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Introduction

Hawthorn (Crataegus sp.) has been used as both food and traditional remedy around the world for centuries. The Chinese used it widely as tonic, digestive and for amelioration of hyperlipidemia, poor circulation and dyspnea. It was included in Tang Ben Cao - the world’s first known official pharmacopoeia in 659 A.D. [1-9]. In Europe the fruits, leaves and flowers have been extensively utilized as a cardiotonic, diuretic and tonic drug. Its use can be traced back to Dioscorides (1st century A.D.), who actually named one of the species “Οξυακανθα”, a name which was retained by Linnaeus in the name C. oxyacantha. The authoritative herbalist, physician, astrologist and apothecary from the 17th century Nicholas Culpeper (1606-1654) included hawthorn in its famous treatise “Complete Herbal” -an influential work, which was issued in 1653 and had significant impact over the medicine in post-Renaissance Europe. Culpeper outlined the efficacy of hawthorn in diverse ailments, including dropsy – a primordial designation of congestive heart failure [10].

Since 1800 the application of hawthorn as cardiovacular drug was widely accepted throughout Europe, up until now. Hawthorn has gained exceptional popularity in the USA too after 1896 [1, 4, 6, 11]. Diverse hawthorn-based products have been widely used in Germany, France and Eastern Europe as cardiotonic in neurogenic-induced functional disturbances of the ventricular function, as well as in a variety of other cardiological conditions [1, 8, 12-15]. Hawthorn is also widely used as a nutraceutical in Canada and USA. Nowadays the efficacy of hawthorn leaf and flower has been well corroborated by the variety of controlled and open clinical trials, which firmly indicate its efficacy in low grade (NYHA I-II stages) chronic heart failure [1, 3, 4, 16]. Both experimental and clinical data however, indicate its efficacy as antiarrhythmic, vasodilator and cardioprotective agent. Hawthorn has the potential to be a valuable natural alternative treatment for mild heart disease as well as a safe promoter of general cardiovascular health [5, 6, 17].

The available preparations are based on the official drug Crataegi Folium cum Flore (Ph. Eur.), which is composed of dried leaves and flowers from hawthorn - Crataegus monogyna and/or Crataegus oxyacantha [18]. The presented review is a concise outline of the literature findings regarding the phytochemistry, mechanism of action, preclinical pharmacokinetic, pharmacodynamic and toxicological findings for hawthorn.
Phytochemical peculiarities

The active ingredients of hawthorn leaf and flower extract have not been elucidated and it is thought that a mixture of them is obligatory for optimal therapeutic effect. The major chemical substituents of hawthorn leaf and flower are briefly outlined below (representative compounds are summarized in Figure 1).

The major chemical constituents of the Hawthorn leaf and flower, which most probably greatly contribute to the pharmacological properties of the corresponding medicinal products, are the flavonoids, namely vitexin, vitexin-2''-rhamnoside, acetylvetexin-2'-rhamnoside, apigenin, luteolin as well as related proanthocyanidins, although the quantity of the latter is lower in comparison with the hawthorn berries [15, 19-23].

Fig 1. Characteristic flavonoid (left) and triterpenoid compounds from hawthorn leaf and flower.

In the inflorescence different flavonol glycosides are abundant too, mainly hyperoside, spiraeoside and rutin. The predominant flavonoid derivatives in the leaves are epi-catechin and or catechin as well as the procyanidins formed via condensation of 2-8 monomeric units of the aforementioned catechins, together with oligomeric procyanidin derivatives [4, 24, 25]. The hawthorn based products contain also some simple phenolic acids such as chlorogenic and caffeic acid. Among the non-phenolic characteristic compounds are some pentacyclic triterpenoids, namely oleanolic acid, crataegolic acid (α-hydroxy oleanolic acid) and ursolic acid [21].

A minor fraction of biologically active compounds, which in line with their potent pharmacological activity probably at least partly contribute to the pharmacological properties of the plant are some biogenic amines such as phenethylamine, methoxyphenethylamine, dopamine, tyramine, ethylamine, choline and acetycholine [19, 26].

The Hawthorn leaves and flowers contain also a small fraction of phytosterols, namely β-sitosterol, cycloartenol, 24-methylen-24-dihydrolanosterol and butyrospermol [19, 26]. Minor constituents include some neutral polysaccharides (an arabinono-xyloglucan and a highly branched arabinogalactan), purine derivatives (adenine, adenosine and uric acid), and minerals with a high potent of potassium [19, 26].

Pharmacology

Hawthorn has a long record of use as a cardiac tonic, hypotensive, coronary and peripheral vasodilator and antiarrhythmic [1, 3, 4, 9, 25-29]. Although Hawthorn’s phytochemistry is exceptionally well-documented the actual contribution of the individual components to the overall pharmacological effects of the plant is still not fully ascertained [24]. Hereby the pharmacological properties of hawthorn leaf and flower are briefly summarized in the light of the existing nonclinical data. Even though generally beyond the scope of the present review, some clinical trial information is also outlined as it represents the ultimate manifestation of the pharmacological properties of hawthorn.

