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Differential role of endothelium in hawthorn fruit extract-induced relaxation of rat cerebral, coronary, carotid, and aorta

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SUMMARY

The present study was aimed to examine the role of endothelium in the relaxant effect of hawthorn fruit extract of *Crataegus pinnatifida* in four different types of rat arteries, posterior cerebral communicating artery, right descending coronary artery, common carotid artery, and aorta. In 9,11-dideoxy-11α,9α-epoxy-methanoprostaglandin $F_{2\alpha}$ (U46619)-preconstricted arterial rings except for aorta, the extract produced endothelium-independent relaxations with similar potency. This relaxation was unaffected by pretreatment with 100 μM N^G-nitro-L-arginine methyl ester (L-NAME, the nitric oxide synthase inhibitor), 3 μM 1H-[1,2,4]oxadiazolo[4,2-α]quinoxalin-1-one (ODQ, the guanylate cyclase inhibitor), or 10 μM indomethacin (the cyclooxygenase inhibitor). Putative K^+ channel blockers (charybdotoxin plus apamin or glibenclamide) did not affect the extract-induced relaxation in cerebral or coronary artery rings. In contrast, in rat aortic rings the extract produced significantly smaller relaxant response in endothelium-denuded rings than that in endothelium-intact rings. Pretreatment with L-NAME or ODQ abolished the extract-induced endothelium-dependent aortic relaxation, whilst indomethacin (3 μM) had no effect. The present results indicate that hawthorn fruit extract possesses a vasorelaxing effect in cerebral, coronary and carotid arteries and this effect is independent of the presence of a functional endothelium. However, the extract-induced endothelium-dependent relaxation in rat aorta was mediated through endothelial nitric oxide and cyclic GMP-dependent mechanisms, suggesting that active components in the extract may act on endothelium to stimulate release of nitric oxide in large conduit arteries of the rats.

Key words: *Crataegus pinnatifida*; Endothelium; Nitric oxide; Relaxation; Artery; Rat

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INTRODUCTION

Many species of hawthorns in the genus *Crataegus* have a long history in both Oriental and European herbal medicines (Huang, 1999; Holubarsch *et al.*, 2000; Weiss and Fintelmann, 2000). The dried fruits of *Crataegus pinnatifida* are commonly used as a popular snack food in China since it is believed to reduce food stagnancy and to stimulate appetite. As an herbal medicine in China, the dried fruits of *C. pinnatifida* are employed to combat hypertension, angina pectoris, and hypercholesterolemia (Huang, 1999). Hawthorn extract is well recognized in Europe as an antihypertensive remedy, particularly useful in the treatment of mild forms of cardiac

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insufficiency and angina pectoris which are usually associated with decreased coronary blood flow (Weiss and Fintelmann, 2000). Animal studies with the isolated heart have verified that hawthorn extract increased coronary blood flow (Mävers and Hensel, 1974). The cardioprotective effect of hawthorn extract was also reported on the ischemic-reperfused rat heart (Nasa *et al.*, 1993). This protection may be partly associated with the antioxidative activity of hawthorn extract, which inhibits formation of free radicals and subsequent damage to the cardiac tissue (Bahorun *et al.*, 1994; Bahorun *et al.*, 1996).

The medicinally used parts of the hawthorn include the flower, the leave and the fruit. We prepared an ethanol extract from the dried fruits of *C. pinnatifida*, which was collected from Xingling County of Hebei Province, China. We isolated a series of flavonoids from the dried fruits of *C. pinnatifida* and observed that all phenolic compounds except for ursolic acid were protective to human low-density lipoprotein (LDL) from Cu^{2+}-mediated oxidation (Zhang *et al.*, 2001). These results suggest that part of the cardioprotective effect of hawthorn extract may be attributable to the direct protection to human LDL from oxidation or indirect protection through maintaining the concentration of α-tocopherol in human LDL.

