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Full Length Research Paper

Cardio-tonic effect of the aqueous extract of whole plant of *Crataegus aronia* syn: *azarolus* (L) on isolated Rabbit's heart

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The aim of this work is to study the effect of aqueous extract of whole plant of *Crataegus aronia* on the force of contraction and heart rate of isolated rabbit's heart. Six Isolated rabbit's hearts were perfused through aorta in a Langendorff mode. Heart rate and contractility were determined for 5 min in the presence of 8 concentrations of *C. aronia* aqueous extract (1, 2, 5, 10, 20, 40, 100 and 200 mg/ml) or adrenaline (0.05 mM) as control drug. The changes after each treatment were compared with their baseline values. Data were collected with the help of PowerLab data acquisition and analyzed by Labchart Pro 7 software. At all time intervals recorded, there were no significant changes in the force of contraction nor the heart rate (HR) after infusion of low concentrations of the extract (1, 2, 5 and 10 mg/ml). The maximum increase in force of contraction occurred at a dose of 40 mg/ml, while the maximum decrease in HR occurred at a dose of 20 mg/ml (P< 0.01). The highest doses of the extract (100 and 200 mg/ml) caused hardening of the heart, stopping of the perfusion fluid from entering and stopped the beating of the heart. Therefore, the aqueous extract of whole plant of *C. aronia* syn: *azarolus* (L) showed a positive inotropic and negative chronotropic effects on isolated rabbit's heart.

Key words: *Crataegus aronia*, cardiovascular, rabbits.

INTRODUCTION

Cardiac disorders are of serious medical concern, and are increasing throughout the world (Brauwald et al., 2001). Several drugs with therapeutic value in congestive heart failure and those with positive inotropic effect such as cardiac glycosides and phosphodiesterase inhibitors (PDI), have several side effects that limit their use (Packer et al., 1991; Uretsky et al., 1990). Medicinal plants have over the years constituted indispensable tools for research and development of new drugs (Bonati, 1980). Coupled with the fact that there are still many plants whose medicinal values have not been exploited, it is reasonable to describe the plant kingdom as a sleeping giant for potential drug development (Harvey, 2000).

Hawthorn (*Crataegus*) is a plant native to Mediterranean region, North Africa, Europe and Central Asia. The medicinal use of its extracts date back to ancient times and now listed officially as herbal drugs in pharmacopoeias of countries, such as Germany, France, China and England (Chang et al., 2002b). There are more than 200 species of hawthorn worldwide, but only very few were tested and used medicinally to treat cardiovascular diseases, such as *C. oxycantha*, *C. laevigata, C. monogyna*, C. *orientalis* and *C. pinnatifida* (Bahorun et al., 2003). These studies showed that some of these species increase contraction of the heart, dilates coronary and peripheral blood vessels, and improves blood supply to the heart, thereby help in treating heart disease and mitigating symptoms in early stage of heart failure (Rewerski, 1967; Petkov et al., 1981; Wagner and Grevel, 1982; Leuchtgens, 1993; Reutxer, 1994; Rigelsky and Sweet, 2002). *Crataegus aronia* syn: *azarolus* (L), the predominant species which populates the mountains of the Mediterranean basin, has not been subjected to adequate scientific research. Several ethnobotanical and ethnopharmacological surveys on the therapeutic use of indigenous plants in Jordan and the Palestinian area

revealed the use of *C. aronia* in the Arab traditional medicine to treat cardiovascular diseases, as well as cancer, diabetes and sexual weakness (Ali-Shtayeh et al., 2000; Said et al., 2002).

Despite the extensive researches of the different species of hawthorns on cardiovascular system, most of these studies were carried out *in vivo*. At a practical level, the isolated heart, especially from small mammals, provides a highly reproducible preparation which can be studied quickly and in large numbers at relatively low cost (Hearse and Sutherland, 2000). It allows broad spectrum of biochemical, physiological, morphological and pharmacological indices to be measured. These measurements can be made in the absence of the confounding effects of other organs, such as systemic circulation and circulating neuro-hormonal factors. Thus, it reflects the intrinsic responsiveness of the heart independent of the systemic hemodynamic and neuro-hormonal alterations. In addition, there is a deficient supporting evidences from previous researches on the effect of hawthorn on isolated heart, which is consider a limitation for previous researches. Only very few studies using the most popular hawthorn species (*C. oxycantha*) or standardized commercial extracts, were studies on isolated heart (Popping et al., 1995; Almak et al., 2009).

To the best of our knowledge, no single evaluation study of *C. aronia* on cardiovascular system has been reported prior to this report either in an *in vivo* or *in vitro* study. Therefore, the aim of our current study is to examine the effect of *C. aronia* aqueous extract on isolated rabbit's heart by investigating its effect on the force of contraction (FC) and heart rate (HR).

MATERIALS AND METHODS

Preparation of the extract

This study was performed during the month of August 2011, in the Research laboratory of Physiology Department at the Medical School of King Khalid University. Fresh *C. aronia* syn: *azarolus* (L) whole plant (stems, leaves and flowers) was purchased from a local market in Jordan (Middle-east). The plant was identified, dried and extracted in the Department of Pharmacognosy at the College of Pharmacy of King Khalid University, Abha, Saudi Arabia. The dried plant material was ground to a powder and extracted by maceration using distilled water (1 kg/1 L, w/v) for 3 days at 37°C (Abdul et al., 2009). The extract was filtered and evaporated under reduced pressure in a rotary evaporator. The resulting residue (28 g; the aqueous extract) was stored at 4°C. The residue was re-constituted in Ringer-Locke solution to obtain the various concentrations (1, 2, 5, 10, 20, 40, 100 and 200 mg/ml) used in this study.

Experimental animals

Six adult white albino male rabbits weighing between 2 and 3 kg were used for the experiments, with the approval of Ethical Committee (REC-2011-05-01) of the medical School, King Khalid University, Abha, Saudi Arabia. The animals were obtained from the animal house of the College of Medicine of King Khalid University where they were fed with standard rabbit pellets and allowed free access to water. They were housed at a controlled ambient temperature of 25 ± 2°C and 50 ± 10% relative humidity, with 12-h light/12-h dark cycles. All studies were conducted in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals (National Institute of Health, 1996).

