Cardiovascular Effects of Aqueous-Ethanolic Extract of *Achillea Wilhelmsii* in Rabbit

Niazmand S[1,2*], Esparham M[3]

[1] Cardiovascular Research Center, Mashhad University of Medical Sciences (MUMS)

[2] Department of Physiology, Medical School, Mashhad University of Medical Sciences (MUMS)

[3] Department of Biology, Sciences School, Azad University of Mashhad

*Corresponding author: Saeed Niazmand, E-mail: niazmands@mums.ac.ir

Tel: +985118828565, Fax: +985118828564

Running title: Cardiovascular effects of *Achillea wilhelmsii*

Summary

For many years in herbal medicine the antihypertensive and lowering blood lipid properties of *Achillea wilhelmsii* have been suggested. In the present study the impacts of the plant extract on rabbit's blood pressure, heart rate and intraventricular pressure have been investigated.

Twenty four NWZ rabbits weighed (2-3 kg) were randomly divided into four groups. Two groups of 6 rabbits received jugular injection of either *A. wilhelmsii* extract (20, 40 and 80 mg/kg) or normal saline for blood pressure effects and two groups for intraventricular pressure. Blood pressure, heart rate and intraventricular pressure were measured via carotid cannula using pressure transducer connected to a power lab system.

The blood pressure was significantly decreased in 80 mg/kg dose of the extract. However, there were not any significant effects on heart rate in other doses of the extract or normal saline. Although, the extract was significantly decreased (dp/dt) max and left ventricular developed pressure (LVDP), it increased (dp/dt) min. Therefore, it seems that although A. *wilhelmsii* extract had no effect on heart rate, it showed a negative inotropic effect. Furthermore, the extract reduced blood pressure which may partly due to the negative inotropic effect of the extract.

Keyword: *Achillea wilhelmsii*, Heart rate, Blood pressure, Intraventricular pressure, Rabbit

Introduction

The prevalence of cardiovascular diseases is very high and increases dramatically worldwide. Hypertension is a very important risk factor for development of other cardiovascular diseases such as myocardial infarction and heart failure (1). These conditions are the most important causes of hospital admissions which are responsible for high mortality rates, disabilities and costs. Therefore, heart failure treatment particularly; lowering blood pressure by pharmacological treatment is an essential step for preventing cardiovascular diseases. In the recent years, herbal medicine applications for the prophylaxis and treatment of cardiovascular diseases has been increased and scientists have been paying more attention to investigate the cardiovascular effects of herbs (2).

Achillea, is one of the most important genera of the *compositae* family and comprises more than 120 species. Several pharmacological effects of *Achillea* such as anti-inflammatory (3), antibacterial (4,5), antitumor (6,7). Antispasmotic (8,9), choleretic (10), antiulcer (11), antibacterial (Helicobacter pylori) (12) and hepatoprotective (9) have been reported. Moreover, there are some reports on cardiovascular effects of *Achillea* such as changing of electrocardiogram and cardiac enzymes[13]. *Achillea wilhelmsii* is the major species which is grown in Iran (it is called "Boomadaran" in Iran) and widely used in Iranian traditional medicine. It has chemical components including alkaloids (achilleine), cineol, borneol, α and β pinen, luteolin, apigenin, lignans, camphor, caryophllene, thujene, rutin and carvacrol (14-17). Recently the antihypertensive and antihyperlipidemia effects of *A. wilhelmsii* were demonstrated (18). However, there is no comprehensive study to specify the pharmacological activities of *A. wilhelmsii* extract on cardiovascular system. Therefore, the present study was conducted to investigate the effects of aqueous-ethanol extract of *A. wilhelmsii* on cardiac and blood pressure *in vivo*.

