Hawthorn (Crataegus spp.) extracts have long lasting and well established position as cardiotonic and cardioprotective remedies used in traditional medicine since ancient times. First descriptions of Hawthorn beneficial action on the heart come from first century A.D. and since then products evaluated from its berries, leaves and flowers gained growing popularity among herbalists and medics around the world (1, 2). Nowadays Hawthorn preparations are widely available in various forms (from tinctures to extracts) as prescription drugs or over-the-counter medications. Extracts are produced from the plant material using predominating hydroalcoholic solvent equivalent in strength to a minimum of 45% ethanol. The most studied and popular among them are WS 1442 and LI 132, both derived from leaves and flowers of the plant.

Crataegus special extract WS 1442 is standardized aqueous alcoholic (45% ethanol) Hawthorn extract containing 18.75% oligomeric procyanidins (OPC), which have beneficial cardioprotective values and play a role as free-radicals scavengers, that protect the ischemic heart tissue from neutrophile elastase action successions. Moreover, WS 1442 also carries proven vasorelaxant activity, via affecting eNOS synthase, and prevents ischemic heart tissue swelling by influence on calcium signaling pathways, and thus detain hyperpermeability of endothelium. Actions of WS 1442 special extract were investigated in in vitro as well as in vivo studies including large clinical trials. In this review authors present current state of knowledge about possible beneficial effects of WS 1442 special extract on cardiovascular system.

**Key words:** hawthorn extract, cardiovascular system, oligomeric procyanidins, cardioprotection, intracellular calcium/nitric oxide

Crataegus special extract WS 1442 pharmacological activity and recent experiences in experimental models (Table 1)

OPC contained in Crataegus special extract WS 1442 act as free radicals scavengers. They are products of the ‘plant aromatic pathway’, which consists of three main sections: the shikimate segment that produces the aromatic amino acids phenylalanine, tyrosine and tryptophan, the phenylpropanoid segment that produces the cinnamic acid derivatives that are precursors of flavonoids and lignans, and the flavonoid route that produces the diverse flavonoid compounds. Their anticipated interaction with biological systems originates primarily from their characteristically ability to form complexes, both with metal ions and with macromolecules such as proteins and polysaccharides, and from their anti-oxidative scavenging properties. All those interactions at the basis of their physiological and pharmacological interactions, are in principle directly derived from the physical and chemical properties of the polyphenolic skeleton.

The main, and best known effect of OPCs administration is free radical scavenging. They participate in the process of scavenging as donors of electrons of hydroxyl groups to form stable forms of radicals, instead of free radicals. They transfer an unpaired electron by reacting with other antioxidants or by binding metals. The antioxidative effect depends on local environment and the concentration and composition of the antioxidant extract.

They are also proven to inhibit human neutrophil elastase which is released from accumulated and activated leukocytes after blood flow restoration in previously ischemic myocardium.
This action could be one of important mechanisms explaining cardioprotective properties of WS 1442 extract, along with well described endothelium function improvement causing increase of coronary flow.

Vasorelaxant effect is induced by increased release of nitric oxide (NO) from vascular endothelium and it was previously suggested that this increase is due to activation of endothelial nitric oxide synthase (eNOS) in isolated rat heart (5). It has been described that certain plant extracts reveal vasodilatory action mediated by NO and Akt cellular signalling \textit{in vivo} as well as \textit{in vitro} (6, 7). More recent studies confirmed that indeed WS 1442 
\textit{Crataegus oxyacantha} special extract induces an endothelium-dependent, NO-mediated vasorelaxation via eNOS-phosphorylation at Serine 1177 also in isolated human mammary artery (8). Interestingly, new findings suggest that the intracellular formation of superoxide anions is a trigger-factor which begins the cascade of events leading to an enhanced endothelial formation of NO by increasing the phosphorylation of eNOS via the Src/PI3-kinase/Akt pathway (9). It could be hypothesized that endothelium-mediated action is not the sole participation of WS 1442 extract in NO-formation, as latest studies revealed its influence on recently discovered NO synthase of red blood cells (rbcNOS) (10). It was shown that 
\textit{Crataegus} extract activates rbcNOS and causes NO-formation in red blood cells without altering their deformability (11).