Experimental procedure

This experiment was carried out in accordance with the Langendorff (1985) procedure. Each rabbit was injected with 1000 IU of heparin intravenously through the marginal ear vein. Five minutes later, a blow on the neck of the rabbit made them unconscious. The chest was opened and the heart was dissected out with about 1 cm of aorta attached, and washed quickly as possible with oxygenated Ringer-Locke solution (NaCl; 45.0 g, $NaHCO_3$; 1.0 g, D-glucose; 5.0 g, KCl; 2.1 g, $CaCl_2.2H_2O$; 1.6 g, in 5 L of distilled water). The isolated heart was gently squeezed several times to remove as much residual blood as possible. The heart was then transferred to the perfusion apparatus (Radnoti isolated heart system, AD Instruments, Australia) and tied to a stainless steel cannula through the aorta. The perfusion fluid was Ringer-Locke solution, which was continuously bubbled with a mixture of 95% oxygen and 5% carbon dioxide and was applied at a constant perfusion pressure of 70 mm Hg (Chlopicki et al., 2003). Temperature was continuously monitored by a thermo-probe inserted into the perfusion fluid tank and maintained between 36.5 and 37.5°C. The hearts were allowed to stabilize for 30 min before any drug interventions. Briefly, 1 ml of adrenaline (0.05 mM) was given before the beginning of the experiment procedure to record the sensitivity of the heart. Then, 1 ml of Ringer-Locke solution containing different concentrations of the extract (1, 2, 5, 10, 20, 40, 100 and 200 mg/ml) was injected over 30 s with the aid of 1 ml syringe through the perfusion line above the aortic line, and the changes in the cardiac parameters were recorded (Figures 1 and 2). After each treatment, the hearts were washed by the perfusion fluid for 10 min until the baseline recording is achieved and the second dose was then given. The recording before the direct perfusion of adrenaline or each extract dose was considered as baseline reading for each dose. Parameters measured were: 1) mean force of contractions (mean cycle height in g); 2) heart rate (beats/min) and 3) an electrocardiogram (ECG) for rhythm monitoring.

The mechanical responses of spontaneously contracting, isolated hearts were recorded by attaching one end of a thread to the apex of the heart using a Palmer clip and the other end of the thread to a force transducer ((MLT 844; AD Instruments, Australia). ECG and HR were recorded by attaching three spring clip electrodes directly to the heart surface (MLA1210, AD Instruments, Australia). The signal from the force transducer and the ECG electrodes were filtered and amplified and sent to an analog-to-digital converter (PowerLab data acquisition and analysis system: AD Instruments, Australia) attached to a computer. The signals recorded were saved for later analysis. FC, HR and ECG were recorded and analyzed with the help of Labchart Pro7 software (AD Instruments, Australia).

Determination of dose response curve

Dose response curve of the effect of *C. aronia* extract on force of contraction (g) was plotted by plotting the log of each dose against its resulted increase in the force of contraction (difference between the maximum increase in force of contraction after dose perfusion and its baseline force of contraction). EC_{50} which resulted in half maximum effect was calculated using dose response curve module installed in LabChartpro7 software (AD Instruments, Australia)The percent of change in FC or HR for each dose from its baseline

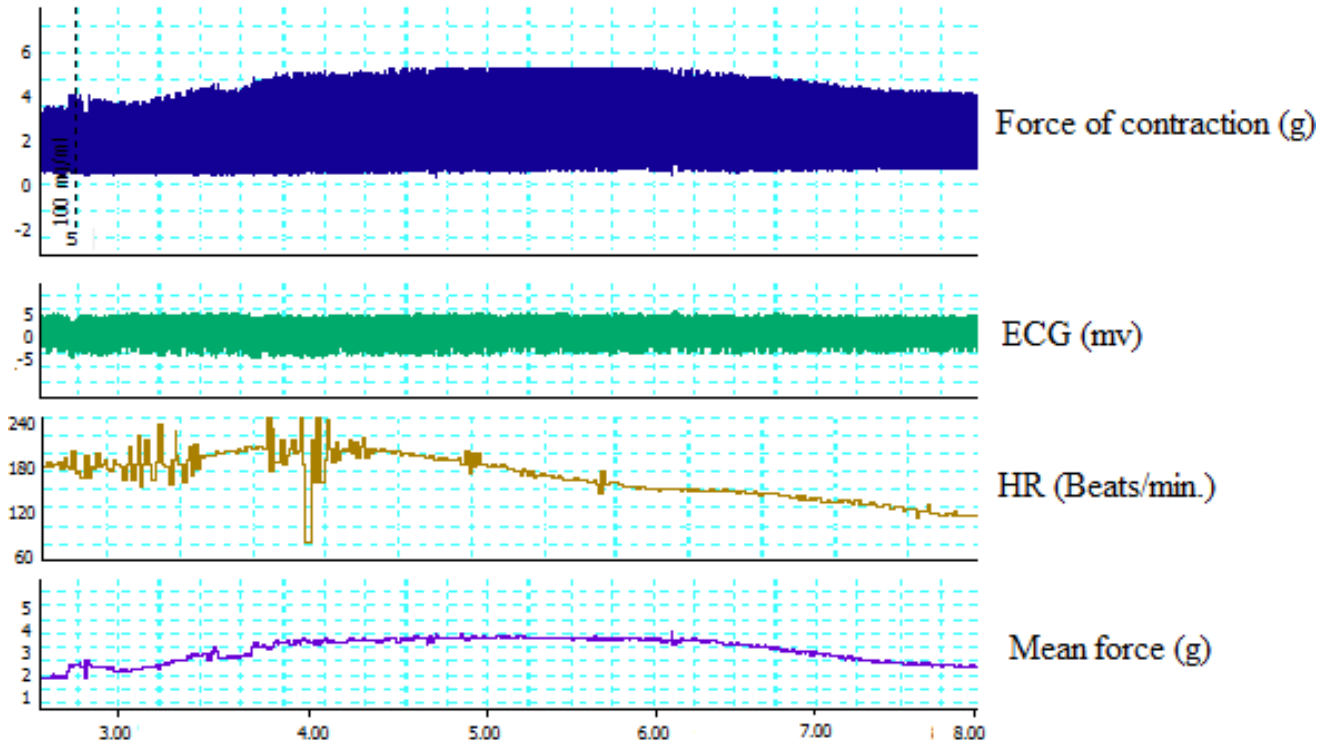

Figure 1. The effect of *Crataegus aronia* syn: *azarolus* (L) aqueous extract (20 mg/ml) on heart rate; ECG and force of contraction on rabbit's isolated heart. The signal from the force transducer and the ECG electrodes were filtered and amplified and sent to an analog-to-digital converter (PowerLab data acquisition and analysis system: AD Instruments, Australia). Data were collected and analyzed by Labchart Pro7 software (AD Instruments, Australia).

