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HAWTHORN REVISITED: TIME- AND DOSE-DEPENDENT CARDIOPROTECTIVE ACTION OF WS-1442 SPECIAL EXTRACT IN THE REPERFUSION-INDUCED ARRHYTHMIA MODEL IN RATS *IN VIVO*

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Standardized WS-1442 extract from *Crataegus oxycantha* (hawthorn) leaves and berries is one of the most widely studied preparations received from hawthorn. This popular substance is known from its positive influence on the cardiovascular system. The current research aimed to evaluate the optimal dose of standardized WS-1442 extract and the most beneficial period for its use. The study analysis was based on experiments previously conducted on male Sprague-Dawley rats (n = 152). The animals were divided into subgroups to examine the relationship between the dose-dependent (n = 96) and time-dependent (n = 56) effects of the mentioned extract. The research was performed based on the modified early reperfusion-induced arrhythmias model *in vivo*. The following parameters were assessed during the study: efficiency of mortality index reduction, reduction of ventricular arrhythmias incidences as well as the influence of standardized WS-1442 extract on hemodynamic parameters and amount of biochemical marker of cardiac tissue damage (creatine kinase). The current study revealed the dose- and time-dependent cardioprotective effect of standardized WS-1442 extract. It was expressed by mortality index reduction, decrease in the incidence and duration of severe ventricular arrhythmias and decline in the total amount of creatine kinase. Analyzed data coming from a model of reperfusion-induced arrhythmias in rats suggests that standardized WS-1442 extract is a potent cardioprotective agent whose action depends on both dose and intake time.

Key words: *hawthorn, cardioprotection, reperfusion-induced arrhythmias, creatine kinase, mean arterial blood pressure, ventricular fibrillation, oxidative stress*

INTRODUCTION

Cardiovascular disease and its main complications: myocardial infarction, cardiac rhythm disturbances and heart insufficiency remain leading causes of mortality and disablement globally. Even though considerable progress has been made in pharmacotherapy, urgent need of new cardioprotective drugs remains.

The main underlying pathology of cardiovascular disease is the generation of thrombogenic plaques in vessels. Arteriosclerosis causes serious dysfunction of vessel wall endothelium and gradually disrupts coronary blood flow. This pathomechanism leads to myocardial ischemia and provokes life-threatening severe arrhythmias (1). Myocardial ischemia and reperfusion coexisting with the processes mentioned above damage heart tissue, increase oxidative stress and promote reactive oxygen species (ROS) (2-5). That is why cardioprotective drugs with anti-oxidative activity are recognized as exceptionally valuable (6, 7).

The extracts received from the leaves and flowers of *Crataegus oxycantha* belong to the most frequently used medicaments in traditional and natural medicine. Consumption of berries and preserves such as tinctures, jams, wines and teas have been known in many regions of the world for centuries (8). This is due to their traditionally recognized positive influence on cardiovascular, digestive and respiratory systems, as well as anxiolytic and sleep-regulating properties (9).

The standardized extract WS-1442 is the most investigated and recognized preparation among those received from hawthorn (10). This extract is obtained from the dry leaves and flowers of *Crataegus oxycantha* and dissolved in 45% ethanol. The standardized content of oligomeric proanthocyanidins (OPC) is recognized as an essential feature of the positive cardioprotective action of this extract (11, 12). Among the main known active flavonoids and OPC's of WS-1442, there are hyperoside, ramnoside, vitexin, catechin and epicatechin (13). On the other hand, the beneficial action of WS-1442 in cardiovascular diseases is not fully understood. However, in some experimental studies, it has been proposed that this influence comes out of a number of pleiotropic effects.

Schussler *et al*. observed a significant increase of coronary flow and positive inotropic action of flavonoids derived from *Crataegus* species in a Langendorff model of isolated guinea pig hearts (14). In this study it was also suggested that the effects mentioned were dose-dependent and correlated positively with higher concentrations of flavonoids.

Positive inotropic action of *Crataegus* is most probably cAMP-independent. It has been shown that WS-1442 special

extract increases the contraction strength of cardiomyocytes by influence on Na^+/K^+ ATP-ase and blockage of the sodium pump, as well as an increase of Ca^{2+} transport in cardiac cells (15, 16). Results coming from *in vitro* studies also suggest negative-chronotropic action of *Crataegus* extracts. Such influence has been demonstrated in a cultured cardiomyocyte assay and was proven to be independent of beta-adrenergic receptors (17). It was later suggested that this effect is caused by muscarinic-receptor activation; however, this hypothesis does not fully explain the negative-chronotropic action of *Crataegus* extracts (18). Significant data about the influence of WS-1442 on endothelium and vessels homeostasis come from other *in vitro* studies. In more recent reports it has been shown that WS-1442 stimulates vascular relaxation, caused by an increase of nitric oxide (NO) release by endothelium (19). This process is probably dependent on the activation of endothelial nitric oxide synthase (eNOS) caused by serine Ser-1177 phosphorylation (20). This might suggest that the protective influence of WS-1442 is similar to the action of other naturally-derived, polyphenol-rich cardioprotective assets such as red wine extract (21, 22).