We also reported that hawthorn extract produced relaxation in isolated rat mesenteric artery rings and this relaxation was mainly mediated by endothelium-derived vasoactive factors such as nitric oxide (Chen *et al.*, 1998). Crataegus extract contains a mixture of flavonoids and procyanidins. Procyanidins may be an active ingredient responsible for Crataegus extract-induced endothelium/nitric oxide-dependent vasorelaxation (Kim *et al.*, 2000). Although hawthorn extract is well documented to be one of most successful herbal remedies in the treatment of coronary heart disease, the exact mechanisms underlying its vasodilator action remains incompletely understood. There is no report on whether hawthorn fruit could relax smaller vessels and whether the endothelium might play a role in the beneficial effect of hawthorn in the treatment of vascular disease. The present study was therefore aimed to examine the possible vasodilator effect of hawthorn fruit extract in isolated rat arteries of different diameters ranging from resistance-sized vessels to conduit artery, and to examine the role of endothelium in the relaxations of these vessels.

MATERIALS AND METHODS

Extraction of hawthorn fruit

Dried hawthorn fruits (*C. pinnatifida*) were purchased from Xingling County of Hebei Province, China. The method used for extraction was described elsewhere (Chen *et al.*, 1998). Briefly, following removal of the seeds, the fruit flesh was freeze-dried and ground into powder in a coffee grinder. The fruit powder (100 grams) was extracted three times using 80% ethanol at room temperature for 12 hours. The pooled ethanol fraction was filtered and then concentrated to approximately half volume in a vacuum rotary evaporator followed by addition of two volumes of double-distilled water and cooling overnight at 4°C. Following filtration, ethanol was evaporated under vacuum and the remainder was freeze-dried to produce about 26% in total weight of the dried powder. The extract was kept at -20°C.

Artery preparation

After approval was obtained from the Animal Research Ethical Committee of the Chinese University of Hong Kong, adult male Sprague-Dawley rats weighing ~300 g were killed by pure CO_2. The brain or heart was rapidly removed and placed in ice-cold Krebs solution containing (mM): 119 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 25 $NaHCO_3$, 1.2 KH_2PO_4, and 11 d-glucose. The posterior cerebral communicating artery or right descending coronary artery was dissected out, cleaned of the adhesive tissues, and cut into cylindrical segments, ~2-mm in length. The common carotid artery and aorta were also dissected and cut into ring segments ~3-mm in length. Each cerebral or coronary artery ring was mounted in a Multi Myograph System (Danish Myo Technology A/S, Denmark) for recording of changes in vessel tension. Briefly, two tungsten wires (each of 40 μm diameter) were passed through the segments lumen and each wire fixed to the jaws of the myograph. Segments of the common carotid artery

or aorta were suspended horizontally between two stainless steel parallel hooks for isometric force measurement (Grass Instruments, USA) in organ baths filled with Krebs solution. The segments were stretched between the wires under a previously determined optimal tension of 300 mg for cerebral artery, 500 mg for coronary artery, 500 mg for carotid artery and 1500 mg for aorta. Two to three ring-segments were prepared from each artery. The bath solution was continuously bubbled with a mixture of 95% O_2 and 5% CO_2 at 37°C to give rise to a relatively constant pH of 7.2-7.4.

In some artery rings, the endothelium was mechanically removed by rubbing the luminal surface of the ring several times with a small stainless steel wire. Removal of a functional endothelium was confirmed by the lack of a relaxant response to 1 μM acetylcholine at the start of each experiment.

Force measurement

Thirty minutes after being set up in the organ baths, each ring was first constricted by 1 μM phenylephrine to test its contractility and subsequently relaxed by 1 μM acetylcholine to verify the integrity of endothelium. The ring was then rinsed several times until baseline tone was restored. A steady contraction of the arterial ring with endothelium in response to 10-30 nM 9,11-dideoxy-11α,9α-epoxy-methanoprostaglandin $F_{2\alpha}$ (U46619) was induced, and the hawthorn fruit extract was added cumulatively to evoke concentration-dependent relaxations. In experiments that examined the role of endothelium-derived vasoactive factors, the rings were exposed for 30 min to various inhibitors (100 μM N^G-nitro-L-arginine methyl ester, 3 μM ODQ, 10 μM indomethacin, 1 μM glibenclamide or 100 nM charybdotoxin plus 100 nM apamin) before they were contracted by U46619 to establish a sustained tone. The extract was then applied cumulatively. The effect of the vehicle was also tested. The effect of hawthorn fruit extract was also examined in endothelium-denuded rings. Since pretreatment with inhibitors of nitric oxide-mediated relaxation or removal of endothelium significantly enhanced the U46619-induced contraction, the concentration of U46619 was lowered to match the initial tone in control vessels.