Inotropic effect

The application of Hawthorn leaves and flower as cardiotonic in functional ventricular disunction and low grade (NYHA I-II) chronic heart failure is the best characterized by scientifically sound clinical evidence indication for this medicinal plant [3-6, 8, 15, 27]. As a matter of fact, apart from the cardiac glycosides Hawthorn is the most well documented plant-derived inotropic agent. As with other plant extracts however its effects are multimodal and the precise mechanism of action is still not thoroughly elucidated [5, 8].

The evaluation of the cellular and molecular aspects of Hawthorn’s inotropic effect show that at least several putative mechanisms are involved namely, inhibition of the Na⁺/K⁺ adenosinetriphosphatase (Na⁺/
K+ pump); catecholamine-like cAMP-dependent effects; inhibition of the phosphodiesterase (Figure 2).

Similar to the digitalis glycosides Hawthorn has been found to inhibit the Na+/K+ adenosine triphosphatase, thus affecting the sodium outward and potassium inward fluxes [25]. This in turn indirectly hampers the activity of the Na'/Ca2+ antiport leading to increased intracellular calcium levels, facilitation of the interactions between the contractile elements and ultimately to positive inotropic effect [11]. Conversely some unidentified Hawthorn constituents have been found to displace radio-labeled ouabain from its binding sites indicating a digitalis-alike pharmacological basis of the positive inotropic activity.

Hawthorn derived polyphenols have been found to possess a catecholamine-like activating effect upon the adenylyl cyclase and to increase the level of cAMP, which in turn, activates protein kinase A and evokes a series of signal events associated with increased contractility of the cardiomyocytes [1, 4, 6, 21]. Siegel et al. investigated the electrophysiological effects of Hawthorn extracts (with flavonoid concentrations of 10^-7 to 10^-5 mol/l) on canine papillary muscle action potential. The authors noted an increase in the maximal upstroke velocity and overshoot of the action potential, indicating an enhancement of the inward fast Ca2+ current. This action is similar to beta-adrenergic stimulation. The enhancement of the Ca2+ channel in this case is caused by stimulation of adenylate cyclase, rise in intracellular cAMP, which in turn activates protein kinase A, and hence stimulates the phosphorylation of calcium-activatable K+ channels. This chain of events causes an increase in the open state probability of the channel. It is noteworthy however, that the effects of Hawthorn differ from the beta-adrenergic stimulation in that there is also slowing of the final repolarization phase [30].

**Fig. 2.** Molecular mechanisms of the positive inotropic effects of Hawthorn leaf and flower – comparison with the common conventional inotropic drugs in clinical use – digitalis glycosides, beta-agonists (e.g., dobutamine) and phosphodiesterase inhibitors (e.g, milrinone). (1) Hawthorn active principles bind to and inhibit the Na+/K+ pump, causing an indirect hinder of the Ca++/Na+ exchange, with subsequent rise in intracellular calcium. (2) Hawthorn activates the beta-receptor responsive signal pathways, namely the adenylyl cyclase (AC)/cAMP –dependent protein kinases (e.g. PKA), which phosphorylate calcium channels and cause an increased calcium influx; (3) Hawthorn’s inotropic effects are partly mediated by inhibition of the phosphodiesterase (PDE) with a subsequent rise in intracellular cAMP and augmentation of the cAMP-evoked cellular responses; these three alternative mechanisms converge on a common event - they cause eventually rise in the intracellular concentration of free cytosolic calcium and hence increased contractility, i.e. positive inotropic effect.
This study adds further controversy regarding the precise role of cAMP-dependent signaling pathways for the inotropic effects of Hawthorn; it firmly demonstrates that its actions on the myocardium cannot be explained purely by adrenergic properties. Hawthorn may activate protein kinase A (PK-A), but it also affects targets not phosphorylated by PK-A or other PK-A agonists. These in vitro data, demonstrating the ability of Hawthorn to affect ion channels in the membrane of cardiac and smooth muscle cells may account for the decreased ventricular arrhythmias, the positive inotropic effect and the decrease in diastolic blood pressure seen in the in vivo studies with Hawthorn [30].

The inotropic effects of different compounds in Hawthorn were tested in Langendorff perfused isolated guinea pig hearts at a constant pressure of 70 cm H2O, whereby luteolin-7-glucoside, hyperoside and rutin (66%) showed superior activity as compared to vitexin, vitexin-rhamnoside and monoacetyl-vitexin-rhamnoside. The inotropic activity of the compounds was abolished by addition of propranolol or reserpine, thus indicating an adrenergic mechanism [30].

At least some of the cardio-vascular effects of Hawthorn extracts are mediated by inhibition of phosphodiesterase as evidenced by different in vitro studies. This effect is consistent with reduced rate of cAMP breakdown and hence sustained effects on protein kinase A, and the cAMP–dependent calcium channels and signaling cascades in cardiomyocytes [11].