Materials and Methods

Plant and extract

The aerial part of *A. wilhelmsii* was collected from Nishabour city (Khorasan Province, Iran) and was dried at room temperature. The plant was identified by the Ferdowsi University Herbarium (voucher No. 164-2218-2). Three hundred grams of aerial part of *A. wilhelmsii* were macerated with ethanol (50%) at 30°C for 24 hours and shaken intermittently. The solution was then filtered and dried in oven at 40°C. The average w/w yield was 13%. The dried extract was dissolved in the distilled water to make 20, 40 and 80 mg/kg concentrations.

Animals and procedures

Twenty four NWZ rabbits weighed (2-3 kg) were randomly divided into four groups (n=6 in each group) as following: 1- Control group for blood pressure which received jugular injection of normal saline with the same volume of the extract 2- Test group of blood pressure which received jugular injection of *A. wilhelmsii* extract (20, 40 and 80 mg/kg) 3- Control group of intraventricular pressure which received jugular injection of normal saline with the same volume of the extract 4- Test group of intraventricular pressure which received jugular injection of *A. wilhelmsii* extract (20, 40 and 80 mg/kg). The extract doses were chosen on the base of the dose which had the minimum effect on the cardiovascular parameters in pilot study.

In control groups, normal saline (37˚C) with similar volumes of the extracts used to rule out the effects of blood volume changes on cardiovascular parameters. The rabbits were kept in standard conditions at 20 ± 2˚C and fed with standard diet. The study was permitted by the Institutional Ethics Committee of Mashhad University of Medical sciences (MUMS).

Animals were anaesthetised using sodium thiopental (50 mg/kg, ip) and then jugular vein and carotid artery were cannulated. *A. wilhelmsii* extracts were introduced through the jugular vein. Blood pressure, heart rate and left ventricular pressure (LVP) were measured by a power lab machine (AD Instruments, Australia) via a pressure transducer (UFI 1050.1) which was connected to carotid cannula[19]. The data were recorded by computer. The indexes for myocardial function were left ventricular developed pressure (LVDP), which was defined as left ventricular systolic pressure minus diastolic pressure, heart rate (HR), (dp/dt) max and (dp/dt)min.

Statistical analysis

The changes in blood pressure and intraventricular pressure before and after injections were analyzed by paired t-test. The results represent as mean±SEM and the differences were considered significant if $P<0.05$.

Results

There was no significant differences in the normal saline treatment groups for mean arterial blood pressure (MAP), heart rate, left ventricular develop pressure (LVDP), (dp/dt)max and (dp/dt)min; so injection of normal saline with the same volume of *A. wilhelmsii* extract did not affect on cardiovascular parameters (Table 1).

Table 1: Effect of normal saline injection by the same volume of *Achillea wilhelmsii* extract on intraventricular pressure, mean arterial pressure and heart rate. (n=6 for intraventricular pressure control group, n=6 for mean arterial pressure control group)

Parameters	Before injection of normal saline	After injection of normal saline	P-value
(dp/dt)max (mmHg s^{-1})	2197.56±120.53	2144.14±140.54	0.41
(dp/dt)min (mmHg s^{-1})	-1151.33±137.4	-1186±143.2	0.65
LVDP (mmHg)	97.3±6.5	96.9±7.1	0.75
MAP (mmHg)	77.75±5.9	78±6.1	0.79
H.R (beat/min)	217±24.9	219±22.1	0.51

LVDP (left ventricular develop pressure), (dp/dt)max (maximum rate of rise of left ventricular pressure during ventricular contraction), (dp/dt)min (maximum rate of fall of left ventricular pressure during left ventricular relaxation), MAP (mean arterial pressure), H.R (heart rate)

Treatment with *A. wilhelmsii* extract at 80 mg/kg significantly, reduced the mean arterial blood pressure (96.9 *vs* 80.2 mmHg) (Fig.1). Moreover, in the 20 and 40 mg/kg doses of *A. wilhelmsii* extracts the mean blood pressure reduced, but the changes were not reached to a significant value (Fig.1).

