Standardized Extracts from Hawthorn Leaves and Flowers in the Treatment of Cardiovascular Disorders – Preclinical and Clinical Studies

Authors   **Egon Koch**[1], **Fathi Abdul Malek**[2]

Affiliations   [1] Preclinical Research, Dr. Willmar Schwabe GmbH & Co. KG, Karlsruhe, Germany
[2] Clinical Research, Dr. Willmar Schwabe GmbH & Co. KG, Karlsruhe, Germany

Abstract
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Extracts from different parts of hawthorn plants (*Crataegus spp.*) are used worldwide for the treatment of cardiovascular diseases. So far, almost all clinical studies have been conducted with standardized hydroalcoholic extracts from leaves and flowers. These trials with more than 4000 patients have provided evidence for clinical benefits in the therapy of mild chronic heart failure. Besides cardiotonic effects, recent pharmacological investigations indicate that hawthorn extracts also possess cardio- and vasoprotective properties. Thus, these extracts may also be employed in the prophylactic and therapeutic treatment of such conditions as endothelial dysfunction, atherosclerosis, coronary heart disease, or prevention of restenosis/reocclusion following peripheral endovascular treatment. In this review the pharmacological and clinical data relating to these standardized extracts are summarized.

Correspondence
Dr. Egon Koch
Preclinical Research
Dr. Willmar Schwabe
GmbH & Co. KG
Willmar-Schwabe-Strasse 4
76227 Karlsruhe
Germany
Phone: + 49 72 14 00 53 56
Fax: + 49 72 14 00 51 50
egon.koch@schwabe.de

Introduction
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The hawthorn plant, *Crataegus spp.* (Rosaceae), is used worldwide as an herbal remedy in the treatment of chronic heart failure (HF). Hawthorn preparations are available in various forms ranging from infusions and tinctures to standardized extracts and may be available variously as authorized prescription drugs, over-the-counter (OTC) medications, authorized herbal medicinal products, dietary supplements, or unregulated herbal remedies depending on the regional regulatory requirements. Extracts may be prepared using hydroalcoholic (methanol or ethanol) or water-based extraction and are derived from various plant parts including, most commonly, berries or leaves and flowers. While many preparations are poorly characterized, the pharmacological properties of some standardized extracts have been thoroughly investigated in *in vitro* experiments, in animal studies, and in human clinical trials. To date, almost all clinical and most pharmacological investigations have been performed with aqueous alcoholic extracts from leaves and flowers such as LI 132 (70% methanol extract) and WS® 1442 (45% ethanol extract) [1]. This article is intended to briefly summarize the current pharmacological and clinical data relating mainly to these two extracts.

Plant Material and Extract Preparation
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Hawthorn species (*Crataegus spp.*; family Rosaceae) grow as shrubs or trees with thorny twigs throughout the temperate zones of the Northern hemisphere. According to the European Pharmacopoeia (EP), hawthorn leaves and flowers consist of the whole or cut, dried, flower-bearing branches of *C. monogyna* Jacq. (Lindm.), *C. laevigata* (Poir.) DC. (*C. oxyacanthoides* Thuill.) or other European *Crataegus* species, including *C. pentagyna* Waldst. et Kit ex Willd., *C. nigra* Waldst. et Kit, and *C. azarolus* L. as well as their hybrids.

The source material contains a range of pharmacologically active substances, the most important being flavonoids (1.5% to 2%, calculated as hyperoside equivalence according to EP) such as vitexin, hyperoside, rutin, or vitexin-2″-O-α-L-rhamnoside (○ **Fig. 1**), and catechin/epicatechin derived oligomeric procyanidins (OPC) (○ **Fig. 2**) with a varying degree of polymerization (1% to 3%, cyanidine chloride equivalence per EP). Further constituents are triterpenic acids (approximately 0.6%), e.g., ursolic, oleanolic, and crataegolic acid, and phenol carboxylic acids such as chlorogenic and caffeic acid, as well as various amines [2–5].