WS 1442 has also positive effect on endothelial permeability dysfunction, protecting from endothelial hyperpermeability which is crucial in edema formation and inflammatory processes in heart failure (12). This action is mediated by affecting endothelial calcium signaling. The most recent studies revealed that 
\textit{Crataegus} extract WS 1442 increases intracellular calcium concentrations by inhibiting the sarcoplasmic/endoplasmic reticulum \textit{Ca}^{2+} ATPase (SERCA) and by activating the inositol 1,4,5-trisphosphate (IP(3)) pathway. Importantly, WS 1442 did not induce store-operated calcium entry (SOCE), but even irreversibly prevented histamine-induced SOCE, preventing intracellular calcium concentration associated cytotoxicity (13). This observations additionally explain mechanisms underlying positive influence of WS 1442 in treating heart failure.

Interestingly, there are suggestions that postulated positive effect of Hawthorn extracts on endothelium may be more complex. Apart from important role in NO-synthesis increase, positive role of WS 1442 may be due to stimulation of releasing endothelium-derived hyperpolarizing factor (EDHF). EDHF is an important factor taking part in regulation of vascular homeostasis and angiorelaxation (14). Exact mechanism of action of EDHF is not fully understood. However, its role in endothelial regulation is undisputable and confirmed in many experimental studies with different species of animals as well as in humans (15). EDHF takes part in vasodilatation pathway which is independent from NO release as well as prostacyclin (16-18). Dysfunction of this mechanism is strictly connected with age-related endothelial dysfunction (19, 20). Idris-Kodhja \textit{et al.} proved protective effect of WS 1442 on endothelial dysfunction related to age as well as oxidative stress (21).

Moreover, protective action of WS 1442 on endothelial layer surface (ELS) was proposed. ELS plays pivotal role in maintaining endothelial homeostasis, by regulating vascular permeability as well as inactivating pro-coagulative and pro-inflammatory processes in subendothelium (22-24). It was shown, that high sodium levels damages glycocalyx of endothelium (25). Peters \textit{et al.} demonstrated, that WS-1442 standardized extract diminishes permeability for sodium ions in ELS. This effect is provided by WS-1442 ability to perform conformational alterations in ELS what results in transition from densely packed to the loosely packed state of ESL. As known
fact, functional parameters of ESL, e.g., the sodium permeability, strongly depend on the state of ESL (26). Also WS-1442 have been reported to cause endothelium protection (26).

When it comes to direct effects on myocardium, Crataegus special extract is proven to have positive inotropic effect, resulting in increase of heart contractile force. It was also shown that mechanism of this action is cAMP-independent (27). In models of isolated cardiomyocytes and hearts, a prolongation of the refractory period was observed after treatment with Crataegus extracts, and those observations suggested antiarrhythmic potential (28, 29). Moreover, in vivo model oral treatment of rats with WS 1442 for 7 days effectively reduced reperfusion induced arrhythmias and mortality as well as decrease in the total amount of heart injury biochemical markers. More recent observations confirmed also reduction of the extent of ST segment elevation in the electrocardiogram, as well as the incidence of ventricular arrhythmias as well as decrease in the total amount of heart injury biochemical markers.

Table 1. Experimental and in-vitro models investigating cardiovascular effects of Crataegus standardized extract WS-1442.