It is worth mentioning that the first report about the anti-arrhythmic, protective influence of *Crataegus* species extracts come from a paper by Thompson *et al.* published back in 1974 (23). The authors described a significant decrease of aconitine-induced arrhythmias incidence in rabbits after treatment with *Crataegus monogyna* extract. More suggestions on the anti-arrhythmic effects of *Crataegus* species extracts come from later *in vivo* experiments (24-26).

Experimental data that appeared promising on possible cardioprotective effect of *Crataegus* species extracts resulted in a number of clinical studies in the past. A pilot study including a small number (n = 36) of patients suffering from mild, essential hypertension has revealed significant hypotensive action of *Crataegus* species extracts in monotherapy as well as in addition to other drugs (600 mg Mg once daily) (27). Another study, which included patients treated for hypertension and type-2 diabetes, has shown the hypotensive action of *Crataegus laevigata* leaves, fruits and flowers extract (28). Another positive cardiovascular action of hawthorn extracts was suggested in clinical studies concerning hyperlipidemia. Hawthorn extracts were effective in lowering LDL-cholesterol as an additional therapy to statins (29).

Despite promising reports on cardioprotective action coming from previous studies, there is no exact data about the effects of hawthorn extracts on arrhythmias in the acute model of heart ischemia *in vivo*. Our study aimed to establish optimal dosage and treatment time with standardized hawthorn extract WS-1442 in the model of early reperfusion-induced arrhythmias in rats *in vivo.*

MATERIALS AND METHODS

Animals, drugs and reagents

Male Sprague–Dawley rats (n = 152; Central Animal Farm, Medical University of Silesia, Katowice, Poland) weighing approx. 315 ± 13 g and maintained under standard condition (ambient temperature 21 – 23°C; with 12 h/12 h dark/light cycle) with *ad libitum* access to food (standard LSM diet, Poland) and tap water served as experimental animals. The animals were fasted overnight before the experiment.

The study was performed with the approval of the Local Bioethical Committee and all experiments were conducted in accordance with NIH regulations for animal care described in the "Guide for the Care and Use of Laboratory Animals" (NIH publication, p. 2–107, revised 1996).

Standardized WS-1442 *Crataegus oxycantha* extract containing 18.75% of oligomeric procyanides was provided by Dr Willmar Schwabe Pharmaceuticals (Karlsruhe, Germany). All other reagents (pentobarbital, agar solution) were of the highest purity and were supplied by Sigma Chemical Co. (Deisenhofen, Germany).

Procedure and experimental protocol

For this study an improved preparation previously described by Selye *et al*. and a modified method of Clark *et al*. and Crome *et al*. was used (30-32). The Lambeth Conventions were used as the guidelines for this research (33, 34). The complete experimental procedure was performed according to procedures described elsewhere (35, 36).

In brief, the rats were anesthetized with pentobarbital (60 mg/kg, intraperitoneally, pentobarbital sodium salt, Sigma-Aldrich, Deisenhofen, Germany). In the external jugular vein, a PE 50 catheter was placed for lethal anesthesia (always pentobarbital) at the end of the experiment. Rectal temperature was maintained at approximately 38°C. The trachea was cannulated to allow artificial ventilation with room air (Rodent VENTILATOR-UB 7025, stroke volume 0.8 ml/100 g body weight and rate 54 strokes/min, Hugo Sachs Elektronik, March-Hugstetten, Germany). The chest was opened by a left thoracotomy. After opening the pericardium, the heart was not exteriorized and a sling (PROLENE 6/0, EH 7245H, Ethicon GmbH, Germany) was placed around the left anterior descending coronary artery (LAD) close to its origin.

During the stabilization period (15 min) any rat with dysrhythmias or a sustained drop in mean arterial blood pressure (MABP) below 70 mmHg caused by the procedure was discarded from the study as prescribed by the Lambeth Convention and others. The ligature was passed through a piece of plastic tubing (2 cm long, 2 mm od, 1.2 mm i.d.), and the LAD was occluded for 7 min by applying tension to the ligature while pressing the distal part of the plastic tube onto the surface of the heart. Tension was maintained by clamping the tube and by ligature. Successful occlusion and ischemia was confirmed by a pronounced decrease in arterial pressure and ECG alteration (*e.g.* ST-elevation). Reperfusion (15 min) was initiated by removing the clamp and releasing the tension on the ligature. Reflow was confirmed by significant ECG changes (*e.g.* reversal of ST segment elevation) immediately upon release of the ligature.