Drugs

The following drugs were used: phenylephrine hydrochloride, acetylcholine chloride, indomethacin, N^G-nitro-L-arginine methyl ester (L-NAME), charybdotoxin (CTX), apamin, glibenclamide, 9,11-dideoxy-11α,9-αepoxy-methanoprostaglandin $F_{2\alpha}$ (U46619) (Sigma, St. Loius., MO, USA), and 1H-[1,2,4]oxadiazolo[4,2-α]quinoxalin-1-one (ODQ) (Tocris Cookson Ltd. UK). Glibenclamide, indomethacin, U46619 and ODQ were dissolved in dimethyl sulfoxide (DMSO). 0.2% DMSO did not affect U46619-induced tension. Other chemicals were dissolved in distilled water.

Data analysis

The results were expressed as percentage of the initial tone induced by U46619 and IC_{50} values were calculated as the drug concentration that produced half of the maximum relaxation (E_{max} %). Data were mean ± SEM of experiments from n animals. Difference between groups was assessed using Students two-tailed *t*-tests and analysis of variance. $P < 0.05$ was selected as the criterion for the statistical significance.

RESULTS

Relaxation in posterior communicating arteries

In U46619-contracted endothelium-intact cerebral artery rings, the hawthorn fruit extract induced concentration-dependent relaxant with an IC_{50} of 772 ± 84 μg/ml (n=7). The relaxation response to the extract was unchanged upon removal of endothelium (IC_{50}: 634 ± 61 μg/ml, n=7, $P > 0.05$, Fig. 1a). Pretreatment with 100 μM L-NAME or 3 μM ODQ did not alter the relaxation induced by the extract (IC_{50}: 824 ± 141 μg/ml, n=5 in L-NAME and 781 ± 82 μg/ml, n=5 in ODQ, $P > 0.05$, Fig. 1b). Neither CTX plus apamin, each at 100 nM, nor indomethacin at 10 μM influenced the extract-induced relaxation (IC_{50}: 872 ± 64 μg/ml, n=5 in CTX plus apamin and 664 ± 63 μg/ml, n=6 in indomethacin, $P > 0.05$, Fig. 1c).

Relaxation in coronary arteries

In U46619-contracted endothelium-intact coronary artery rings, the hawthorn fruit extract induced relaxations with an IC_{50} of 962 ± 110 μg/ml (n=7).