Hwang et al evaluated the effects of Hawthorn on cardiac remodeling and left ventricular dysfunction after 1 month of pressure overload –induced cardiac hypertrophy in rats (subject to either Sham operation or aortic constriction). The results of the study clearly indicate that Hawthorn treatment modifies left ventricular remodeling and counteracts myocardial dysfunction in early pressure-overload induced cardiac hypertrophy, a finding which accounts for a favorable pharmacological profile of Hawthorn in cardiac failure [31].

The positive inotropic effects from the nonclinical studies are translated into proved clinical efficacy in patients with NYHA class II-III chronic heart failure as evidenced by the several clinical trials. The results form these studies clearly indicate that Hawthorn leaf and flower extracts are endowed effective as evidenced by different end points such as anaerobic threshold, clinical global impressions, ejection fraction, quality of life and improvement of the subjective symptoms, pressure rate product and exercise tolerance [5, 6, 32]. The results generally indicate that Hawthorn holds promise as adjunctive agent in left-myocardial dysfunction; it significantly improves exercise tolerance and the symptoms of mild to moderate heart failure and favorably augments the ventricular function as measured by the LVEF. The peculiarities of the numerous trials are reviewed elsewhere [3, 4, 6, 16, 32] and are beyond the scope of the present review.

A recent clinical trial, which is worth-mentioning, is SPICE (Survival and Prognosis Investigation of Crataegus Extract WS 1442 in Chronic Heart Failure) as it gives additional information regarding the clinical efficacy of Hawthorn as a positive inotropic agent. The SPICE study, a double-blind and placebo controlled randomized clinical trial enrolled 2681 patients and was designed to demonstrate whether the Hawthorn extract is safe and if it reduces the morbidity and mortality in Patients with chronic heart failure (NYHA II-III stage), on top of standard medical therapy. Although the Hawthorn extract failed to significantly alter the primary endpoint (combined endpoint of cardiac death, non-lethal myocardial infarction and hospitalization due to disease deterioration) it significantly reduced the sudden cardiac death in patients with LVEF ≥ 25%, Moreover WS 1442 was very well tolerated and lacked any significant interactions with the standard therapies, namely diuretics, ACE-inhibitors, cardiac glycosides and β-adrenergic antagonists [32].

Vasodilator, vasoprotective, antihypertensive and antiischemic effects

The vasodilating activity of Hawthorn and the related antiischemic and antihypertensive effects of Hawthorn are firmly established, in both in vivo and in vitro nonclinical studies. The proposed mechanisms include phosphodiesterase inhibition, activation of potassium outward currents with secondary hyperpolarization and indirect prostanoïd- or NO-mediated effects [1, 3, 33, 34]. The polyphenols in Crataegus extracts are endowed by intrinsic vasodilator effects, and moreover trigger the release of prostacyclin (PGL2) a potent endogenous vasodilator, while inhibiting the synthesis of the pressor compound thromboxan A2 (TxA2) [1, 3-6, 11, 31]. Moreover it has distinct endothelium protective properties, mediated by modulation of ionc microhomeostasis and autacoid biosynthesis, pharmacology, and trafficking [33-37].

The mechanism of the vasodilating effect of Hawthorn on human coronary arteries smooth muscle action potential was studied also in an in vitro...
test system - coronary arteries obtained from heart transplant patients suffering from dilated cardiomyopathy or extensive atherosclerosis. Both normal and atherosclerotic vessels were studied. Hawthorn extract caused an increase in the K⁺ conduction and a hyperpolarization of the membrane potential. This hyperpolarization increases the L-type Ca²⁺ channel in the closed position whereby the Ca²⁺ inward current into vascular smooth muscle was decreased by 16.3%, causing a decrease in wall tension and dilation of the vessels [21].

Brixius et al. investigated in detail the mechanism of WS 1442 (Crataegus special extract) on the relaxation of rat aorta or human mammalian artery vessel rings pre-contracted by phenylephrine. WS 1442 effectively dilated the vessel rings whereby its effects were comparable to those of the reference agent papaverine. The mechanistic studies revealed that the vasodilatation is mediated by induction of the endothelial NO-synthase (eNOS) and release of NO-release from the vascular endothelium. Pretreatment with an eNOS inhibitor or mechanical disruption of the endothelium completely abrogated the vasodilatory effects of WS 1442, which further evidences that they are NO-mediated [34].