Immediately after the experiment, 0.2 ml of blood was collected directly from the left ventricle and dissolved in saline (1/1 v/v) to analyze CK concentration (CK Reagent-test, Gilford, Ciba-Cornig, USA) and protein contents in the samples to estimate heart muscle damage (37). Systolic and diastolic blood pressures (BPs, BPd) were measured from the left carotid artery using an ISOTEC transducer and were recorded (Watanabe-Graphtec Thermo recorder, HSE, March-Hugstetten, Germany). ECG was picked up from the limb leads (4 needle electrodes) and recorded on an ECG thermo recorder (E-30, Farum, Poznan, Poland; 50 or 100 mm/s) throughout the experiment. In addition, the following parameters were calculated: mean arterial blood pressure (MABP; BPd + 0.42 × ΔBP), heart rate (HR) calculated from ECG, and pressure rate product (PRP; BPs × HR/1000) as an indirect index of myocardial oxygen consumption according to Baller *et al.* (38). The duration of spontaneously reversible ventricular fibrillation (VF) or ventricular tachycardia (VT) that occurred during the 15 min of reperfusion were measured (using the continuous ECG recordings), and the percentage of VF or VT appearance in the groups as well as the mortality index (MI) were calculated.

Seven minutes of ischemia induced by LAD occlusion followed by 15 min of reperfusion is a simple, reproducible and effective method to induce a large number of severe arrhythmias

resulting in a high rate of mortality in reperfusion. This allows for the effective examination of the antiarrhythmic potential of tested agents (31, 32). Additionally, the short period of 7 min of ischemia followed by reperfusion mimics the series of events that lead to arrhythmias seen in clinical scenarios such as the sudden resolution of coronary spasm or revascularization or thrombolytic procedures. Arrhythmias of such etiologies are an important cause of the sudden death that sometimes occurs after these events. In our study animal mortality was caused by continuous, alternating VF and VT episodes during, at least, the first two minutes of reperfusion linked with a drastic drop in blood pressure (BP). Because of the short time of occlusion, the arrhythmias occurring in this period were negligible as might be seen from the characteristic blood pressure tracings and were, therefore, not taken into consideration (31, 32).

Rats were randomly assigned to the following experimental groups. For the dose-effect study, different doses of WS-1442 (25, 50, 100, or 100 + 50, 100 + 100 mg/kg) or the vehicle were orally administered 1 or 24 h and 1 hour (henceforth mentioned as 24 + 1 hour) before LAD occlusion.

For the time-effect study, 100 mg/kg of WS-1442 was given 4 or 8 hours before LAD occlusion. The respective control groups received 0.2% agar solution (10 ml/kg orally). Each experimental group consisted of 16 rats.

Statistical analysis

The chi-square-test ($\chi 2$) was used to estimate the significance between the incidence of ventricular arrhythmias and mortality in all comparisons. For other comparisons, non-parametric Kruskal-Wallis test was used. In all cases differences were considered significant if $P < 0.05$.

RESULTS

Significant dose- and time-dependent effects of administration of WS-1442 were observed in the model of early reperfusion-induced arrhythmias in rats *in vivo* (*Figs*. *1-4* and *Tables 1*, *2*).

The mortality rate was significantly lower in experimental groups where WS-1442 was administered once in dose 100 mg/kg *per os* (p.o). as well as in twice scheme (100 mg/kg + 50 mg/kg, 100 mg/kg + 100 mg/kg; $P < 0.05$) in comparison to control group and 25 mg/kg group. The dose-dependent cardioprotective effect was also observed regarding severe ventricular arrhythmias. The VF occurrence and duration were significantly lower in groups treated with higher doses of WS-1442 (50 mg/kg, 100 mg/kg, 100 + 50 mg/kg 100 + 100 mg/kg; $P < 0.05$) compared to control group and the group treated with the lowest dose of extract (25 mg/kg). Similarly, the dose-dependent cardioprotective effects were observed in shortening the VT duration and lowering CK activity (*Table 1*). There were no significant differences in hemodynamic parameters, HR or PRP between groups (*Figs*. *1* and *2*).

The dose of 100 mg/kg p.o. was chosen for time-dependent study based on the outcomes coming from dose-dependency observations (*Table 2*). The VT duration was significantly lower ($P < 0.05$) in the group when WS-1442 was administered 4 hours before the procedure in comparison to control as well as the second experimental group (WS 1442 administered 8h before the procedure). No significant influence was noticed on mortality or CK. We have observed higher PRP in experimental groups in comparison to control in the phase of stabilization; however, no differences were seen in the occlusion and reperfusion period. There were no significant differences in hemodynamic parameters or HR between groups (*Figs*. *3* and *4*).