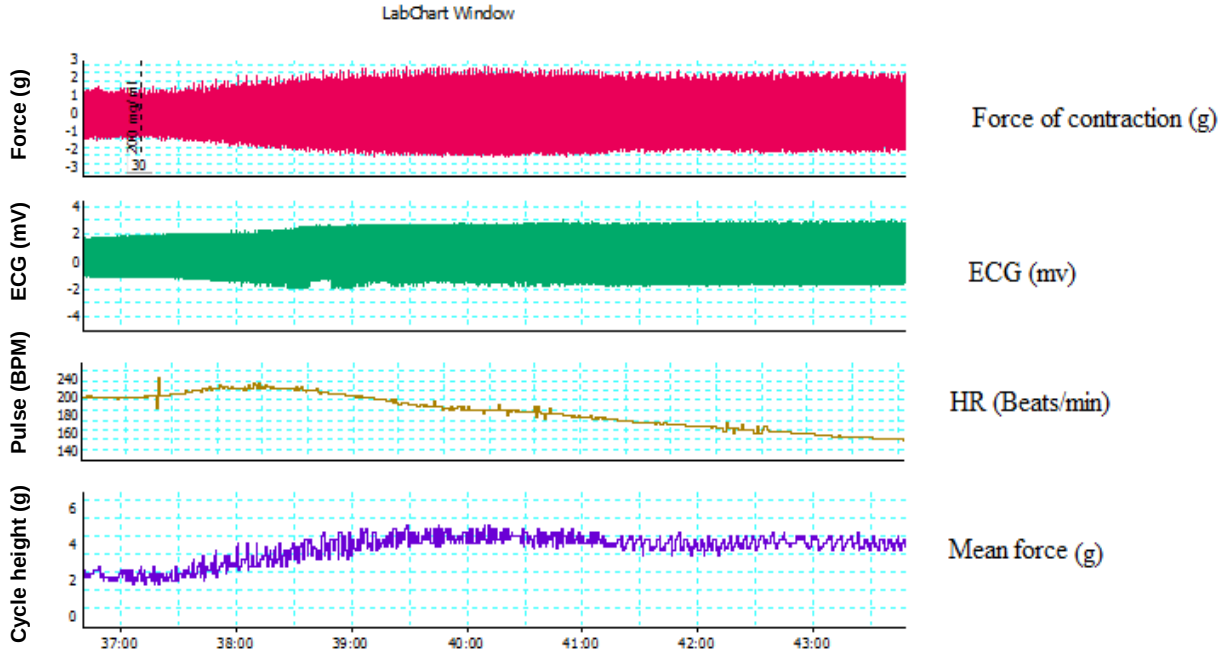

Figure 2. The effect of *Crataegus aronia* syn: *azarolus* (L) aqueous extract (40 mg/ml) on heart rate; ECG and force of contraction on rabbit's isolated heart. The signal from the force transducer and the ECG electrodes were filtered and amplified and sent to an analog-to-digital converter (PowerLab data acquisition and analysis system: AD Instruments, Australia). Data were collected and analyzed by Labchart Pro7 software (AD Instruments, Australia).