Extracts are produced from the herbal material using either water or a hydroalcoholic solvent
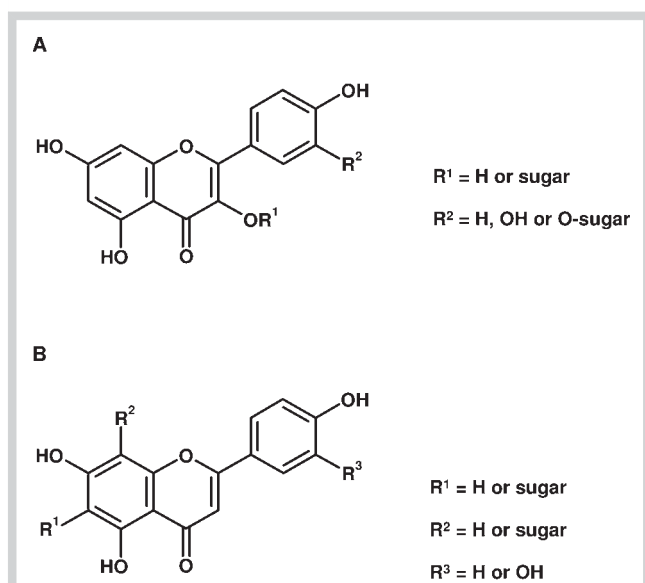
Fig. 1 In *Crataegus* leaves and flowers a series of flavonols (**A**) and flavones (**B**) have been identified. The flavone series is mainly based on apigenin and luteolin whereas quercetin, kaempferol, and 8-methoxykaempferol represent the basic structures of the flavonol series. These flavonoids almost exclusively occur as glycosides. As sugar residues, β-D-glucose, α-L-rhamnose, α-L-4-acetyl-rhamnose, xylose, arabinose, rutinose, and neohesperidose have been observed. Apigenin and luteolin have been found to form C- and O-glycosides while for flavonols only O-glycosides have been described [5].

Fig. 2 Example of an oligomeric procyanidin (OPC) consisting of three epicatechin monomers.

equivalent in strength to a minimum of 45 % ethanol. Aqueous extracts contain a minimum of 2.5 % flavonoids and dried hydroalcoholic extracts a minimum of 6.0 % flavonoids expressed as hyperoside. WS® 1442 is a standardized dry extract (extraction solvent 45 % ethanol) adjusted to a content of 18.75 % OPC with a starting plant material/extract ratio of 4 to 7 : 1 (manufacturer: Dr. Willmar Schwabe GmbH & Co. KG) while LI 132 (extraction solvent 70 % methanol) is adapted to a content of 2.2 % flavonoids (manufacturer: MCM Klosterfrau Vertriebsgesellschaft mbH) [3, 4].

Pharmacology

Myocardium

Using isolated rat cardiomyocytes, LI 132 exhibited a positive inotropic effect on the contraction amplitude similar to the beta-adrenergic agonist isoprenaline and the cardiac glycoside ouabain. However, in contrast to these positive inotropic interventions, the effects of the hawthorn extract were significantly more economical with respect to the energetics of the myocytes [6]. A positive inotropic effect was also seen in electrically stimulated canine papillary muscles [7]. It has been suggested that this effect is due to an enhanced intracellular Ca^{2+} sensitivity [6]. In normal human myocardial tissue, WS® 1442 has been shown to increase the contractile force, to raise the cell membrane calcium gradient and to displace ^{3}H-ouabain on cell membranes. As the extract did not influence the activity of adenylyl cyclase, the pharmacological mechanism is suggested to be comparable to the cAMP-independent positive inotropic action of cardiac glycosides which is mediated via inhibition of the sodium pump (Na^+/K^+-ATPase). Intere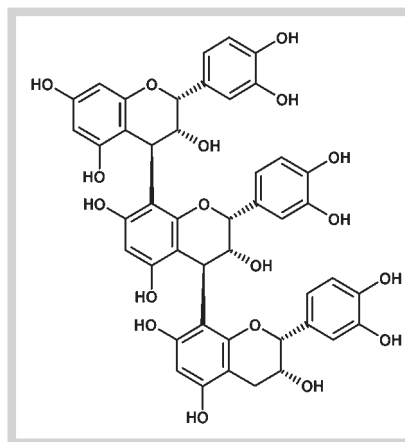stingly, low molecular weight fractions of this extract also displaced ^{3}H-ouabain, but did not show a calcium elevating response [8]. This could indicate different sodium pump binding constituents, or may reflect alternative signaling mechanisms associated with the Na^+/K^+-ATPase. It is now well established that this membrane protein, in addition to classical ion transporting, also mediates extracellular ouabain-binding signaling into the cell through regulation of protein tyrosine phosphorylation. The downstream signals following ouabain-triggered protein phosphorylation events include the activation of mitogen-activated protein kinase (MAPK) signal cascades, mitochondrial reactive oxygen species (ROS) production, as well as activation of phospholipase C (PLC) and the inositol trisphosphate (IP3) receptor [9–11]. In a further study, a significant concentration-dependent effect of WS® 1442 on the contraction of electrically stimulated myocytes isolated from right atria and left ventricles of failing human hearts has been reported [12].