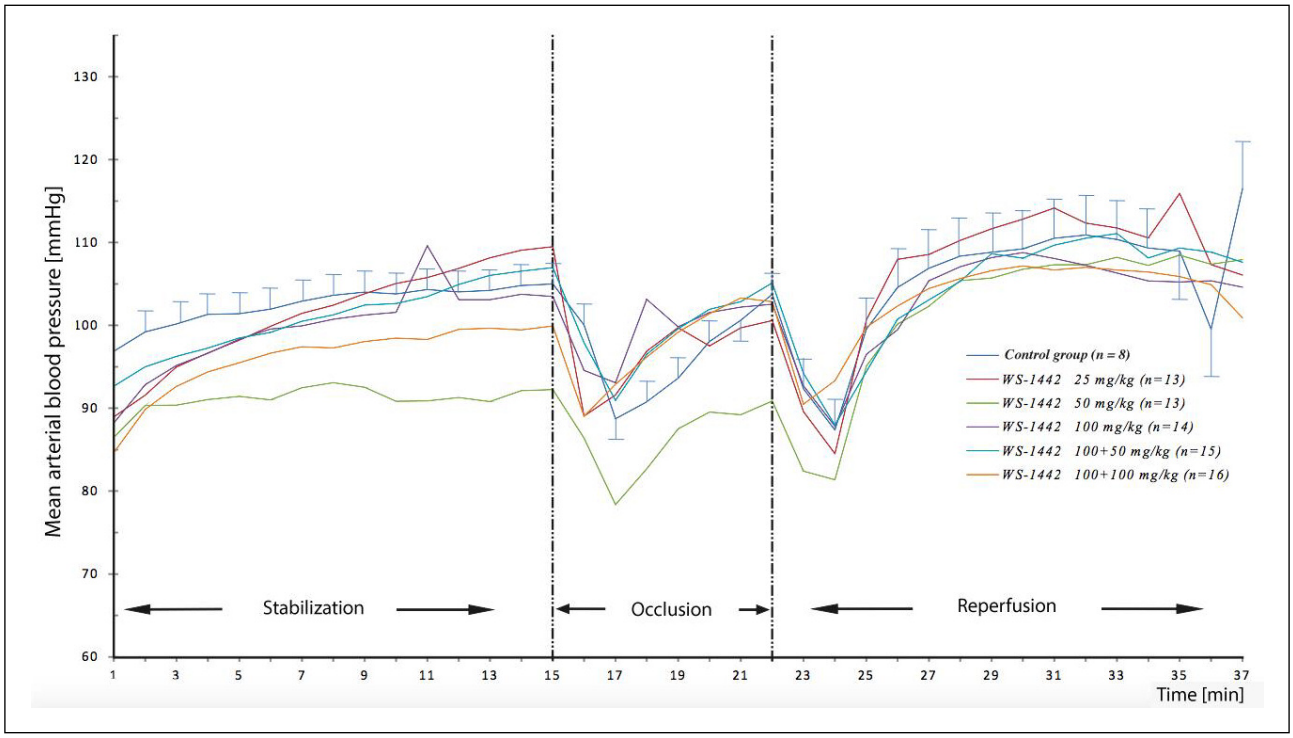

Fig. *1*. Dose-dependent effects of oral administration of WS-1442 extract on the mean arterial blood pressure in a model of reperfusion-induced arrhythmias in rats *in vivo*. Traces of the mean values of surviving animals from each group are shown, and points marked with $*P < 0.05$ are significantly different from control group (non-parametric Kruskal-Wallis test). For the sake of simplicity, SEM values are depicted by the vertical lines in the entire trace of control but in the traces of the treated groups only when values achieved significance.