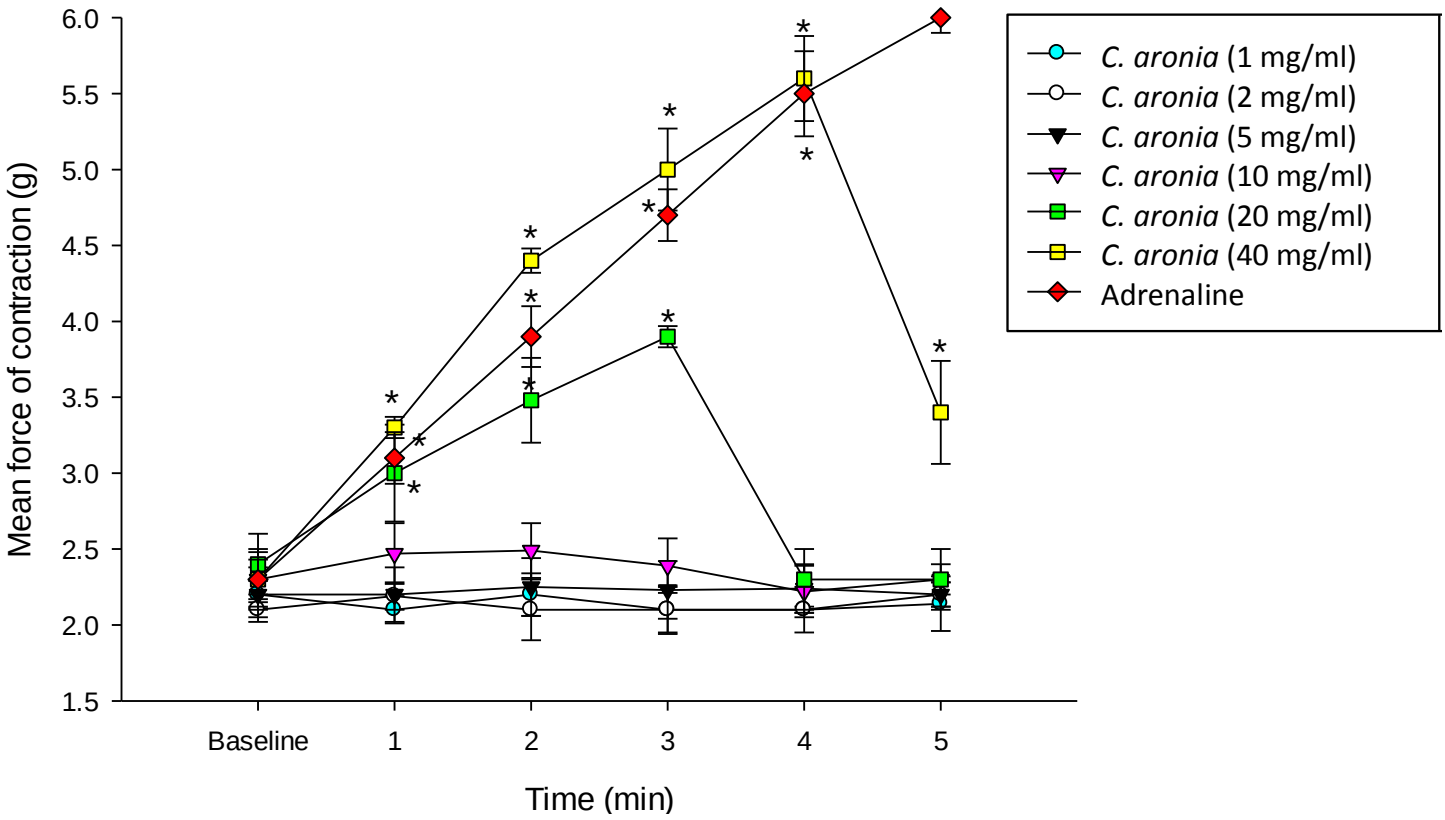

Figure 3. Effect of *Crataegus aronia* syn: *azarolus* (L) aqueous extract (1, 5, 10, 20 and 40 mg/ml) and adrenaline on the force of myocardial contraction of isolated rabbit's heart. Values are given as Mean ± SD for groups of 6 rabbit's hearts each. Analysis by one way ANOVA; *: significantly different from baseline at P< 0.05. #: significantly different when compared to 20 mg/ml reading at same time interval at P< 0.05. and : significantly different when compared to Adrenaline reading at same time interval at P< 0.05.

reading at different time intervals (5 min. intervals) was calculated as follows:

$$\% \text{ of change} = \frac{\text{Mean FC (g) or HR (b/min.) at a given min - mean baseline FC or HR}}{\text{Mean baseline FC or HR}} \times 100$$

Statistical analysis

Results were expressed as the mean value ± SD. Statistical differences between groups were assessed using the SPSS software version 16 by One way ANOVA test. Values of P<0.05 were considered significantly different (95% Confidence interval).

RESULTS

The recordings of FC, HR, and ECG after the administration of 20 and 40 mg/ml of the extract over 5 min are shown in Figures 1 and 2, respectively. The extract produced a notable increase in force of contraction with an initial increase in heart rate at 1 min mainly with 20 mg/ml, followed by a parallel decrease in heart rate at concentrations of 20 and 40 mg/ml thereafter (Figures 3 and 4). The changes in the force of contraction and heart rate recorded at all time intervals after perfusion of *C. aronia* aqueous extract in concentrations of 1, 2, 5, 10 mg/ml were not significantly different from their baseline values. The highest doses of the extract (100 and 200 mg/ml) caused hardening of the heart, stopped the perfusion fluid from entering and stopped the contraction of the heart; therefore no data are shown at these concentrations. The analysis was done for the first 5 min after extract perfusion as our recording and data revealed the diminishing effects on both heart rate and force of contraction after these minutes.

The perfusion of *C. aronia* at a concentration of 20 mg/ml (Figure 3) significantly increased the force of contraction of the isolated rabbit's heart at 1st, 2nd and 3rd minutes only with percents of increases of 25, 45 and 62%, respectively, when compared to the baseline (Table 1). This dose did not cause any significant change in the force of contraction at the 4th and 5th minutes; rather, at these two time intervals, the positive inotropic effect of the extract started to return to its baseline value. The perfusion of *C. aronia* at the higher concentration (40 mg/ml) produced the most profound positive inotropic effect at all minutes of the recording (Figure 3). Percents