Different compounds in Hawthorn were tested for their effects on coronary flow, heart rate and ventricular systolic and diastolic pressure in Langendorff perfused isolated guinea pig hearts at a constant pressure of 70 cm H₂O. An increase in coronary blood flow was caused by luteolin-7-glucoside (186%), hyperoside (66%) and rutin (66%). There was an increase in

![Fig. 3. Molecular mechanisms of the vasodilator activities of Hawthorn. (1) Hawthorn active principles have been found to activate the endothelial NO-synthase (eNOS) and hence to evoke NO-dependent signaling pathways, i.e. activation of guanylyl cyclase, increase in cGMP levels, increased calcium sequestration and potassium efflux leading to hyperpolarization and relaxation of the vascular smooth muscle.; (2) Hawthorn stimulates the synthesis of the vasodilating prostacyclin and (3) it inhibits the synthesis of both angiotensin II and thromboxane A2, both potent vasoconstrictors; (4) At least partly Hawthorn’s vasodilating effects are mediated by inhibition of the phosphodiesterase (PDE) with subsequent rise in intracellular cGMP.](image-url)
relaxation velocity by luteolin-7-glucoside (104%), hyperoside (62%) and rutin (73%). The major effects of these compounds were at 0.5 mmol/l. Vitexin, vitexin-rhamnoside and monoacetyl-vitexin-rhamoside had similar actions, but to a lesser extent [30].

Additional mechanisms underlying the vasodilating activity of Hawthorn include inhibition of the angiotensin-converting enzyme and of phosphodiesterase type III [4].

The application of Hawthorn in hypertension has long history especially in Europe, but recently its antihypertensive efficacy has been demonstrated in clinical trials too [3, 6, 27, 32, 38]. More precisely, a double blind pilot role indicated a promising role of Hawthorn in mild essential hypertension [25].

The antihypertensive activity of Hawthorn is due to its vasodilator, beta-adrenoreceptor blocking effects, cholinergic modulation as well as due to its effects upon the renin-angiotensin–aldosterone system, presumably via inhibition of ACE by the procyanidins fraction of the extract [3, 21, 25, 26, 39] (1). Hawthorn is also a mild diuretic (see section 2.2), which is at least partly implicated in its antihypertensive mode of action.

Walker et al investigated the effects of Hawthorn for hypertension in patients with type 2 diabetes taking prescribed drugs within a randomized controlled trial. In this study patients with type 2 diabetes (n = 79) were randomized to daily 1200 mg Hawthorn extract (n = 39) or placebo (n = 40) for 16 weeks. At baseline and outcome a well-being questionnaire was completed and blood pressure and fasting blood samples taken. A food frequency questionnaire estimated nutrient intake. Hypotensive drugs were used by 71% of the study population with a mean intake of 4.4 hypoglycemic and/or hypotensive drugs. There was a significant group difference in mean diastolic blood pressure reduction, whereby the Hawthorn group showed greater reductions than the placebo group. There was no group difference in systolic blood pressure reduction from baseline. No herb-drug interaction was found and minor health complaints were reduced from baseline in both groups. In this randomized controlled trial the hypotensive effect of Hawthorn in patients with diabetes taking medication was firmly demonstrated [32].

The heart antischemic and cardioprotective effect of Hawthorn has been well documented in different test systems and the underlying mechanisms include; antioxidant effects, modulation of mitochondrial oxidative phosphorylation, coronary arterial dilatation and the stimulatory effects upon heart-revascularization [26, 40-42]. Additional mechanisms which most probably contribute for these effects are the well documented inhibition of ACE and the pronounced anti-inflammatory effects, mediated by inhibition of the release of histamine, prostaglandins, proteases and leukotrienes [26]. Moreover Hawthorn extracts have anti-platelet properties via inhibition of the synthesis of TxA2 – a potent inflammatory mediator, vasoconstrictor an platelet aggregator [28].

Due to the high content of polyphenols, capable of binding heavy metal ions, scavenging reactive oxygen species or lipid-derived free radicals and having low redox-potentials condition the exceptional antioxidant properties of Crataegus extracts, which have been well demonstrated in different experimental and read-out systems [4, 30, 43].

Different extracts of Hawthorn from fresh and dried flowers and pharmaceutical preparations were analyzed for their antioxidant activity in vitro by Bahorun et al. These preparations were also analyzed for their content of total phenols, total proanthocyanidin and total flavonoid. Antioxidant activity was determined by measuring scavenging ability of hydrogen peroxide, superoxide anion and hypochlorous acid. The authors concluded that fresh young leaves, fresh floral buds and pharmaceutical preparations of dried flowers all exhibited in vitro antioxidant activities using all alternative test systems to assess antioxidant activity in vitro. The activity appeared to be connected to the total phenolic proanthocyanidin and flavonoid contents. The concentrations needed for the pharmaceutical preparations were slightly higher than those in the fresh preparation [30].