In an attempt to get information on the mechanism responsible for the positive inotropic action of LI 132, no influence on the L-type calcium current was detected. Thus, an inhibition of phosphodiesterases or a beta-sympathomimetic action, which had previously been proposed to account for the cardiotonic action of hawthorn extracts, may be excluded as both of these activities would be associated with an increase in the L-type calcium current [13].

In rat cardiomyocytes [6] and in isolated guinea pig hearts [14], a prolongation of the refractory period was observed in the presence of hawthorn extracts providing evidence for an antiarrhythmic potential. Generally, an unusual profile has been obtained since in addition to exerting negative chronotropic effects (without β-adrenoceptor antagonism), the extracts act antiarrhythmically, and can induce rhythmicity in quiescent cardiomyocytes [15]. Fractionation of extracts by molecular size shows that these activities are present in different fractions and therefore are attributable to multiple substances. These authors also observed a decreased contraction frequency of neonatal murine cardiomyocytes in the presence of hawthorn extracts. As this effect was blocked by atropine and himbacine it appears to be mediated via muscarinic M2 receptor activation [16].

In guinea pig papillary muscles, LI 132 significantly increased action potential duration and time required for recovery of the maximum upstroke velocity, indicating class III and class I antiarrhythmic effects, respectively. Prolongation of the action potential duration may be due to a weak blockade of both delayed and inward rectifier potassium currents [13].

Ischemic reperfusion injury is associated with a substantial release of reactive oxygen species following reoxygenation of the ischemic tissue. This is linked with extensive localized cellular destruction, inflammatory reactions, activation of redox signaling, and induction of apoptosis, which in turn releases further free radicals and prolongs this circulus vitiosus. Reperfusion can also cause a loss of Ca^{2+} control and an uncontrolled influx of Ca^{2+}, leading to the development of cardiac arrhythmias.

Both LI 132 and WS® 1442 have been shown to be cardioprotective against ischemic reperfusion injury. In rats fed a diet containing 2% LI 132 for 3 months, tissue damage following left coronary artery occlusion and subsequent reperfusion was determined by lactate dehydrogenase (LDH) release. LDH release was reduced by > 50% in treated vs. control animals (1777 ± 452 mU/min vs. 3795 ± 512 mU/min) [17]. Oral therapy of rats with WS® 1442 for 7 days effectively reduced reperfusion induced arrhythmias, mortality, and hypotensive crises resulting from occlusion of the left coronary artery, but the increase of creatine kinase remained unaffected [18]. In a similar study, cardiac ischemia in rats was extended over 240 minutes with a 15-minute reperfusion period. The extent of ST segment elevation in the electrocardiogram (ECG), the incidence of ventricular arrhythmias, the size of the infarction zone, and mortality were significantly reduced in animals pretreated with WS® 1442 [19].

The cardioprotective activity of WS® 1442 appears to be mainly associated with the OPC fraction, which exhibits both strong antioxidative properties and inhibits neutrophil elastase [20]. Since restoration of blood flow into previously ischemic tissue is associated with the formation of oxygen free radicals and the accumulation as well as activation of leukocytes, it is suggested that these effects in combination with the improvement of endothelial functions (see below) present reasonable explanations for the cardioprotective properties of WS® 1442.

In a recent publication, it has been suggested that the reduced apoptotic incidence seen after treatment of rats in an experimental ischemia-reperfusion model with a *C. oxycantha* extract is mediated by the regulation of signaling pathways comprising the serine-threonine kinase Akt and hypoxia-inducible factor 1 (HIF-1) [21].

Elevated blood pressure (BP) results in compensatory remodeling of the left ventricle (LV) wall. This is known as cardiac hypertrophy (CH) and serves as an adaptive response in the short term, enabling the heart to cope with a greater load. Over longer periods, however, CH leads to irreversible changes in the left ventricular wall, which becomes harder, thinner, and weaker, resulting in a larger LV volume and ultimately leading to cardiac insufficiency. Calcineurin signaling is an important trigger of cardiomyocyte growth. *In vitro*, WS® 1442 inhibits the phosphatase activity of calcineurin, suggesting that it may block growth signaling *in vivo*, and therefore could reduce CH in hypertension. Significantly elevated BP and CH can be induced in rats by aortic constriction (AC) or treatment with deoxycorticosterone (DOCA) and NaCl/KCl supplementation of the drinking water. Treatment of these hypertensive rats with WS® 1442 reduced the BP and inhibited the development of CH in a dose-dependent manner. Interestingly, BP remained unchanged (normal) in WS® 1442-treated control rats without aortic constriction or DOCA-salt treatment [22]. Similarly, LV remodeling resulting from AC in Sprague-Dawley rats was significantly modified by WS® 1442 treatment. The LV : body weight ratio increased by 34% within 4 weeks after surgery for both WS® 1442-treated and untreated rats. However, the ventricular walls of WS® 1442-treated rats