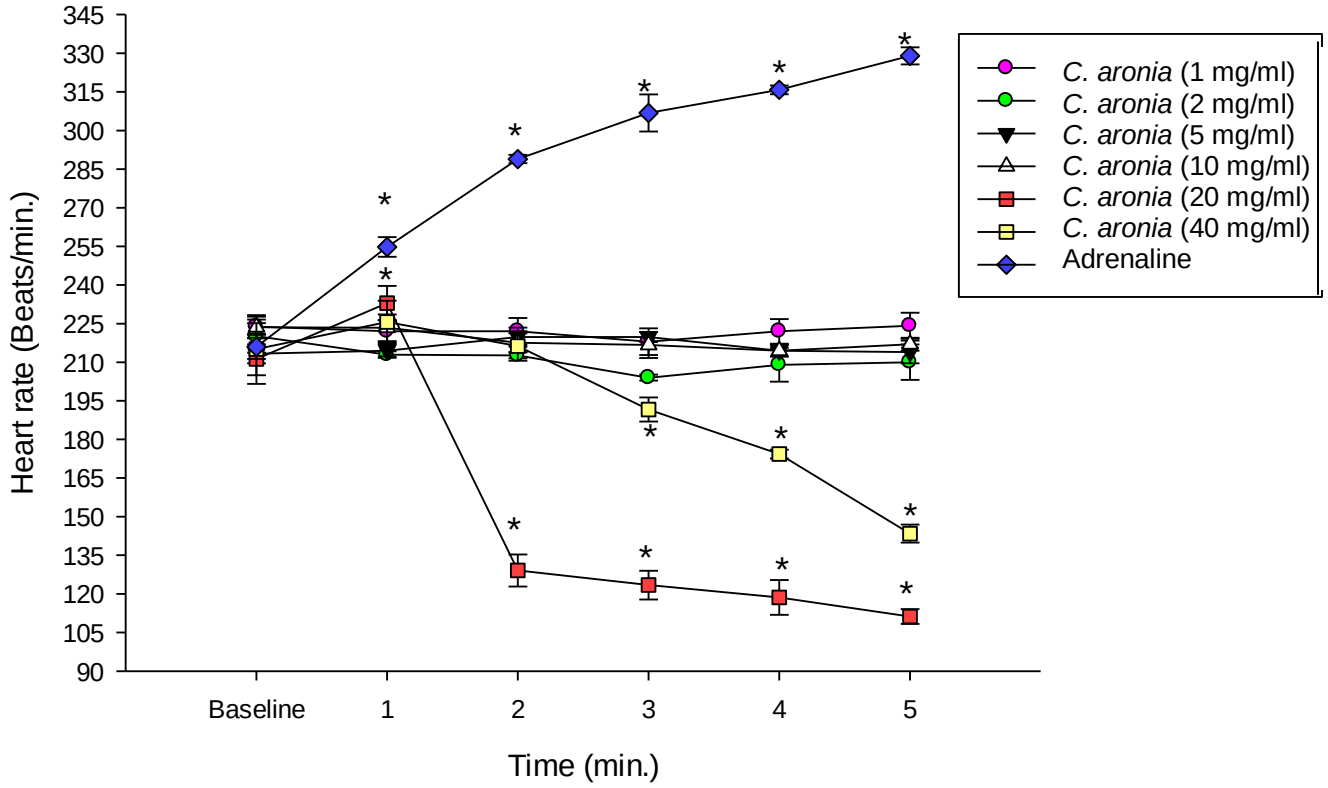

Figure 4. Effect of *Crataegus aronia* syn: *azarolus* (L) aqueous extract (1, 5, 10, 20 and 40 mg/ml) and adrenaline on the heart rate of isolated rabbit's heart. Values are given as Mean ± SD for groups of 6 rabbit's hearts each. Analysis by one way ANOVA; *: significantly different from its baseline at P< 0.05. #: significantly different when compared to 40 mg/ml reading at same time interval at P< 0.05. &: Significantly different when compared to 20 and 40 mg/ml reading at same time interval P< 0.05.

of increases in force of contraction due to *C. aronia* at this dose were 43.47, 91.3, 94.6, 145.5 and 47.8% at the 1st, 2nd, 3rd, 4th and 5th minutes, respectively, when compared to baseline FC (Table 2). At the fifth minute of 40 mg/ml extract dose perfusion, the FC started to declined to its baseline reading. The ANOVA test revealed that the maximum increase in FC at the dose of 20 mg/ml occurred at the 3rd minute (62%), while the maximum inotropic effect of the dose 40 mg/ml occurred at the 4th minute (145%).

Moreover, adrenaline produced a significant prolonged increase in force of contraction at all time intervals and produced 34.7, 69.5, 104.3, 139.3 and 140% increases in the force of contraction at 1st, 2nd, 3rd, 4th and 5th minute. Comparing the positive inotropic effect produced by adrenaline and the extract at the concentration of 40 mg/ml, the ANOVA test revealed that: a) increases in FC at 1st, and 2nd minutes were significantly higher after perfusion of extract; b) the effects produced by adrenaline and the extract at 3rd and 4th minutes were not significantly different; C) at the 5th minute, the force of contraction was significantly higher after adrenalin perfusion. The dose response curve analysis of the effect of the extract on force of contraction showed that the concentration of the extract that produced 50% increase in FC (EC_{50}) is 27.0 ± 1.23 mg/ml (Figure 5).

There were no significant change in heart rate doses of extract at 1-10 mg/ml while, the perfusion of 20 mg/ml of *C. aronia* aqueous extract produced an initial significant increase in HR at 1min (percent of change +10.27%), followed by a progressive significant decrease ($p < 0.0001$) at 2nd, 3rd, 4th and 5th minutes (Figure 4) with percents of inhibition of 38.76, 41.6, 43.87 and 47.37%, respectively (Table 2). On the other hand, the perfusion of *C. aronia* aqueous extract at a dose of 40 mg/ml produced a significant decrease ($p < 0.0001$) in heart rate after 3rd, 4th and 5th minutes, with the percents of inhibition of 11.0, 18.96 and 33.4%, respectively (Figure 4). The ANOVA test revealed that the negative chronotropic effect produced by the dose of concentration of 20 mg/ml on heart rate during the first 5 min intervals after extract perfusion was significantly higher than the effect produced by the dose of concentration of 40 mg/ml. Adrenaline produced a significant positive chronotropic effect with percents of increases of 17.0, 33.75, 42.0, 64.0 52.3% at 1st, 2nd, 3rd, 4th and 5th minutes, respectively (Table 2). No abnormal rhythm was recorded during the period of study.

Table 1. Percent of changes in force of contraction of isolated rabbit's heart during the first 5 min after perfusion of different *Crataegus aronia* syn: *azarolus* (L) aqueous extract doses and adrenaline.

Dose (mg/ml)	Percent of changes (%)				
	1st min	2nd min	3rd min	4th min	5th min
1	-4.76	0.0	-4.76	-4.7	-2.72
2	+4.2	0.0	0.0	0.0	+4.76
5	0.0	+2.27	+1.36	+1.81	0.0
10	+7.4	+8.26	+3.9	-4.3	0.0
20	+25	+45	+62.5	-4.16	-4.16
40	+43.47	+91.3	94.6	+143.5	+47.8
Adrenaline (0.05 mM)	+34.7	+69.5	+104.3	+139.3	+140

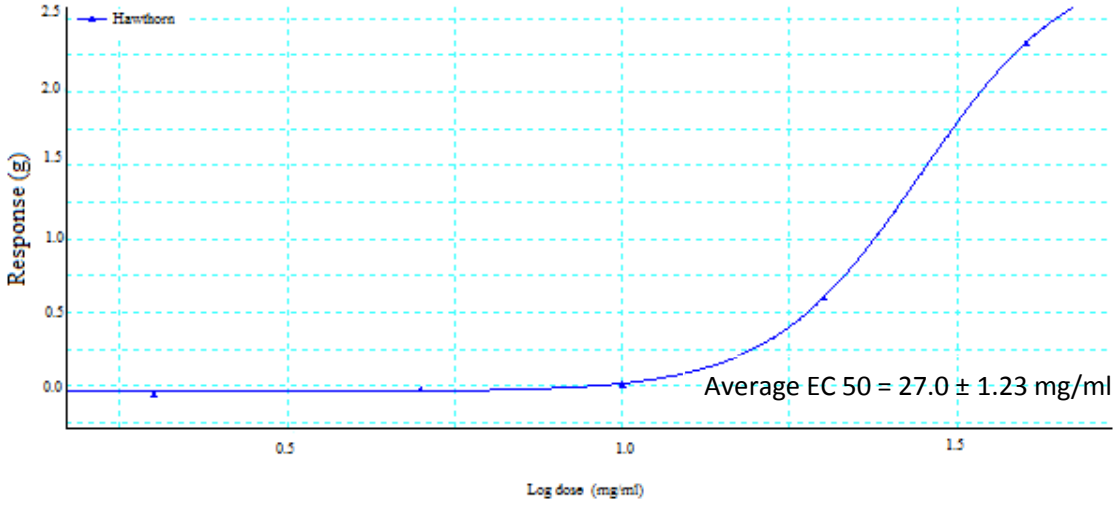

Figure 5. Dose response curve for the effect of *Crataegus aronia* syn: *azarolus* (L) aqueous extract on force of contraction of isolated rabbit's heart. EC_{50} was given as mean ± SD for group of 6 rabbit's hearts each. $EC_{50=}$ 27.0 ± 1.23 mg/ml, Hill slop= 1.67. Dose response (g) for each dose was calculated as the average increase in force of contraction during the first 5 min of extract perfusion, Pre-dose baseline correction was applied during the calculation. Data were collected and analyzed by Labchart Pro7 software (Dose response module, AD Instruments, Australia).