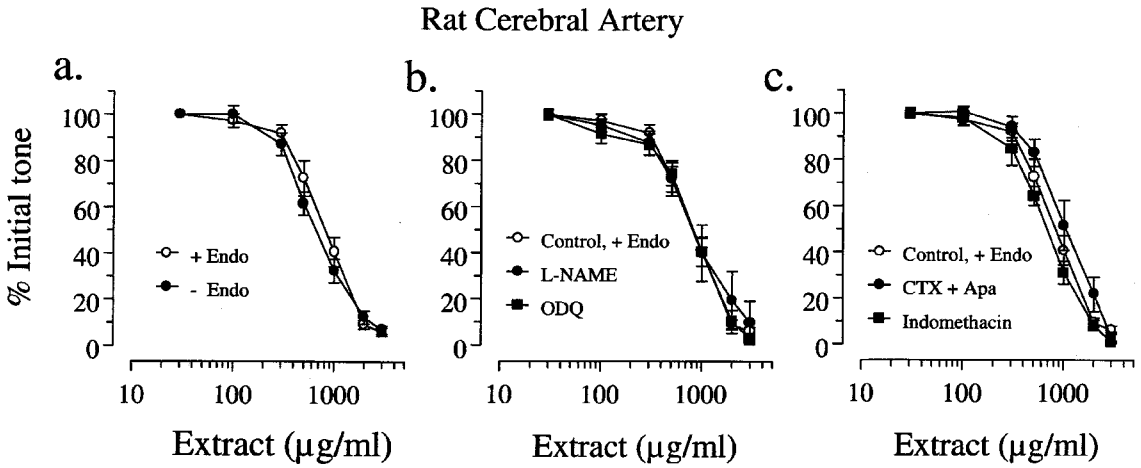

Fig. 1. Relaxant effect of hawthorn fruit extract on U46619-contracted posterior cerebral communicating artery rings in (a) control with endothelium (○) and in endothelium-denuded rings (●); (b) in the presence of 100 μM L-NAME (●) or 3 μM ODQ (■); (c) in the presence of 100 nM CTX plus 100 nM apamin (●) or 10 μM indomethacin (■). Data are mean ± SEM of 5-7 experiments.

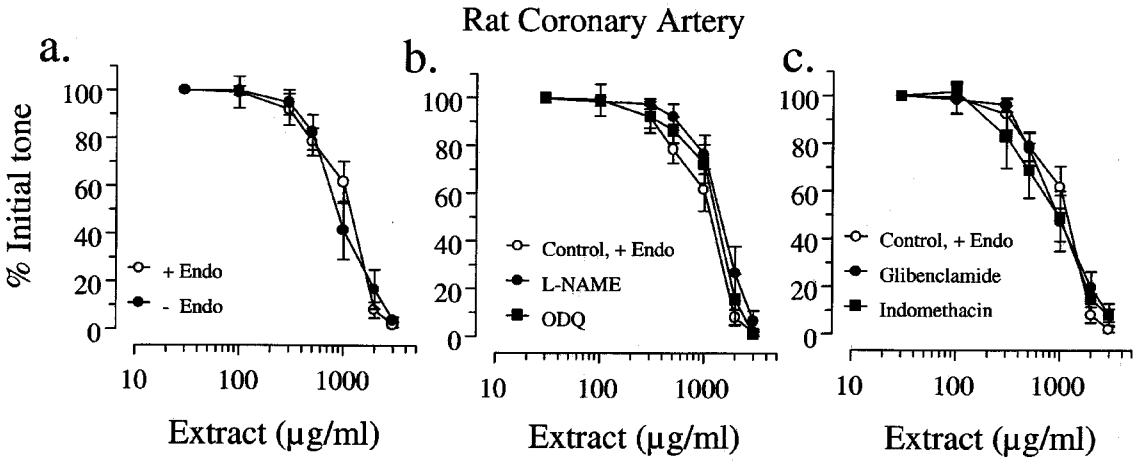

Fig. 2. Relaxant effect of hawthorn fruit extract on U46619-contracted right descending coronary artery rings in (a) control with endothelium (○) and in endothelium-denuded rings (●); (b) in the presence of 100 μM L-NAME (●) or 3 μM ODQ (■); (c) in the presence of 1 μM glibenclamide (●) or 10 μM indomethacin (■). Data are mean ± SEM of 4-7 experiments.

The relaxant response to the extract was unchanged upon removal of endothelium (IC_{50}: 993 ± 21 μg/ml, n=7, $P > 0.05$, Fig. 2a). Pretreatment with 100 μM L-NAME or 3 μM ODQ did not alter this relaxation (IC_{50}: 1392 ± 194 μg/ml, n=5 in L-NAME and 1292 ± 210 μg/ml, n=5 in ODQ, $P > 0.05$, Fig. 2b). Neither 1 μM glibenclamide nor 10 μM indomethacin affected the extract-induced relaxation (IC_{50}: 824 ± 130 μg/ml, n=4 in glibenclamide and 915 ± 152 μg/ml, n=5 in indomethacin, $P > 0.05$, Fig. 2c).