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Crataegus derivative, dose range</th>
<th>Aim of study</th>
<th>Outcomes</th>
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<tbody>
<tr>
<td>Schlegelmilch et al., 1994 (37)</td>
<td>WS-1442, up to 3 000 mg/kg p.o.</td>
<td>To assess toxic potential and safety of high doses of WS-1442 extract</td>
<td>* no signs of toxicity observed after p.o. application of doses up to 3000 mg/kg * LD50 for i.p. administration was established (1750 mg/kg)</td>
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<td>Chatterjee et al., 1997 (47)</td>
<td>WS-1442, 20 mg/kg/day - 100 mg/kg/day</td>
<td>To establish the mechanism of action involved in cardioprotective and free-radicals scavenging constituents of WS-1442</td>
<td>* human neutrophil elastase inhibition is a key pathway of antioxidant activity of WS-1442</td>
</tr>
<tr>
<td>Schwinger et al., 2000 (24)</td>
<td>WS-1442, 0.1 μg/ml, 100 μg/ml (applied to organ bath containing human myocardium strips)</td>
<td>To investigate the mode of inotropic action in human myocardium from patients with congestive heart failure</td>
<td>* mechanism is likely to be similar as cardiac glycosides * inotropic action is cAMP-independent</td>
</tr>
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<td>Brixius et al., 2006 (5)</td>
<td>WS-1442 fractions application, (3-100 μg/ml)</td>
<td>To investigate influence of extract on the relaxation of rat aorta and human mammary artery</td>
<td>* WS-1442 induces endothelium-dependent, NO-mediated vasorelaxation via eNOS phosphorylation</td>
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<td>Rieckehofer et al., 2011 (8)</td>
<td>WS-1442, 25 – 100 μg/ml (incubated with blood)</td>
<td>To determine whether extract is able to induce NO-formation in red blood cells</td>
<td>* WS-1442 activates red blood cell NO-synthase</td>
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<tr>
<td>Willer et al., 2012 (10)</td>
<td>WS-1442, 100 μg/ml (incubation with cell cultures)</td>
<td>To examine how WS-1442 affects intracellular calcium concentration</td>
<td>* WS-1442 increases intracellular calcium concentrations in the human endothelium * this dose did not cause endothelial contraction, hyperpermeability or toxicity * WS-1442 blocks SERCA and activates the IP3 pathway</td>
</tr>
<tr>
<td>Idris-Khodja et al., 2012 (18)</td>
<td>WS-1442, 100 mg/kg/day, 300 mg/kg/day</td>
<td>To evaluate whether WS-1442 prevents the development of aging-related endothelial dysfunction</td>
<td>* WS-1442 prevents aging-related endothelial dysfunction by reducing prostanoid-mediated contractile responses * improvement of increased oxidative stress is most likely underlying mechanism</td>
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</table>

Another aspect of Crataegus cardioprotective properties is influence on lowering blood cholesterol, and several mechanisms of this action have been proposed. Tinctures prepared from its berries reduced total cholesterol as well as all cholesterol fractions (VLDL, LDL, HDL) by increasing cholesterol liver uptake, degradation and decreasing synthesis (40). Outcomes of more recent studies on explaining mechanisms of those beneficial action brought up to light significant decrease of intestinal CoA: cholesterol transferase pathway inhibition and inhibition of Ca2+ dependent protein kinase C isoforms (33, 34), however following observations did not confirm antiplatelet effect in healthy volunteers (35, 36).
acyltransferase (ACAT) accompanied with increased expression of liver cholesterol-7-α-hydroxylase in animals fed with high cholesterol diet (41). Promising results coming from animal models encouraged researchers to study effects of *Crataegus* extracts on lipid metabolism in human cell cultures. Indeed, significant increase in LDL receptors as well as decrease of apolipoprotein B100 were found in human HepG2 hepatocytes cells line treated with WS 1442 (42). These particular molecules play a key role in systemic lipid homeostasis, remaining important targets in treatment of hyperlipidemia.