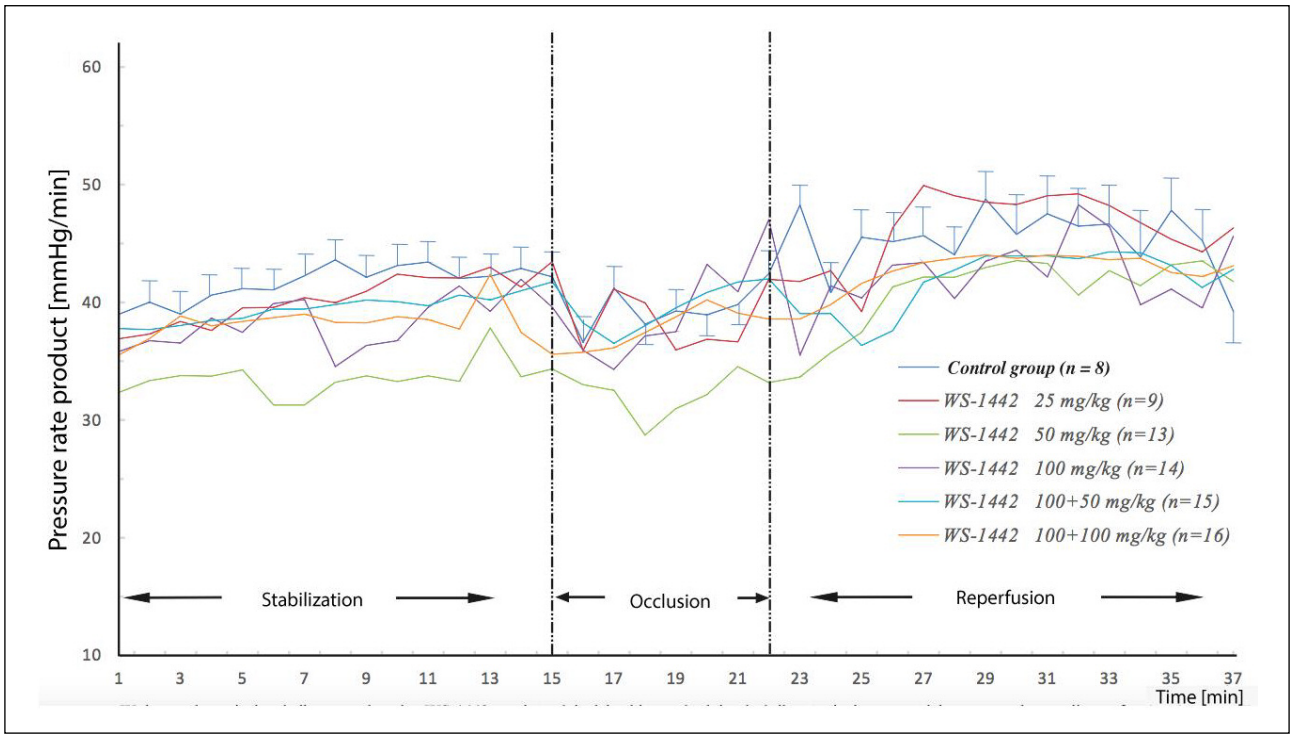

Fig. 2. Dose-dependent effects of oral administration of WS-1442 extract on pressure rate product in a model of reperfusion-induced arrhythmias in rats *in vivo.* Traces of the mean values of surviving animals from each group are shown, and points marked with *P < 0.05 are significantly different from the control group (non-parametric Kruskal-Wallis test). For the sake of simplicity, SEM values are depicted by the vertical lines in the entire trace of control but in the traces of the treated groups only when values achieved significance.

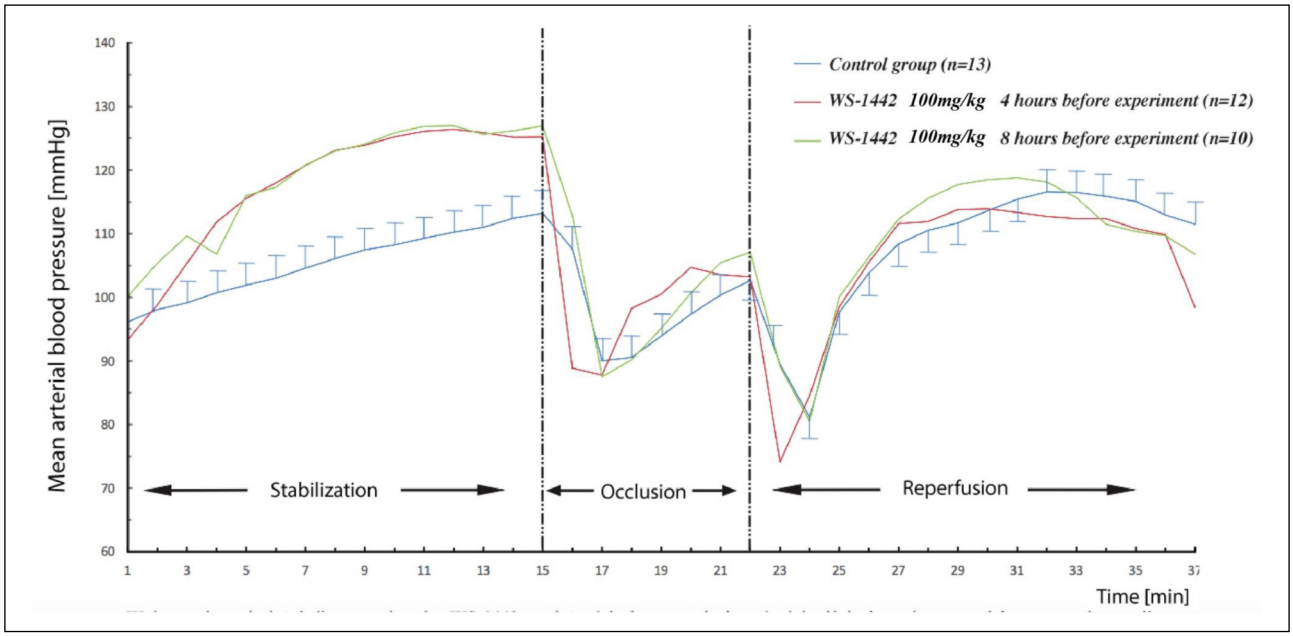

Fig. 3. Time-dependent effects of oral administration of WS-1442 extract on mean arterial blood pressure in the model of early reperfusion-induced arrhythmias in rats *in vivo*. Traces of the mean values of surviving animals from each group are shown, and points marked with *P < 0.05 are significantly different from the control group (non-parametric Kruskal-Wallis test). For the sake of simplicity, SEM values are depicted by the vertical lines in the entire trace of control but in the traces of the treated groups only when values achieved significance.

