive molecules. Moreover, the investigation of the risk of tissue accumulation, as well as appreciation of the risk/benefit ratio in humans, will be extremely beneficial. The recommended daily intake should also be established.

Conflict of interest

"The authors declare no conflict of interest."

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