**Effects of Crataegus special extract WS 1442 in clinical studies**

Mentioned above outcomes on cardioprotective, positive influence observed in pre-clinical studies are combined with particularly good tolerance of *Crataegus* special extract WS 1442 (Table 2). No signs of toxicity were observed after oral application of doses up to 3000 mg/kg and LD50 for intraperitoneal administration was described as 1750 mg/kg in animal studies (43). Data coming from previous experimental studies finally led to introducing WS 1442 also into number of clinical trials exploring clinical potential of *Crataegus* extract. Positive results of *Crataegus* treatment such as reduced shortness of breath, ankle edema etc. combined with considerably better quality of life for the patient, in particular with respect to mental well-being were observed in a multicenter, placebo-controlled double-blind study in patients with NYHA II class patients (44). Also in study comparing the three month therapy of patient in NYHA II class heart failure with WS 1442 special extract 450 mg twice daily on submaximal exercise capacity at 6 months as determined by the 6 min walk test. Secondary outcomes investigated in this trial were quality of life measures, peak oxygen consumption and anaerobic threshold during maximal treadmill exercise testing, NYHA classification, left ventricular ejection fraction (LVEF), neurohormones, and measures of oxidative stress and inflammation. Significant improvement of LVEF was noted in WS 1442 treated patients. On the other hand, due to no positive influence on secondary outcomes, authors concluded that *Crataegus* special extract provides no clinical benefit as addition to standard optimal therapy. On the other hand, due to no positive influence on secondary outcomes, authors concluded that *Crataegus* special extract provides no clinical benefit as addition to standard optimal therapy.

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<td>Leuchtmanns, 1993 (46)</td>
<td>WS-1442, 2 x 450 mg/day</td>
<td>To assess alterations in exercise tolerance, heart rate and arterial blood pressure after treatment</td>
<td>- advantage in pressure rate product</td>
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<td>Weikl et al., 1996 (38)</td>
<td>WS-1442, 900 mg/day, 1800 mg/day</td>
<td>To investigate the efficacy of WS-1442 in patients with NYHA II chronic heart failure</td>
<td>- improvement in main symptoms (shortness of breath, tiredness, ankle oedema etc.)</td>
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<tr>
<td>Holabarschn et al., 2008 (43)</td>
<td>WS-1442, 900 mg/day</td>
<td>To investigate the efficacy and safety of and add-on treatment with WS-1442 patients with NYHA II and NYHA III chronic heart failure</td>
<td>potential reduction of sudden cardiac death in patients with less compromised left ventricular function</td>
</tr>
<tr>
<td>Zick et al., 2009 (42)</td>
<td>WS-1442, 2 x 450 mg/day</td>
<td>To determine whether hawthorn improves exercise capacity in NYHA II-III chronic heart failure</td>
<td>only modest LVEF improvement</td>
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</table>

Table 2. Clinical and randomized studies on human subjects investigating cardiovascular effects of *Crataegus* standardized extract WS-1442.
extract WS 1442 (51). Significant improvement of exercise tolerance as well as reduction of symptoms in groups of patients with modest cardiac insufficiency were also described by other researchers in randomized clinical trials (52).

Blood pressure lowering effect of Crataegus extracts remains controversial. However data coming from experimental studies suggested potential hypotensive cardioprotective action of Crataegus extracts (5, 8-10, 47) mediated by endothelial increase of NO release, outcomes of clinical observation published by Asher et al. did not confirm blood pressure lowering effect which could be mediated via an NO-dependent mechanism (53).

What is necessary to mention, Crataegus products are very safe to use drugs. The major adverse effects of Crataegus products are vertigo, dizziness, nausea, fatigue, sweating, palpitations, headache and epistaxis (54). The only reported contraindication for use of Crataegus products is hypersensitivity to its compounds. These products are not recommended for use during pregnancy, they can cause uterine stimulation. Also, it is not recommended for children and breastfeeding mothers. But yet, adverse effects and side effects of Crataegus products can be reported as very rare.

CONCLUSION

Data coming from experimental and clinical studies on Crataegus special extract WS 1442 suggests that it can be successfully used as an addition to optimal treatment of chronic heart failure. What is more, WS 1442 has a range of vasoactive and cardio active properties that could be possibly useful in treatment of other diseases of cardiovascular system like endothelial dysfunction, coronary disease, arrhythmias, or prevention of restenosis after endovascular treatment. More information about optimal dose regimen for those purposes still needs to be provided.

Conflict of interests: None declared.

REFERENCES