Relaxation in common carotid arteries

In U46619-contracted endothelium-intact carotid rings, the hawthorn fruit extract induced relaxations with an IC_{50} of 764 ± 111 μg/ml. Removal of endothelium did not affect the relaxation (IC_{50}: 713 ± 102 μg/ml, $P > 0.05$, n=8, Fig. 3a). The relaxation was unaffected by pretreatment with 100 μM L-NAME (IC_{50}: 824 ± 52 μg/ml, $P > 0.05$, n=6), 3 μM ODQ (IC_{50}: 693 ± 104 μg/ml, $P > 0.05$, n=6), or 10 μM indomethacin (IC_{50}: 644 ± 63 μg/ml, n=6, $P > 0.05$, Fig. 3b).

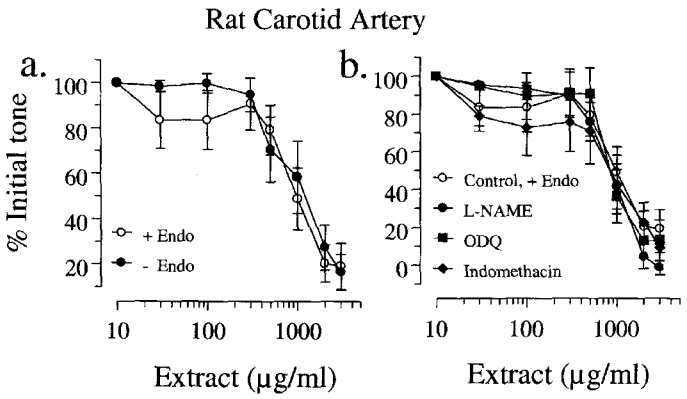

Fig. 3. Relaxant effect of hawthorn fruit extract on U46619-contracted common carotid artery rings in (a) control with endothelium (○) and in endothelium-denuded rings (●); (b) in the presence of 100 μM L-NAME (●), 3 μM ODQ (■) or 10 μM indomethacin (◆). Data are mean ± SEM of 6-8 experiments.

Relaxation in aorta

The hawthorn fruit extract produced greater reduction of U46619-induced contraction in the endothelium-intact than in endothelium-denuded aortic rings. The IC_{50} values were 661 ± 44 μg/ml (n=6) and 1133 ± 62 μg/ml (n=5) in the presence and absence of endothelium, respectively ($P < 0.05$, Fig. 4a). Pretreatment of endothelium-intact rings with 100 μM L-NAME or 3 μM ODQ significantly attenuated the relaxing potency of the extract (IC_{50}: 1201 ± 43 μg/ml, n=6 in L-NAME and 1243 ± 64 μg/ml, n=6 in ODQ, $P< 0.05$, Fig. 4b). In contrast, 10 μM indomethacin was without an effect (IC_{50}: 574 ± 44 μg/ml, $P > 0.05$, n=5, Fig. 4b). The maximal relaxation induced by the extract was unaffected by L-NAME or ODQ (Fig. 4b).

DISCUSSION

The results of our investigation show that the extract from hawthorn fruit (*C. pinnatifida*) concentration-dependently relaxed the four types of rat isolated arterial rings: posterior cerebral communicating artery, right descending coronary artery, common carotid artery and aorta, with similar potency. In response to hormonal stimuli which trigger Ca^{2+} influx, the endothelial cells in intact arteries regulate the contractility of the underlying arterial smooth muscle cells by releasing vasoactive factors, which produce relaxation via different mechanisms. Nitric oxide, prostacyclin, and endothelium-derived hyperpolarizing factors are the best-known relaxing agents produced in

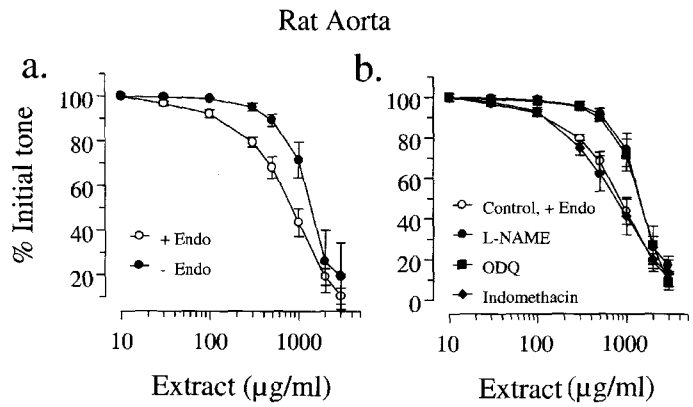

Fig. 4. Relaxant effect of hawthorn fruit extract on U46619-contracted aortic rings in (a) control with endothelium (○) and in endothelium-denuded rings (●); (b) in the presence of 100 μM L-NAME (●), 3 μM ODQ (■). Data are mean ± SEM of 5-6 experiments.