DISCUSSION

Our results for the first time show significant, dose- and time-dependent cardioprotective action of WS-1442 hawthorn extract in the model of early reperfusion-induced arrhythmias in rats *in vivo*. The most pronounced and strongest cardioprotective effect was seen in groups in which WS-1442 was administered in higher doses (100 + 50 mg/kg, 100 + 100 mg/kg) and shortly before ischemia (100 mg/kg 4 hours before procedure). This beneficial effect was manifested in diminishing mortality,

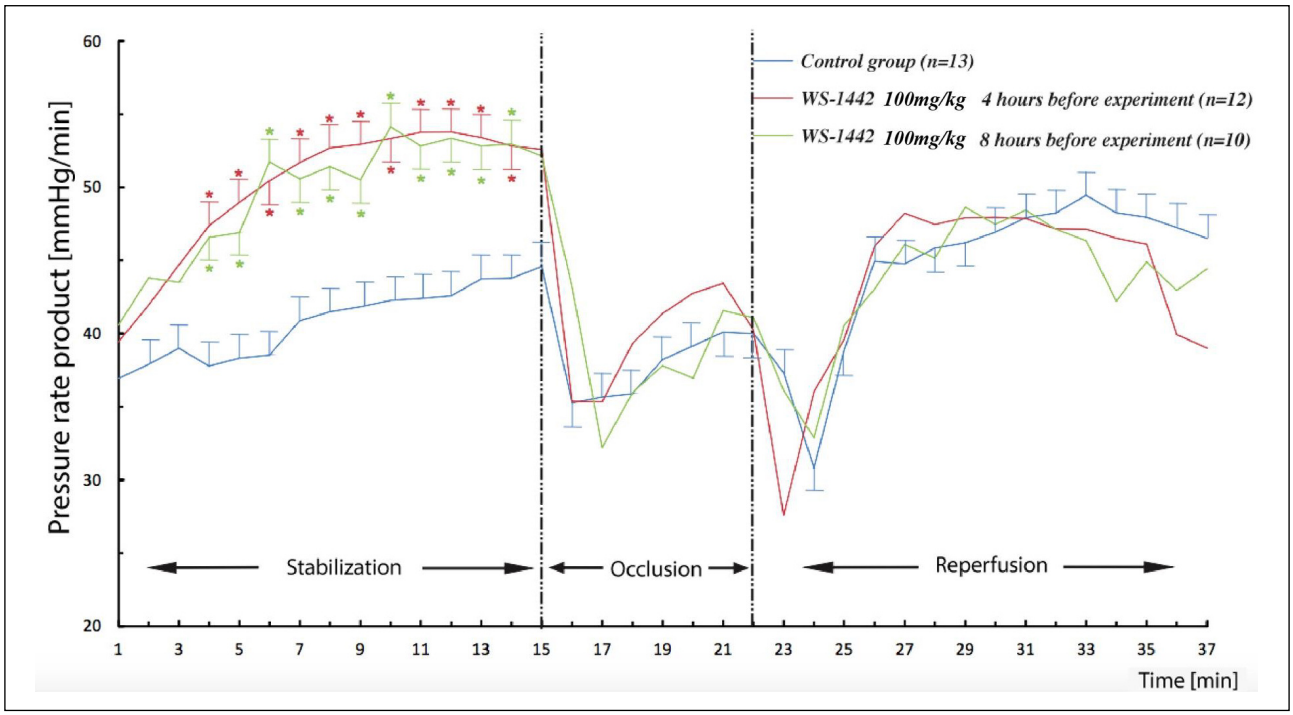

Fig. 4. Time-dependent effects of oral administration of WS-1442 extract on pressure rate product in the model of early reperfusion-induced arrhythmias in rats *in vivo*. Traces of the mean values of surviving animals from each group are shown, and points marked with *P < 0.05 are significantly different from the control group (non-parametric Kruskal-Wallis test). For the sake of simplicity, SEM values are depicted by the vertical lines in the entire trace of control but in the traces of the treated groups only when values achieved significance.