were markedly thicker and the LV volumes significantly lower than those of the sham-treated controls [23]. This result was extended using an overload period of 5 months. Again, in both WS® 1442-treated and sham-treated animals, the LV : body weight ratio increased markedly (53% over non-AC controls). Just as with the shorter-term treatment, LV volume and size increased more in sham-treated animals (> 20%) than in WS® 1442-treated animals (< 10%). The reduction in contraction rate (i.e., weakening of cardiac muscle) was also lower in animals treated with higher doses of WS® 1442 [24]. On a molecular level, AC increased the mRNA expression for atrial natriuretic factor (10-fold) and fibronectin (1.8-fold) in untreated animals. WS® 1442 treatment diminished the enhanced gene expression by 80% and 50%, respectively.

Circulating interleukin (IL)-1, IL-2, IL-6, or IL-10 levels were not altered in long-term studies designed to identify possible immunomodulatory mechanisms for the therapeutic effects of WS® 1442 against cardiac failure [25].

Vascular endothelium

Hawthorn extracts have repeatedly been reported to increase coronary flow [14, 26] apparently due to an enhanced release of the vasorelaxant nitric oxide (NO) from the vascular endothelium. In rat tissue, the vasodilating effect could be completely abrogated by N-nitro-L-arginine (L-NNA), a nitric oxide synthase (NOS) inhibitor, and the soluble guanylyl cyclase inhibitor ODQ. Indomethacine, a cyclooxygenase inhibitor, and aminoguanidine, a specific inhibitor of inducible NOS (iNOS), did not negate the hawthorn-induced vasodilation. This suggests that WS® 1442 stimulation of NO release is mediated by endothelial NOS (eNOS) [27]. The endothelium-dependent vasorelaxant effect of WS® 1442 has further been demonstrated in the human mammary artery. In human tissue, WS® 1442 activates eNOS via phosphorylation at serine 1177. The NO mediated vasodilating mechanism appears to be largely associated with the OPC fraction [28]. Since the effects of the NO donor sodium nitroprusside was significantly augmented by WS® 1442, even in the presence of L-NNA, the antioxidative effects of WS® 1442 may also inhibit the metabolism of NO, thus increasing its potency [27]. Further studies with pig coronary artery rings indicate that eNOS activation may be secondary to the release of reactive oxygen species (ROS), which then serves to trigger the src/PI3/Akt signal cascades resulting in phosphorylation and thereby activation of eNOS [29]. These authors also obtained evidence that the endothelium-derived hyperpolarizing factor, besides release of NO, may contribute to the vasorelaxing activity of WS® 1442.

Rat models suggest that hawthorn extracts may as well be helpful in preventing atherosclerosis and restenosis following angioplasty. Treating rats with WS® 1442 from day 2 before to day 13 after angioplasty reduced neointima formation (vascular smooth muscle growth) and improved luminal volume compared with controls. Pathogenic neointima formation has been linked to platelet-derived growth factor (PDGF)-β in atherosclerosis and restenosis, which can be inhibited by polyphenols, a class of substances abundant in hawthorn extracts. In cell cultures, WS® 1442 inhibited PDGF receptor β phosphorylation (activation) and reduced DNA synthesis in a concentration-dependent fashion [30]. Taken together, this indicates a clear therapeutic potential for WS® 1442 in restenosis prevention.

Oxygen-derived free radicals and iNOS activation are also implicated in the pathology of endotoxin shock. In a rat model, WS® 1442 administered 1 hour prior to endotoxin injection provided

protection against reduced cardiac output and an increase in peripheral resistance without affecting the heart rate or the mean arterial BP. These effects are likely to be related to the antioxidative properties of WS® 1442 [31].