the endothelium. In order to elucidate the possible involvement of endothelium in the extract-induced vasorelaxation, inhibitors of endothelium-derived relaxing factors were used in the present study. The extract-induced relaxation was the same in both endothelium-intact and denuded rings prepared from cerebral, coronary and carotid arteries. L-NAME (an inhibitor of nitric oxide synthase), ODQ (an inhibitor of nitric oxide-activated soluble guanylate cyclase), or indomethacin (an inhibitor of cyclooxygenase) did not affect the extract-induced relaxant response, thus discounting a role of endothelial nitric oxide or prostacyclin. In contrast, the extract produced both endothelium-dependent and -independent relaxation in rat aortic rings. Removal of a functional endothelium partially, but significantly, blunted the extract-induced aortic relaxation without affecting the maximal relaxation. This indicates the involvement of the endothelium-derived factors. Pretreatment of endothelium-intact rings with L-NAME or ODQ reduced the extract-induced relaxation to the same degree as observed in endothelium-denuded rings. However, exposure to indomethacin, an inhibitor of endothelial prostacyclin biosynthesis, had no effect. These results point to a primary role of nitric oxide but not the relaxing prostanoids in the endothelium-dependent aortic relaxation induced by hawthorn extract. These data suggest that the bioactive component(s) of hawthorn fruit extract may act on the aortic endothelium to release nitric oxide that subsequently elevates cyclic GMP levels in the adjacent smooth muscle and that cyclic GMP-dependent mechanism is likely responsible for an endothelium-dependent relaxation. The present results with aortic rings are consistent with our previously reported endothelium/nitric oxide-dependent effect of the extract in rat isolated mesenteric arteries. In mesenteric arteries, the presence of endothelium contributed over 75% towards the extract-induced maximal relaxation (Chen *et al.*, 1998). It is currently unknown why the extract could still produce a full relaxation of rat aorta in the absence of endothelium. Kim et al. have recently described the similar but more potent effect of *Crataegus* extract and procyanidins, a bioactive component extracted from hawthorn on rat aorta (Kim *et al.*, 2000). They found that *Crataegus* extract from *C. oxyacantha*, L. and *C. monogyna* Jacq., induced endothelium-dependent aortic relaxation (Kim *et al.*, 2000). However, we were only able to demonstrate a partial endothelium-dependent response to the hawthorn extract from *C. pinnatifida*, another *Crataegus* species widely available in China. This discrepancy may be due to differences between two species in the relative composition of active ingredients such as procyanidins, which may be responsible for the endothelial nitric oxide-mediated relaxation.

It is known that the endothelial nitric oxide is the major mediator of the endothelium-dependent relaxation of large conduit arteries such as aorta, while endothelium-derived hyperpolarizing factors or relaxing prostanoids play a more important role in endothelium-induced relaxation of smaller resistance-sized vessels. Combined treatment with CTX and apamin, inhibitors of large- and small-conductance Ca^{2+}-activated K^+ channels used to block K^+ efflux through endothelial Ca^{2+}-activated K^+ channels (Edwards *et al.*, 1998) did not affect the extract-induced relaxation in rat cerebral arteries, suggesting that CTX/apamin-sensitive endothelium-derived hyperpolarizing factors are not involved. Similarly, glibenclamide, an inhibitor of arterial ATP-sensitive K^+ channel (Standen *et al.*, 1989), failed to alter the extract-induced relaxation in the rat coronary artery, indicating that this channel plays no role. ATP-sensitive K^+ channel is importantly involved in the regulation of coronary blood flow (Daut *et al.*, 1994).