Guendjev et al investigated the effect of a Hawthorn-derived flavonoid Crataemon®, on reperfusion of the myocardium after ligation of a coronary vessel. The left coronary artery of 67 female Wistar rats was ligated. Two days before and 10 days after the ligation, 34 of the experimental rats were treated with 150 mg/kg Crataemon®, a purified flavonoid extract from Crataegus monogyna leaves. The other 33 rats were not treated. In this study the mortality was decreased in the treated group compared with the control group. Twenty-seven of the control and 32 of the experimental rats survived the procedure and were sacrificed 10 days post-ligation. Histological sections demonstrated that the necrotic focus was smaller in the experimental rats than in the controls. It was also noted that there were significantly more capillaries, venules and arterioles in the experimental rats compared with controls. The ratio of venous vessels to arterial vessels was similar in both experimental and
control animals. The authors suggest that decreased post-operative mortality in the experimental animals might be due to the capillary strengthening effect of Crataemon®. The high level of revascularization produced by Crataemon® causes an immediate high blood flow, containing many phagocytic cells, to the necrotic tissue, thus decreasing the dimension of the necrotic focus [30].

**Antihyperlipidemic effects**

Crataegus extracts have been shown to exert prominent effects on lipid and cholesterol metabolism both in preclinical studies and in human trials. When applied to rats fed a hyperlipidemic diet the Hawthorn extracts effectively prevented the rise in plasma lipid levels, the total cholesterol, LDL-fractions [44, 45]. Mechanistic studies revealed that these effects are mediated by up-regulation of the hepatic LDL receptors, resulting in greater influx of plasma LDL into the liver. Hawthorn extracts also alter cholesterol levels by enhancing its breakdown in bile acids, promoting bile flow and suppressing cholesterol biosynthesis.

In a series of elegant studies it was demonstrated that Hawthorn extracts exert also a fibrate-like effects, mediated by interactions with peroxisome proliferator activating receptor (PPAR) elements, leading eventually to activation of lipoprotein lipase and amplified lipoprotein breakdown [43, 46]. The authors investigated the effects of different natural medicines upon lipid metabolism via regulation of lipoprotein lipase (LPL) expression. To meet this objective a green fluorescent protein (GFP) gene was constructed downstream of the peroxisome proliferator response element (PPRE) and the constructed plasmid was microinjected into Xenopus oocytes to establish a PPRE regulatory reporter system [43]. Using this system, Hawthorn flavonoids were quickly selected from a panel of natural medicines and found to up-regulate GFP expression by an effect on PPRE. To confirm the effect of Hawthorn flavonoids, the authors treated mice orally with water (control), Hawthorn flavonoids, and pioglitazone and measured the LPL levels in serum, adipose tissue, and muscle by an ELISA. The serum LPL levels were no different from the controls after treatment with either Hawthorn flavonoids or pioglitazone, but LPL increased significantly in muscular tissues and decreased in adipose tissues, which implies that Hawthorn flavonoids mediate LPL expression in mice with tissue-specific differences. A novel PPRE regulatory report system was established for rapid and effective selection and evaluation of LPL-mediating drugs [46].

Chen et al. investigated the Hawthorn’s ability to reduce body weight, body fat and blood lipids in rats and humans. The animal experiments were performed on 37 Sprague–Dawley male rats divided into three groups; one was given a Hawthorn drink containing 4–6% sugar, another water containing 8% sugar and the third group received tap water. Body weights of the rats were lower in Hawthorn-fed rats, but the decrease in body weight was not statistically significant. Nevertheless the Hawthorn group did have significantly decreased body fat, total cholesterol levels and triglyceride level compared with the two control groups. Moreover the HDL level was greater in the Hawthorn group than in those drinking sugar water, but was less than in the group drinking tap water. The human portion of the study enrolled 30 volunteers diagnosed as hyperlipidemic. After consuming Hawthorn for 1 month, average cholesterol levels decreased from 7.3 mmol/l to 6.2 mmol/l and serum triglyceride levels decreased from 1.9 to 1.7 mmol/l. Hawthorn did not raise HDL levels in the human study. Hawthorn was also shown to decrease serum levels of lipid peroxide malonic dialdehyde levels, suggesting a strong antioxidative effect [30].

**Anti-arrhythmic effects**

The anti-arrhythmic effects of Hawthorn have been established in different experimental test systems [6, 29, 47, 48]. The mechanistic aspects of the anti-arrhythmic effects are multimodal and include antiischemic properties due to the high polyphenol content and also some direct electrophysiological effects of Hawthorn. It has been found that Hawthorn extracts prolong the action potential, presumably due to modulation of potassium channels, thus causing an increase of the myocardial refractory period in a manner analogous to the electrophysiological effects of Class III antiarrhythmic drugs [3, 25].