DISCUSSION

In this study, higher extract concentrations (100 and 200 mg/ml) caused ballooning of the heart, stopping of perfusion solution circulation and stopping of heart beating. One explanation for such effect at these high doses could be due to the high viscosity of the extracts, which caused a blockage in the small coronary vessels (capillaries) with subsequent prevention of the perfusion solution from circulating through its normal pathway in Langendorff preparation and instead flowing back through aortic valve into the left ventricle and thus resulting in ballooning of the left ventricle. Langendorff preparation involves the cannulation of the aorta which is then attached to a reservoir containing oxygenated perfusion fluid. This fluid is then delivered in a retrograde direction down the aorta either at a constant flow rate (delivered by an infusion or roller pump) or a constant hydrostatic pressure (usually in the range of 60 to 100 mmHg). In both instances, the aortic valves are forced shut and the perfusion fluid is directed into the coronary ostia, thereby perfusing the entire ventricular mass and then drained into the right atrium via the coronary sinus (Langendorff, 1985).

The positive effect of *C. aronia* aqueous extract on the force of contraction of isolated rabbit's heart reported in this study is in agreement to most previously published *in vivo* and *in vitro* studies that showed a similar effect of other extract prepared from other species of hawthorn (Reutxer, 1994; Popping et al., 1995). However, varying results have been observed regarding the effect of hawthorn and its constituents on HR. In majority of *in*

Table 2. Percent of changes in heart rate of isolated rabbit's heart during the first 5 min after perfusion of different *Crataegus aronia* syn: *azarolus* (L) aqueous extract doses and adrenaline.

Dose (mg/ml)	Percent of changes (%)				
	1st min	2nd min	3rd min	4th min	5th min
1	-0.8	-0.8	-2.58	-0.8	0.18
2	-3.25	-3.39	-7.34	-5.07	-5.0
5	+0.56	+3.04	+3.04	+0.56	+0.53
10	-0.02	-2.68	-3.09	-4.07	-2.60
20	+10.27	-38.76	-41.6	-43.87	-47.37
40	+4.8	+0.56	-11.0	-18.96	-33.4
Adrenaline (0.05 mM)	+17.9	+33.75	+42.0	+64.2	+52.3

vitro studies, an increase in HR has been observed, while most of the *in vivo* studies reported a decrease in HR which is similar to our finding (Ammon and Kaul, 1994). In fact, there was an increase in heart rate at 1 min with both doses of extract (20 and 40 mg/ml) but maximum seen with 20 mg/ml followed by quick fall at the 2nd minute, which then continued to fall slowly thereafter. This difference in response of HR could be due to the effect of extract on vagal tone with the *in vivo* studies and possibly the species difference used in our study, as no effect has been previously reported on *C. aronia* syn: *azarolus* (L) extract on isolated heart (Petkov et al., 1981). However, we did not investigate the mechanism of action by which *C. aronia* aqueous extract exerts its positive inotropic and negative chronotropic effects. Other *in vivo* and *in vitro* studies are running now in our laboratory, aiming to demonstrate the mechanism of the inotropic effect of *C. aronia* extract. However, at this stage, we may postulate some of the possible mechanisms based on previously published works.

The mechanism underlying the enhanced FC is an enhanced Ca^{2+} membrane influx (Chang et al., 2002a). In this study, the positive increase in FC after extract perfusion strongly suggests that the *C. aronia* syn: *azarolus* (L) could act on rabbit's heart by opening the membrane L-type Ca^{2+} channels. Inhibition of myocardial Na^{+}/K^{+} ATPase, which is an integral membrane enzyme that maintains cardiac resting potential and inhibition of the enzyme phosphodiesterase (PDE) that ultimately results in an increase in intracellular cyclic nucleotides, have been reported to occur in different studies with different species of hawthorn (Holzl et al., 1988; Reutxer, 1994; Popping et al., 1995). Both ways eventually enhance the opening of L-type Ca^{2+} with subsequent increase in FC.

Flavonoids, tannins, saponins, terpenes and sterols are the main constituents of *C. aronia* syn: *azarolus* (L) aqueous extract (Shatoor, 2011). Most of the pharmacological actions of hawthorn are attributed to the flavonoids contents (Yao et al., 2008). The reported flavonoid contents of C*. orientalis* and *C. oxycantha* are: hyperoside, along with apigenin, apigenin 7-glucoside, ursolic acid, vitexin and vitexin 4′-rhamnoside (Melikoglu et al., 1999). Ursolic acid interacts with the digitaloid binding site for Na^{+}/K^{+} ATPase, while catechin, the flavonoid vitexin and flavonol kaempferol were found to be structurally similar to papaverine and theophylline, the two chemical agents known to inhibit PDE. On the other hand, saponins are mainly plant-derived glycosides, occurring as triterpenoid or steroid saponins. Steroid saponins have been found to have multiple interesting biological and pharmacologic effects including negative chronotropic, positive inotropic, diuretic, antibacterial, anti-inflammatory, hypocholesteremic (Francis et al., 2002; Lacaille-Dubois and Wagner, 1996). Furthermore, administration of oral standardized *C. oxycantha* extract to an ischemic/reperfusion rat model effectively protected animals from reperfusion induced arrhythmias and hypotensive crisis (Krzeminski and Chatterjee, 1993).