As heart failure is accompanied by inflammation leading to endothelial hyperpermeability and edema formation, possible effects of WS® 1442 on endothelial barrier dysfunction were investigated. The extract effectively inhibited endothelial hyperpermeability, which was induced in vitro and in vivo by thrombin and histamine, respectively. A dual mode of action of WS® 1442 was elucidated since it inhibited the barrier-destabilizing Ca++/PKC/RhoA pathway and activated the barrier-protecting cAMP/Rap1/Rac1 signaling network [32].

Lipid metabolism

Hawthorn extracts may also have hypocholesterolemic properties by acting on blood cholesterol levels through a number of mechanisms. In rats fed an atherogenic diet for 6 weeks, reduced concentrations of total as well as low, very low, and high-density lipoprotein (LDL, VLDL, HDL) cholesterol were observed in the animals treated with a tincture prepared from hawthorn berries whose constituents are similar to the ones in leaves and flowers. The reduction in cholesterol was shown to be due to a combination of increased liver uptake, increased degradation, and decreased biosynthesis [33]. In later experiments, this treatment was also shown to inhibit the subsequent development of atherosclerosis [34]. Reductions in cholesterol and triglycerides following treatment with hawthorn berry extract have also been shown in hamsters fed high-cholesterol diets. The changes were accompanied by an increased expression of the liver enzyme cholesterol-7-α-hydroxylase and lower levels of intestinal acyl-CoA:cholesterol acyltransferase (ACAT) [35]. An inhibition of ACAT activity by hawthorn extracts and some constituents such as the triterpenic acid components oleanolic acid and ursolic acid has also been observed in other hamster and cell culture experiments [36].

Treatment of human HepG2 cells (a perpetual human hepatocyte line) with WS® 1442 induced a 5.6-fold upregulation of LDL-receptor (LDL-R) transcription. The activity was found to be mainly mediated by the OPC fraction. The extract also downregulated ApoB synthesis in a concentration-dependent manner. ApoB is a structural component of serum LDL particles, and also a competitive ligand of LDL-R, which is in turn responsible for binding serum LDL for elimination by receptor-mediated uptake [37].

Pharmacokinetics
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In mice, orally administered ^{14}C-labelled OPCs and catechins were rapidly adsorbed, with measureable absorption of all labeled substances within 1 hour. Absorption rates ranged from 16% to 40% depending upon the substance (overall 31% of total OPCs). Radioactivity was higher following repeated oral dosing [38]. The oral bioavailability of vitexin rhamnoside was assessed using a combination of chromatographic and mass spectroscopic techniques. Bioavailability was only 3.6% indicating either poor absorption or extensive first-pass metabolism [39]. A similar low oral bioavailability was observed for vitexin glucoside in a subsequent study by the same authors [40].

Toxicity
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Following oral application of WS® 1442, no signs of toxicity were observed for doses up to 3000 mg/kg. The LD_{50} dose for intraperitoneal administration was measured for mice and rats as 1170 mg/kg and 750 mg/kg, respectively (Schlegelmilch, personal oral communication, 1996). No clinical, chemical, hematological, morphological, or histological abnormalities could be identified in either dog or rats following 26 weeks of oral treatment at doses of 30 mg, 90 mg, or 300 mg/kg/day. There was no evidence of genotoxicity of any kind including mutagenicity or clastogenicity. Teratogenicity was not apparent in rats or rabbits following oral dosing with up to 1600 mg/kg WS® 1442. In rats, neither peri- or post-natal development was influenced, nor was male or female fertility of treated animals or their F1 offspring [41]. Similarly, no adverse effect on fetal development was seen following daily gavage with 2.8 g/kg of a leaf preparation at either days 1 to 8 or 8 to 15 of pregnancy [42]. Postmarketing surveillance as well as animal and clinical studies has shown no safety signals for carcinogenicity.

Clinical Efficacy
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Hawthorn extracts are recommended in the treatment of chronic heart failure corresponding to a functional grading of New York Heart Association (NYHA) stage II (slight, mild limitation of activity; the patient is comfortable at rest or with mild exertion) [3,4, 43–45]. A considerable body of evidence, including a large number of clinical studies, largely with randomized, placebo-controlled, double-blind study designs, supports the clinical efficacy of hawthorn extracts, specifically of the standardized hydroalcoholic extracts LI 132 and WS® 1442.

Statistically significant efficacy has been demonstrated in the treatment of heart failure in over 4000 patients. Treatment resulted in significant reductions in patients' subjective discomfort ratings, improved left-ventricular ejection fraction (LVEF) and cardiac efficiency (reduction in rate-pressure product). In addition, there was an increase in physical stress tolerance (elevation of anaerobic threshold), as well as improved cost/benefit performance. These conclusions are supported by smaller short-term studies of placebo-controlled, double-blind [46–54], or open-label designs [55,56], as well as by a large open-label study with over 700 patients [57].