Al Makdessi et al. studied the protective effect against reperfusion arrhythmias of a 3-month oral pretreatment with a dried extract of *Crataegus oxyacantha* (LI 132)(standardized to 2.2% flavonoids). The Langendorff heart of the rat after global no-flow ischemia was used as experimental model. According to pilot experiments two durations of global no-flow ischemia were chosen for the study: 20 minutes (group 20) in which ventricular fibrillation (VF) was the predominant form of arrhythmias, and 18 minutes (group 18) in which the prevalence of VF was markedly lower despite the small difference in the duration of ischemia. *Crataegus* pretreatment significantly reduced the average prevalence of malignant
arrhythmias (VF + Flutter) as observed during the 20-min-period of reperfusion as follows: group 20: from 89% to 51% and in group 18: from 48% to 8%. In the former group, ventricular tachycardia (VT) could be observed only in the treated group, because of the predominance of VF in the control group. LI 132 pretreatment reduced the average prevalence of VT in group 18 in spite of the identical percentage of occurrence (6 out of 8 rats, with and without treatment) due to a shorter duration of the VT episodes. Thus, in this study, effective prevention against reperfusion arrhythmias by *Crataegus* pretreatment was evident [47].

**Secondary pharmacodynamics**

The pharmacological evaluation of a flavonoid-enriched fraction derived from Hawthorn flower extract in dogs showed that it is endowed by diuretic activity when administered at 50 mg/kg [21].

Hawthorn has centuries-long record of ethnopharmacological use as an antispasmodic agent for diverse gastrointestinal conditions in China and Europe [3, 12-14, 45, 49]. A series of recent *in vitro* experiments of Hawthorn flower extract indicate that it is endowed by significant antispasmodic effect in isolated rabbit intestine. The extract has been found to inhibit barium chloride-, histamine- and nicotine-induced contractions and to be sparingly effective against acetylcholine or serotonin-induced spasms. Intravenously administered flavonoid-enriched fraction from Hawthorn leaf and flower extract (20 mg/kg) inhibited intestinal contraction in cats, whereas i.p. injection (400 mg/kg) inhibited acetic acid-induced writhing in mice [21].

Zhang et al. investigated the neuroprotective effects of *Crataegus*-derived flavonoids on brain ischemic insults in a Mongolian gerbil stroke model. The rationale of the study is based on the potent antioxidant properties of flavonoids on one hand and on the crucial role of free radical production in the pathogenesis of stroke is during the reperfusion period, ton the other [50]. Pretreatment of the animals with the flavonoids decreased reactive oxygen species (ROS) production, thiobarbituric acid reactive substances content, and nitrite/nitrate concentration in brain homogenate, increased the brain homogenate-associated antioxidant level in a dose-dependent manner. Flavonoid pretreatment increased the amount of biologically available NO by scavenging of superoxide anion produced during reperfusion. At the same time, in the process of ischemia/reperfusion brain damage, the content of nitrite/nitrate (the end product of NO) increased, and of NO detected by ESR decreased. Oral pretreatment with flavonoids decreased the nitrite/nitrate content in the brain homogenate and increased the biologically available NO concentration in a dose-dependent manner. The authors attribute the effect on NO to the scavenging effect on superoxide anion, which could react with NO into peroxynitrite. iNOS was implied in delayed neuron death after brain ischemic damage and it was found that pretreatment with CF could decrease the protein level of tumor necrosis factor (TNF)-alpha and nuclear factor-kappa B (NF-kappaB), and increase the mRNA level of NOS. More neurons survived and fewer cells suffered apoptosis in the hippocampal CA1 region of CF treated animal brain. These results suggest that oral administration of this Hawthorn extracts increases the antioxidant level in the brain and protects the brain against delayed cell death caused by ischemia/reperfusion injury [50, 51].

Hosseinimehr and colleagues has assessed the radioprotective effect of Hawthorn (*Crataegus microphylla*) extract against genotoxicity induced by gamma irradiation in mouse bone marrow cells. A single intraperitoneal administration of Hawthorn extract at doses of 25, 50, 100 and 200 mg/kg 1h prior to gamma irradiation (2 Gy) reduced the frequencies of micronucleated polychromatic erythrocytes (MnPCEs). The maximum reduction in MnPCEs was observed in mice treated with extract at a dose of 200 mg/kg. Administration of amifostine at dose 100 mg/kg and Hawthorn at dose 200 mg/kg reduced the frequency of MnPCE almost 4.8 and 5.7 fold; respectively, after being exposed to 2 Gy of gamma rays, compared with the irradiated control group. *Crataegus* extract exhibited concentration-dependent activity on 1,1-diphenyl 2-picrylhydrazyl free radical showing that *Crataegus* contained high amounts of phenolic compounds and the HPLC analysis determined that it contained chlorogenic acid, epicatechin and hyperoside. It appeared that Hawthorn extract with antioxidant activity reduced the genotoxicity induced by gamma irradiation in bone marrow cells [52]. The same group has also revealed the radioprotective and antigenotoxic effects of Hawthorn in other model test systems [53, 54].

**Safety pharmacology and pharmacodynamic drug interactions**

Larger doses in animal studies with various species were reported to cause sedation after intragastric administration of leaf and flower extracts. A 60% methanol extract of the flowers (at a dose of 800 mg/
kg) has been found to increase hexobarbital-induced sleeping times, and to decrease the spontaneous motility and exploratory behavior in female mice [11, 21]. Moreover the flavonoids abundant in Hawthorn have been purportedly advocated to have a sedative effect in humans, the clinical impact of this data however remains unknown [3, 11].