Table 1. Dose-dependent effects of per os administration of WS-1442 extract on mortality index, ventricular rhythm disturbances and creatine kinase activity in the model of early reperfusion-induced arrhythmias in rats *in vivo.*

Experimental group	**Number of animals**	**Mortality index** (%)	**Ventricular fibrillation** (VF)		**Ventricular tachycardia** (VT)		**Creatine kinase activity** (U/g Prot)
			Occurence (%)	Duration (s)	Occurence (%)	Duration (s)	
Control (0.2% agar solution, 10 ml/kg)	n = 16	50 (8/16)	100 (8/8)	32.7 ± 5.47	100 (8/8)	69 ± 6.44	20.01 ± 0.85 (n = 8)
WS 1442 (25 mg/kg)	n = 16	43.75 (7/16)	88.89 (8/9)	15.25 ± 1.85	100 (9/9)	49.87 ± 5.97	20.92 ± 0.57 (n = 9)
WS 1442 (50 mg/kg)	n = 16	18.75 (3/16)	30.77*† (4/13)	4.4 ± 1.52*	100 (13/13)	30.83 ± 7.22	15.06 ± 1.19 (n = 13)
WS 1442 (100 mg/kg)	n = 16	12.5*† (2/16)	7.14*† (1/14)	2.4	100 (14/14)	24.17 ± 6.9*	12.19 ± 0.69*† (n = 14)
WS 1442 (100 + 50 mg/kg)	n = 16	6.25*† (1/16)	0*†♦	0	93.3 (14/15)	18.2 ± 3.39*	11.89 ± 0.68*† (n = 15)
WS 1442 (100 + 100 mg/kg)	n = 16	0*†	0*†♦	0	87.5 (14/16)	13.94 ± 3.31*†	12.24 ± 0.73*† (n = 16)

Data in parentheses are presented as the number of rats with MI, VT or VF/total number of rats in each group. Durations of VF and VT are the mean values (± SEM) of animals with these episodes only. Creatine kinase (CK) concentration was estimated in animals that survived until the 37th minute of the experiment. The chi-square-test ($\chi 2$) was used to estimate the significance between mortality and the incidence of arrhythmias. The differences in VF or VT duration, as well as CK concentration, were calculated using non-parametric Kruskal-Wallis test. Values marked with * were significantly different against the control group (P < 0.05). Values marked with † were significantly different against the WS 1442 = 25 mg/kg group (P < 0.05). Values marked with ¦ were significantly different against WS 1442 = 50 mg/kg group (P < 0.05).

significant reduction of occurrence and duration of ventricular arrhythmias and CK levels. It is worth emphasizing that this positive effect was not accompanied by any considerable influence on hemodynamic parameters or the heart rate. The PRP increase observed in the stabilization period is complementary to previous observations on positive-inotropic

Table 2. Time-dependent effects of *per os* administration of WS-1442 extract on mortality index, ventricular rhythm disturbances and creatine kinase activity in the model of early reperfusion-induced arrhythmias in rats *in vivo*.

Experimental group	**Number of animals**	**Mortality index** (%)	**Ventricular fibrillation** (VF)		**Ventricular tachycardia** (VT)		**Creatine kinase activity** (U/g Prot)
			Occurence (%)	Duration (s)	Occurence (%)	Duration (s)	
Control (0.2% agar solution, 10 ml/kg)	n = 24	45.84 (11/24)	100 (13/13)	35.3 ± 5.47	100 (13/13)	63.9 ± 5.97	20.2 ± 0.85 (n = 13)
WS 1442 (100 mg/kg, 4 h before experiment)	n = 16	25 (4/16)	33.34*† (4/12)	11.6 ± 0.1*	100 (12/12)	39.2 ± 5.97	19.5 ± 0.57 (n = 12)
WS 1442 (100 mg/kg, 8 h before experiment)	n = 16	37.5 (6/16)	80 (8/10)	17.8 ± 0.2	100 (10/10)	49.7 ± 7.22	18.3 ± 1.19 (n = 10)

Data in parentheses are presented as the number of rats with MI, VT or VF/total number of rats in each group. Durations of VF and VT are the mean values (± SEM) of animals with these episodes only. Creatine kinase (CK) concentration was estimated in animals that survived until the 37th minute of the experiment. The chi-square-test ($\chi 2$) was used to estimate the significance between mortality and the incidence of arrhythmias. The differences in VF or VT duration, as well as CK concentration, were calculated using non-parametric Kruskal-Wallis test. Values marked with * were significantly different against the control group ($P < 0.05$). Values marked with † were significantly different against WS-1442-administered 100 mg/kg 8 hours before experiment ($P < 0.05$).

action of hawthorn extracts (14). On the other hand, this effect was not seen in the occlusion and reperfusion period. This suggests the stabilizing effect of WS-1442 on hemodynamic parameters and the direct cardioprotective effect that reflects in the diminishing of CK activity.