The mechanisms which may account for the slow diastolic depolarization, seen between two successive action potentials of myocardial pacemaker cells, may include one of the following: 1) a slow inward Na^{+} current, I_f, the so-called 'funny current' that is induced by cell hyperpolarization; 2) a temporal decrease of the outward K^{+} current due to a time-dependent decay of the membrane K^{+} conductance; 3) a low background K^{+} outward current; 4) an inward Na^{+}/Ca^{++} exchange current, and 5) an inward T-type and L-type Ca^{2+} current (Lipsius et al., 1996). The individual contributions of these currents to pacemaker function are controversial. To decrease the HR, one or more of the aforementioned mechanisms, could be altered. HR is tightly coupled to myocardial oxygen consumption (Laurent et al., 1956; Braunwald, 1971). Hence, myocardial oxygen demand is reduced through bradycardia in patients with severe heart failure (HF) treated with digitalis (Erdmann, 1998). Furthermore, slowing of HR, prolong diastolic period and improve diastolic flow through coronary arteries.

Therefore, HR reduction became well established strategy for the treatment of various ischemic heart diseases (IHD), and HF since the introduction of β-adrenoceptor blockers (Gillam, 1965; Goethals et al., 1993; Lechat, 1998). β-Adrenoceptor blockers have in

common, a negative inotropic effect which may worsen HF symptoms and have to be used carefully in patients with impaired left ventricular function. Therefore, the newer agents like Ivabradine, and Zatebradine which specifically inhibit sinus node pacemaker current without direct effect on contraction is gaining more attraction for treating patients with IHD and/or HF (Gillam, 1965; Goethals et al., 1993; Lechat, 1998; Borer et al., 2003).

The currently available inotropic agents such as Dobutamine, milrinone, enoximone and Vesnarinone are limited by the proarrhythmic effects, increased mortality and are not recommended for routine use in patients with HF and/or IHD (Focaccio et al., 1996; Cuffe et al., 2002; Abraham et al., 2005; Dec, 2005). Almost all inotropic agents exhibit their positive inotropic effects by increasing the influx of calcium into the cytosol (Lorell et al., 1988). This process leads to intracellular calcium overload and may trigger serious arrhythmias, while slowing of HR prolongs the diastolic period and contribute to intracellular calcium release (Brutsaert et al., 1993; Martin et al., 2011). It is not yet known how hawthorn handles the intracellular calcium. However, combining inotropic agents such as enoximone which act through inhibition of PDE with β-adrenoceptor blockers improve survival in advanced cases with HF (Shakar et al., 1998). It seems that the presence of multiple active ingredients in the aqueous extract of the whole plant of *C. aronia* syn: *azarolus* (L) may exert the inotropic and chronotropic effects through a multiple mechanisms of action.

Conclusion

The results of this study showed that the aqueous extract of *C. aronia* syn: *azarolus* (L) produced a positive inotropic and negative chronotropic effects on isolated rabbit's heart. Further investigation is therefore needed to define the mechanism of action underlying the enhanced force of contraction and the negative chronotropic effects of the aqueous extract of *C. aronia* syn: *azarolus* (L).

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REFERENCES

Abdul M, Krushna GS, Hussain SA, Devi KL (2009). Effect of Aqueous Extract of Nutmeg on Hyperglycaemia, Hyperlipidaemia and Cardiac Histology Associated with Isoproterenol-induced Myocardial Infarction in Rats. Trop. J. Pharm. Res. 8(4):337-344.

Abraham WT, Adams KF, Fonarow GC, Costanzo MR, Berkowitz RL, LeJemtel TH, Cheng ML, Wynne J, ADHERE Scientific Advisory Committee and Investigators, ADHERE Study Group (2005). In-hospital mortality in patients with acute decompensated heart failure requiring intravenous vasoactive medications: an analysis from the Acute Decompensated Heart Failure National Registry (ADHERE). J. Am. Coll. Cardiol. 46(1):57.

Ali-Shtayeh MS, Yaniv Z, Mahajna J (2000). Ethnobotanical survey in the Palestinian area: a classification of the healing potential of medicinal plants. J. Ethnopharmacol. 73:221-232.

Almak DS, Sweidan H, Muller S, Jacob R, Forsch A, Abdul Kareem M, Krushna GS, Hussain SA, Devi KL (2009). Effect of Aqueous Extract of Nutmeg on Hyperglycaemia, Hyperlipidaemia and Cardiac Histology. Associated with Isoproterenol-induced Myocardial Infarction in Rats. Trop. J. Pharm. Res. 8(4):337-344.

Ammon H, Kaul R (1994). Heat circulation mechanism of action of *Crataegus* extract, flavanoid and procyanidin. Part I. History and workings. Dtsch. Apoth. Ztg. 134:433-436.

Bahorun T, Aumjaud E, Ramphul H, Rycha M, Luximon-Ramma A, Trotin F, Aruoma O (2003). Phenolic constituents and antioxidant capacities of *Crataegus monogyna* (Hawthorn) callus extracts. Nahrung 47:191-198.

Braunwald E (1971). Control of myocardial oxygen consumption. Am. J. Cardiol. 27:416-432.

Brauwald E, Zipes D, Libby P (2001). Heart disease a textbook of cardiovascular medicine. USA: W.B.Saunders. P. 1.

Brutsaert DL, Sys SU, Gillebert TC (1993). Diastolic failure: pathophysiology and therapeutic implications. J. Am. Coll. Cardiol. 22(1):318.

Borer JS, Fox K, Jaillon P, Lerebours G (2003). Antianginal and antiischemic effects of ivabradine, an I(f) inhibitor, in stable angina: a randomized, double-blind, multicentered, placebo-controlled trial. Circulation, 107(6): 817.

Bonati A (1980). Medicinal plants and industry, J. Ethnopharmacol. 2(2):167-171.

Chang CT, Chen YC, Fang JT, Huang CC (2002a). Star fruit (*Averrhoa carambola*) intoxication: an important cause of consciousness disturbance in patients with renal failure. Renal Failure 24:379-382.

Chang Q, Zuo Z, Harrison F, Chow MSS (2002b). Hawthorn. J. Clin. Pharmacol. 42:605-612.

Chlopicki S, Kozlovski VI, Geygewski RJ (2003). Clonidine-induced coronary vasodilation in isolated guinea pig heart is not mediated by endothelial a2 adrenoceptors. J. Physiol. Pharmacol. 54:511-521.