Hawthorn leaf and flower extract have been shown to be less arrhythmogenic than the other inotropic agents, i.e. milrinone, digitalis or sympathomimetics. Nevertheless it has been found to prolong the pacemaker repolarization, indicating an antihypertrophic class III–like activity [25]. Although Hawthorn is not known to be non-torsadogenic the risk of potential interactions with this respect should be considered, when combined treatment with a Q-T prolonging agent has to be performed.

In line with the pharmacodynamic properties of Hawthorn and especially its Na+/K+-pump and phosphodiesterase inhibitory activities it can be presumed that it may have a potentiating effect on digitalis glycosides, beta-blockers and other hypertensive drugs, when co-administrated [1, 24, 25, 55]. These interactions could be related to the sole cardiotonic and hemodynamic effects of Hawthorn.

Increased vasodilation and possibly hypotension could ensue if Hawthorn leaf and flower extract is co-administrated with vasodilator drugs, e.g. theophylline, papaverine, adenosine or nitrates. The effects of digoxin could be potentially potentiated thus necessitating dose reduction [6, 25]. It is noteworthy that certain natural products and phytomedicines could in turn adversely augment Hawthorn’s effects leading to toxicity. These include squill, ginger, cola, mate, guarana, etc [6].

Moreover Hawthorn leaf and flower extract has been found to exert beta-blocking activities and to antagonize the effects of isoprenaline [3], which in turn indicates the theoretic risk of adverse antagonistic interactions with co-administered beta-adrenergic agonists or other sympathomimetic agents. The results form the clinical trials, however failed to demonstrate any significant drug interactions with the majority of drugs used to treat cardiac failure. The available data on Crataegi Folium and Flore indicate that its application is devoid of adverse interactions with various laboratory tests such as serum levels of electrolytes (e.g. sodium chloride, potassium chloride, calcium chloride), serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, γ-glutamyl transpeptidase, total bilirubin, cholesterol and creatinine, and blood glucose levels [21].

**Pharmacokinetics**

Apart from the initial animal studies with radiolabelled procyanidins only few pharmacokinetic studies have been carried out and hence the peculiarities of the Hawthorn-derived ingredients metabolism and elimination remain largely unknown.

Absorption of a 14C–labelled oligomeric procyanidins fraction of standardized leaf and flower extract has been measured in mice after intragastric application (0.6 mg). 20-30% of the total fraction, 40-81% of the trimeric procyanidins and 16-42% of the oligomeric procyanidins were absorbed within 1-7 h after the administration of the preparation. After 7 h 0,6% of the total fraction radioactivity was eliminated by expiration and ca. 6 % were eliminated in the urine. Daily intragastric treatment of radiolabelled procyanidins fraction in mice over 7 days led to an accumulation of radioactivity that was 2-3 times that in mice given a single dose [21].

*Crataegus* extracts do not inhibit the P-glycoprotein efflux pump and hence fail to affect the bioavailability of co-administered digoxin. The flavonoid fraction however has been found to interact (induce or inhibit) the cytochromP450 enzymatic system, which accounts for a theoretic risk of drug interactions [11]. As evident from the clinical trials however, there are no reported clinically significant interactions of Hawthorn with co-administered drugs such as ACE-inhibitors, beta-blockers, digoxin or aldosterone [55].

In an interesting study, among the first to address the human pharmacokinetics of Hawthorn-derived flavonoids Chang et al. investigated the pharmacokinetics of (-)-epicatechin, chlorogenic acid, hyperside, and isoquercitrin—following administration of an extract formulation (as Hawthorn phenolic extract, which contained the active compounds) or equivalent doses of individual pure compound in male Sprague-Dawley rats (n = 5 per group). The rationale of the study is based upon the possible alterations of the pharmacokinetics of a given active herbal substance when administered in an extract form as compared to that when administered as a pure compound. The Hawthorn phenolic extract or pure compounds were administered both orally and intravenously. After the intravenous injection of Hawthorn phenolic extract, higher plasma drug concentration, larger AUC_{0-∞}, longer terminal elimination half-life, smaller Vd, lower Cl_{int}, and higher urinary excretion of each compound were obtained when compared to that after the pure compound. Following the oral administration of either Hawthorn phenolic extract or pure compound,
only epicatechin was absorbed, and their pharmacokinetics were generally not significantly different between these 2 formulations. The differences in the pharmacokinetics of the 2 formulations following intravenous but not oral administration may be attributable to the existence of other co-occurring components in the Hawthorn phenolic extract (which may be present in the body after intravenous but not oral administration) [56].