In summary, the present results show a differential role of endothelial nitric oxide in hawthorn fruit extract-induced relaxation of four different rat arteries. In cerebral, coronary and carotid arteries, the endothelium does not seem to participate in the extract-induced relaxation. In contrast, endothelial nitric oxide and cyclic GMP-dependent pathway mediate the extract-induced endothelium-dependent relaxation in rat aorta. Lack of effect of indomethacin indicates that endothelial relaxing prostacyclin is not involved. Together with the antioxidant and hypolipidemic activities of hawthorn fruit previously reported by us (Zhang *et al.*, 2001; Zhang *et al.*, 2002), the relaxing effect on the carotid and cerebral arteries suggests a potential preventative effect of hawthorn

fruit against cerebral circulation-related disease such as cerebral vasospasm and stroke. Further investigation is warranted to identify bioactive components of hawthorn fruit extract and to elucidate the possible mechanisms underlying the endothelium-independent relaxation in resistance-sized blood vessels.

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REFERNCES

Bahorun T, Gressier B, Trotin F, Brunet C, Dine T, Luyckx M, Vasseur J, Cazin M, Cazin JC, Pinkas M. (1996) Oxygen species scavenging activity of phenolic extracts from hawthorn fresh plant organs and pharmaceutical preparations. *Arzneimittelforschung* **46**, 1086-1089.

Bahorun T, Trotin F, Pommery J, Vasseur J, Pinkas M. (1994) Antioxidant activities of Crataegus monogyna extracts. *Planta Med.* **60**, 323-328.

Chen ZY, Zhang ZS, Kwan KY, Zhu M, Ho WKK, Huang Y. (1998) Endothelium-dependent relaxation induced by hawthorn extract in rat mesenteric artery. *Life Sci.* **63**, 1983-1991.

Daut J, Klieber HG, Cyrys S, Noack T. (1994) K_{ATP} channels and basal coronary vascular tone. *Cardiovasc. Res.* **28**, 811-817.

Edwards G, Dora KA, Gardener MJ, Garland CJ, Weston AH. (1998) K^+ is an endothelium-derived hyperpolarizing factor in rat arteries. *Nature* **396**, 269-272.

Holubarsch CJ, Colucci WS, Meinertz T, Gaus W, Tendera M. (2000) Survival and prognosis: investigation of Crataegus extract WS 1442 in congestive heart failure (SPICE)–rationale, study design and study protocol. *Eur. J. Heart Fail.* **2**, 431-437.

Huang KC. (1999) Anti-hypercholesterolemic herbs. In: *The Pharmacology of Chinese Herbs*, 2nd edn, p. 117-20, CRC Press LLC, Florida.

Kim SH, Kang KW, Kim KW, Kim ND. (2000) Procyanidins in crataegus extract evoke endothelium-dependent vasorelaxation in rat aorta. *Life Sci.* **67**, 121-131.

Mävers WH, Hensel H. (1974) Changes in local myocardial blood circulation following oral administration of a Crataegus extract in non-narcotized dogs. *Arzneimittelforschung* **24**, 783-785.

Nasa Y, Hashizume H, Hoque AN, Abiko Y. (1993) Protective effect of crataegus extract on the cardiac mechanical dysfunction in isolated perfused working rat heart. *Arzneimittelforschung* **43**, 945-949.

Standen NB, Quayle JM, Davies NW, Brayden JE, Huang Y, Nelson MT. (1989) Hyperpolarizing vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle. *Science* **245**, 177-180.

Weiss RF, Fintelmann V. (2000) *Herbal Medicine*, Georg Thieme Verlag, New York.

Zhang ZS, Chang Q, Zhu M, Huang Y, Ho WKK, Chen ZY. (2001) Characterization of antioxidants present in hawthorn fruits. *J. Nutr. Biochem.* **12**, 144-152.

Zhang ZS, Ho WKK, Huang Y, James AE, Lam LW, Chen ZY. (2002) Hawthorn fruit is hypolipidemic in rabbits fed a high cholesterol diet. *J. Nutr.* **132**, 5-10.