Cuffe MS, Califf RM, Adams KF Jr, Benza R, Bourge R, Colucci WS, Massie BM, O'Connor CM, Pina I, Quigg R, Silver MA, Gheorghiade M (2002). Outcomes of a Prospective Trial of Intravenous Milrinone for Exacerbations of Chronic Heart Failure (OPTIME-CHF) Investigators. Short-term intravenous milrinone for acute exacerbation of chronic heart failure: a randomized controlled trial. JAMA 287(12):1541.

Dec GW (2005). Acute decompensated heart failure: the shrinking role of inotropic therapy. J. Am. Coll. Cardiol. 46:65.

Erdmann E (1998). Digitalis therapy – relevance of heart rate reduction. Basic Res. Cardiol. 93: 156-159.

Francis G, Kerem Z, Makkar HPS, Becker K (2002). The biological action of saponins in animal systems: a review. Br. J. Nutr. 88:587-605.

Focaccio A, Peeters G, Movsesian M, Roden R, Eki Y, Krall J, Bristow MR (1996). Mechanism of action of OPC-8490 in human ventricular myocardium. Circulation 93(4):817.

Gillam PMS (1965). Use of propranolol in angina pectoris. Br. Med. J. 2:337-339.

Goethals MA, Raes A, van Bogaert P (1993). Use-dependent block of the pacemaker current If in rabbit sinoatrial node cells by zatebradine (UL-FS 49). Circulation 88:2389-2401.

Harvey A (2000). Strategies for discovering drugs from previously unexplored natural products. Drug Discov. Today, 5:294-300.

Hearse DJ, Sutherland FJ (2000). Experimental models for the study of cardiovascular function and disease. Pharmacol. Res. 41:597-603.

Krzeminski T, Chatterjee SS (1993). Ischemia and early reperfusion induced arrhythmias. Beneficial effects of an extract of *Crataegus oxyacantha* L. Pharmacol. Lett. 3:45-48.

Lacaille-Dubois MA, Wagner H (1996). A review of the biological and pharmacological activities of saponins. Phytomedicine 2:363-386.

Langendorff O (1985). Unverschungen, Uber Pfluger. Arch. Gis. Physiol., 61: 291.

Laurent D, Bolene-Williams C, Williams FL, Katz LN (1956). Effects of

heart rate on coronary flow and cardiac oxygen consumption. Am. J. Physiol. 185:355-358.
Lechat P (1998). Beta-blocker treatment in heart failure. Role of heart rate reduction. Basic Res. Cardiol. 93:148-155.
Leuchtgens H (1993). *Crataegus* special extract WS 1442 in NYHA II heart failure. A placebo controlled randomized double blind study. Fortschr. Med. 111:352-354.
Lipsius SL, Hüser J, Blatter LA (1996). Intracellular Ca2+ release sparks atrial pacemaker activity. News Physiol. Sci. 16:101-106.
Lorell BH, Isoyama S, Grice WN, Weinberg EO, Apstein CS (1988). Effects of ouabain and isoproterenol on left ventricular diastolic function during low-flow ischemia in isolated, blood-perfused rabbit hearts. Circ. Res. 63(2):457.
Martin F, Noble NJ, Denis N (2011). Ca2_-induced delayed afterdepolarizations are triggered by dyadic subspace Ca2_ affirming that increasing SERCA reduces aftercontractions. Am. J. Physiol. Heart Circ. Physiol. 301:H921-H935.
Melikoglu G, Mericli F, Mericli AH (1999). Flavonoids of *Crataegus orientalis*. Boll. Chim. Farm. 138:351-352.
National Institute of Health (1996). "Guide for the Care and Use of Laboratory Animals" [DHEW Publication No. (NIH) 85-23, Revised 1996, Office of Sci. Health Reports, DRR/NIH, Bethesda, MD 20205].
Packer M, Carver JR, Rodeheffer RJ, Ivanhoe RJ, DiBianco R, Zeldis SM, Hendrix GH, Bommer WJ, Elkayam U, Kukin ML (1991). Effect of oral milrinone on mortality in severe chronic heart failure. The PROMISE Study Research Group. N. Engl. J. Med. 325(21):1468.
Petkov E, Nikolov N, Vzunov P (1981). Inhibitory effect of some flavanoids and flavanoid mixtures on cyclic AMP phosphodiesterase activity of Rat heart. Planta Med. 43:183-186.
Popping S, Rose H, Ionescu I, Fischer Y, Kammermeier H (1995). Effect of a hawthorn extract on contraction and energy turnover of isolated rat cardiomyocytes. Arzneim. Forsch. 45:1157-1161.
Reutxer HD (1994). *Crategeus* as cardiac plant. J. Phytother. 15: 73-81.
Rigelsky JM, Sweet DV (2002). Hawthorn, Pharmacology and Theraputic Uses. Am. J. Health Syst. Pharm. 59:417-442.
Said O, Khalil K, Fulder S, Azaizeh H (2002). Ethnopharmacological survey of medicinal herbs in Israel, the Golan Heights and the West Bank region. J. Ethnopharmacol. 83:251-265.
Shakar SF, Abraham WT, Gilbert EM, Robertson AD, Lowes BD, Zisman LS, Ferguson DA, Bristow MR (1998). Combined oral positive inotropic and beta-blocker therapy for treatment of refractory class IV heart failure. J. Am. Coll. Cardiol. 31(6):1336.
Shatoor AS (2011). Acute and Sub-acute Toxicity of *Crataegus Aronia Syn. azarolus* (L.)Whole Plant Aqueous Extract in Wistar Rats. Am. J. Pharmacol. Toxicol. 6(2):37-45, ISSN1557-4962.
Uretsky BF, Jessup M, Konstam MA, Dec GW, Leier CV, Benotti J, Murali S, Herrmann HC, Sandberg JA (1990). Multicenter trial of oral enoximone in patients with moderate to moderately severe congestive heart failure. Lack of benefit compared with placebo. Enoximone Multicenter Trial Group. Circulation 82(3):774.
Wagner H, Grevel J (1982). Cardioactive Drugs IV. Cardiotonic amines from *Crategeus oxycantha*. Planta Med. 45:99-101.
Rewerski W (1967). Some pharmacological properties of flavan polymers isolated from hawthorn. Arzneim Forsch Drug Res. 17:490-491.
Yao M, Ritchie HE, Brown-Woodman PD (2008). A reproductive screening test of hawthorn. J. Ethnopharmacol. 118:127-132.