The recently published SPICE trial was a large, randomized, placebo-controlled, double-blind study, involving a total of 2681 patients with NYHA II or III with LVEF ≤ 35% [58] despite a guideline-conform heart-failure-treatment. While 900 mg WS® 1442 as an add-on therapy did not influence the time to the first cardiac event significantly, cardiac mortality was significantly reduced after 6 months (p = 0.009) and 18 months (p = 0.046). Furthermore, sudden cardiac death was significantly reduced for the subgroup of patients with LVEF ≥ 25% (p = 0.025). Tolerability (assessed as adverse events) was comparable in both groups.

In a randomized, double-blind, placebo-controlled study, 209 heart failure patients with more advanced stage of the disease (NYHA III) were treated with 1800 mg WS® 1442, 900 mg WS® 1442, or placebo, respectively, over 16 weeks [47]. For patients receiving 1800 mg WS® 1442, the maximal tolerated workload during a bicycle exercise test was significantly increased when compared to placebo. Typical heart failure symptoms as rated by the patients were significantly reduced in both WS® 1442 groups

in comparison to placebo. The same significant advantage of active therapy over placebo was observed when the changes in the score sums according to von Zerssen's list of complaints were tested for differences between the groups.

In the HERB CHF trial [46], the effect of 900 mg daily WS® 1442 (2 × 450 mg) add-on therapy upon submaximal exercise capacity was compared with placebo in 120 NYHA II/III patients. The results revealed a statistically significant improvement in LVEF ($p = 0.04$) under WS® 1442 vs. placebo whereas the 6-minute walk test was comparable in both groups.

In a randomized, placebo-controlled trial with 79 type-2 diabetes patients, adjunctive therapy with 1200 mg/day LI 132 over 16 weeks significantly reduced the diastolic blood pressure, with no significant effect upon the systolic blood pressure [59].

According to the results of an open-label cohort study performed in 711 patients, the add-on treatment with WS® 1442 led to significant improvements of quality of life in NYHA stage II coronary heart disease patients and demonstrated substantial and significant reductions in hospitalization costs due to heart failure [57]. The results of the individual clinical studies are further supported by a recent Cochrane review including 14 randomized, placebo-controlled, double-blind, clinical studies and describing the results of meta-analyses based on the 10 studies with data suitable for pooling. The results showed that hawthorn extracts are efficacious adjunctive treatments for CHF NYHA stage II [45]. The authors conclude that hawthorn extracts provided significant beneficial symptom control and physiological outcomes when used as adjunctive treatments for CHF.

Adverse events and drug interactions
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Adverse events (AEs) associated with the use of hawthorn extracts are rare and usually of mild to moderate severity. A comprehensive safety review of clinical study data for hawthorn extract treatments identified 29 clinical studies and examined data relating to AEs or drug-drug interactions from those 24 studies which met the inclusion criteria. The analysis included those 5577 of the 7311 patients for whom data were available. In the meta-analysis, hawthorn extract doses ranged from 160 mg to 1800 mg per day over a period of 3 to 24 weeks. The extracts used were generally either LI 132 or WS® 1442. In all, only 166 AEs were reported. Serious AEs have not been reported in association with WS® 1442 and the incidence of AEs does not appear to be related to dose [60]. There are no case reports on drug interactions with hawthorn extracts [61], and in an interaction study with digoxin no effect on pharmacokinetic parameters by concurrent administration of WS® 1442 was observed [62].

Conclusions
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Hawthorn extracts from leaves and flowers are widely used as an herbal remedy in the treatment of cardiac insufficiency. The extracts have been shown to contain a range of vaso- and cardioactive substances. Clinical studies in over 4000 patients confirm that standarized extracts such as WS® 1442 are effective as an add-on therapy in the treatment of NYHA stage II and III chronic heart failure. In addition, these extracts possess further pharmacological effects which might be successfully applied in the prophylaxis and therapy of other cardiovascular disorders, e.g., endothelial dysfunction, atherosclerosis, coronary heart disease, or prevention of restenosis/reocclusion following peripheral endovascular treatment.

Conflict of interest
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Both authors are employees of Dr. Willmar Schwabe GmbH & Co. KG, the manufacturer of the standardized hawthorn extract WS® 1442.

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