Until now, there has only been a limited number of studies concerning the possible cardioprotective action of hawthorn extracts in reperfusion-induced injury. In an *in vitro* study from 1999, Al Makdeesi *et al*. analyzed the influence of prolonged, 3-months administration of LI 132 extract on the occurrence of reperfusion-induced arrhythmias (39). LI 132 is a standardized extract received from the dried leaves and flowers of *Crataegus oxycantha* containing 2.2% flavonoids. In this observation, the authors reported a diminished occurrence of ventricular arrhythmias induced by ischemia-reperfusion of the isolated heart. However, the mentioned study did not take into consideration the exact dosing of LI 132 that was added to feed received by animals *ad libitum*. Veveris *et al*. performed the first comparison between the effects of different doses of standardized WS-1442 extract on arrhythmias and hemodynamics in a model of prolonged heart ischemia in Wistar rats *in vivo* (40). The extract was administered p.o. for 7 days in doses of 10 mg/kg and 100 mg/kg; after that period, the authors performed a procedure of prolonged ischemia by ligation of LAD (240 minutes) and reperfusion (15 minutes). The authors noticed significant differences between the investigated groups and the control group (mortality rate, ECG changes, area of ischemic lesions in the heart) and suggested anti-ischemic and anti-necrotic, dose-dependent action of WS-1442. In contrast to our study, they reported no significant antiarrhythmic effect of WS-1442.

Based on data coming from previous experimental and preclinical studies, there are several hypotheses on the mechanistic insight of the antiarrhythmic effect of hawthorn extracts. One of them suggests that this effect is similar to class III (Vaughan Williams) drugs (*e.g*. amiodarone, sotalol, vernakalant, dronedarone) and is due to blocking repolarizing potassium currents (41). Another hypothesis interrelates antiarrhythmic action with the content of saponins and flavonoids in hawthorn extracts (42). Standardized extract WS-1442 is characterized by high (18.75%) content of oligomeric procyanidines. This could explain the significant decrease in the occurrence and duration of ventricular arrhythmias observed in our study.

Our study revealed clearly the dose-dependent effect of WS-1442 on the diminishing activity of serum CK in the model of reperfusion-induced arrhythmias in rats *in vivo.* In our model, re-flow of oxygenated blood through previously ischemic heart tissue plays a pivotal role in myocardial damage.

There are several mechanisms driving heart injury in the model of early reperfusion-induced arrhythmias in rats *in vivo*. Among them, the most important are free oxygen species, decrease of the activity of anti-oxidative factors, increase of the activity of neutrophils, edema of cardiomyocytes and local blood extravasation.

The most important postulated mechanisms of the cardioprotective action of hawthorn extracts are: anti-inflammatory action, anti-oxidation, endothelial protection and anti-platelet action. Inflammation plays a crucial role in the etiopathogenesis of cardiac injury during ischemia (43). It was proven that during ischemia there is an increase in the activity of pro-inflammatory cytokines, enzymes and cells (44). This process launches apoptosis and cardiomyocytes death, which escalates during reperfusion (45). It has been observed that *Crataegus pinnatifida* extract has anti-inflammatory action expressed as a significant decrease of the activity of inflammatory mediators COX-2, TNF-α and interleukins (IL 1β and IL-6) in an experimental model of lipopolysaccharide-stimulated inflammation (46).

Increased oxidative stress is an important factor causing myocardial damage. It has been proven that free-radicals scavenging is beneficial and has a cardioprotective effect (47). In the model of isoproterenol-induced cardiac injury, Jayalakshmi *et al*. have demonstrated the beneficial anti-oxidative effect of pretreatment with *Crataegus oxycantha* extract, which was expressed as the lowering of lipid peroxidation (48). What is more, Bernatoniene *et al*. suggested that mitochondrial protection is the key mechanism of the cardioprotective action of

hawthorn extracts, as energetic metabolism of cardiomyocytes is almost exclusively dependent on mitochondria (49).

There are also reports on positive endothelial effects of WS-1442, by modulation of sodium permeability and protection of endothelial layer surface (ELS) (50). ELS function is crucial for endothelial homeostasis and plays an important role in the migration of leukocytes and inactivation of procoagulant factors.

Platelet activation associated with unstable atherosclerotic plaques is a central event in the pathophysiology of acute myocardial ischemia and remains an important therapeutic goal for cardiovascular pharmacology. Preclinical studies revealed the anti-aggregative action of hawthorn extracts and their influence on lowering of thromboxane A_2 synthesis (51). When it comes to clinical studies, Holubarsch *et al*. in 2008 published a large, randomized, double-blind, placebo-controlled multicenter study, which confirmed that WS-1442 was safe to use in patients receiving optimal medication for heart failure. In addition, the outcomes of this study indicated that WS-1442 can potentially reduce the incidence of sudden cardiac death, at least in patients with less compromised left ventricular function (52).

In conclusion, we herein state that the standardized extract WS-1442 has a beneficial cardioprotective dose- and time-dependent action. It diminishes occurrence and duration of life-threatening ventricular arrhythmias and appears to have a stabilizing influence on hemodynamic parameters during ischemia and reperfusion. There is a need to verify these promising experimental outcomes in clinical studies concerning anti-arrhythmic action in human subjects.

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