**Toxicology**

Hawthorn leaf and flower is endowed by an extremely low toxicological potential as evidenced by the tiered toxicological testing, the multy-century safety record of the species as medicinal and edible plant and more importantly by the convincing data from the numerous clinical trials indicating exceptional tolerability, low incidence and transient character of the adverse events [5, 11, 26, 28, 32, 55, 57]. Hereby the available non-clinical toxicological data is briefly outlined.

**Acute toxicity**

The acute toxicity evaluation of a 10% alcoholic extract solution of Hawthorn leaves and fruit shows an oral LD$_{50}$ value of 18.5 ml/kg in mice and 33.8 ml/kg in rats. The extract tested was manufactured by Schwabe and contained 2 or 10% oligomeric procyanidins. Death has been found to occur approximately half an hour after treatment and is caused by excessive sedation and apnea [1, 11]. The murine LD$_{50}$ of the flavonoid fraction following intravenous application is 1.56 g/kg. The corresponding acute toxicity testing of the proanthocyanidin fraction reveals LD$_{50}$ values of 130 mg/kg i.p and 300 mg/kg s.c., in mice [1].

In a non-lethal single dose toxicity studies using a standardized Hawthorn leaf and flower extract (18.75 procyanidin oligomers) oral doses of up to 3 g/kg caused neither mortality nor any detectable signs of toxic effects in either rats or mice [19].

The repeated dose toxicity studies with a standardized Hawthorn leaf and flower extract (18.75 procyanidin oligomers), the daily application of 300 mg/kg p.o. failed to evoke any toxic effects in both rats and dogs [19].

**Genotoxicity and carcinogenicity**

A standard battery for evaluation of the genotoxicity of Hawthorn leaf and flower extract has been carried out, and the results unambiguously indicate the lack of clastogenic and mutagenic effects [11].

The mutagenic potential of Crataegi Flos and Flore standardized extract, containing 18.75% oligomeric procyanidins has been extensively investigated using the *Salmonella typhimurium* test, mouse lymphoma test, cytogenetic analysis in cultured human lymphocytes or in the mouse bone marrow micronucleus test and was found to be devoid of mutagenic or clastogenic properties [21]. In another study some moderate activity has been established with *Salmonella typhimurium* TA98 strain, but only after metabolic activation. The mutagenic potential encountered was attributed to the quercetin present in the extract, however the amount of quercetin ingested in a normal diet is sufficiently higher than the levels attainable after the oral intake of the extract, which points out that the observed effects is of no clinical significance [21]. The available data, and especially the aforementioned lack of mutagenicity/clastogenicity presents no indication of carcinogenic risk [11].

**Reproductive and development toxicity**

The German Comission E states that there are no data for deleterious effects of Hawthorn during pregnancy and lactation, as well as no data for established toxic effects on the embryo/fetus or on fertility and postnatal development.

Experiments carried out with both male and female rats, administered Hawthorn leaves and flower extract at doses up to 1.6 mg/kg body weight, via gastric tube, failed to demonstrate any deleterious effects upon the fertility of the treated animals or their F1 generation [21].

The evaluation of the teratogenic potential of Hawthorn leaf and flower extract in rats and rabbits, (administered high doses p.o.) revealed that it is completely devoid of dismorphogenic or fetotoxic effects [21]. The thorough assessment of the pregnancy associated, non-teratogenic events has shown the total lack of peri- or postnatal toxicity in rats treated with 1.6 g/kg standardized Hawthorn leaf and flower extract [11, 21].

**Conclusions**

The complex pharmacological properties of Hawthorn (leaf and flower) could be generally ascribed to its polyphenolic content, and in particular to the flavonoids. Pharmacological effects documented in both animal and human studies support the traditional and ethnopharmacological application of Hawthorn as an inotropic, hypotensive and coronary
vasodilator, associated with few, clinically insignificant adverse effects. On account of its efficacy and safety Hawthorn has made its place in British Pharmacopoeia and European Pharmacopoeia as well as in numerous authoritative compendia. The German commission E has also approved the use of Hawthorn as a cardiological medicinal product.

The available data from the toxicological studies of Hawthorn leaf and flower products it to be of low toxic potential after oral intake. The clinical trials indicate very low incidence of adverse effects. Data from available investigations do not give support for a genotoxic, mutagenic, teratogenic or carcinogenic hazard in patients receiving recommended clinical doses.

In distinction to the cardiac glycosides Hawthorn leaf and flower products are not arrhythmogenic, and do not exert positive bathmotropic effects. Moreover the constellation of secondary cardiovascular activities including vasotonic and antihyperlipidemic, anti-hypertensive, ACE-inhibiting and mild diuretic could be quite beneficial for the overall cardiac function and outline this natural product as a novel inodilator drug. In contrast to the phosphodiesterase-III-inhibitors, however Hawthorn is devoid of tachicardic effects and does not induce blood dyscrasias. These data clearly indicate that Hawthorn leaf and flower extracts constitute an attractive cardiotonic and a safer alternative of the existing drugs in patients with mild, functional cardiac dysfunction.

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