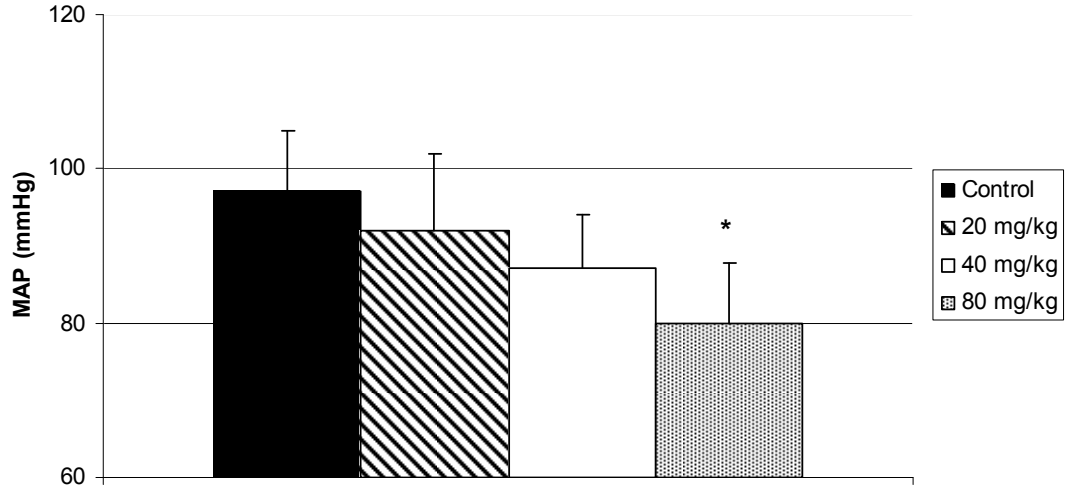
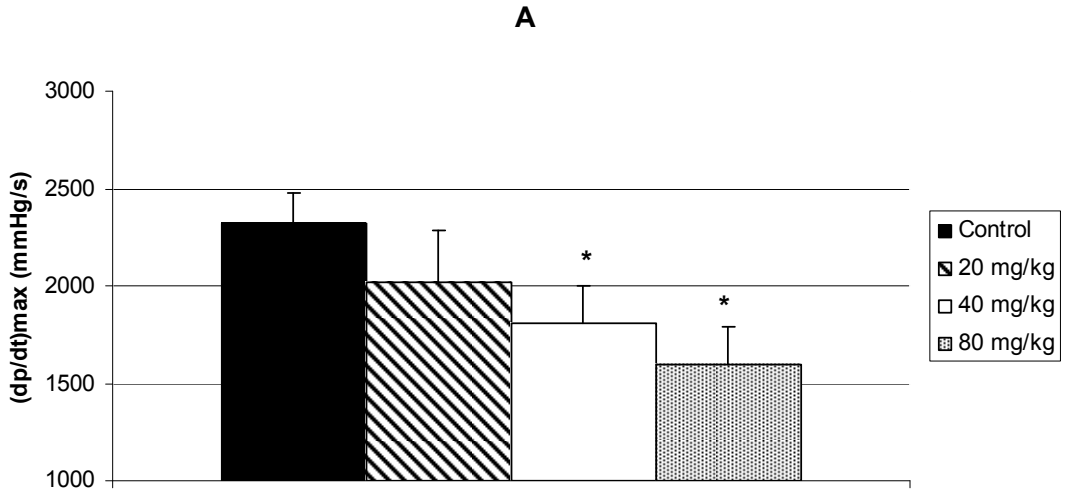


Fig 1: Effect of *Achillea wilhelmsii* extract on mean arterial pressure (MAP). (n=6, * P<0.05)

A. wilhelmsii extract treatments decreased the mean of (dp/dt) max in 40 mg/kg(2324 *vs* 1806 mmHg s^{-1}) and 80 mg/kg (2324 *vs* 1596 mmHg s^{-1}) (P<0.05) (Fig 2A). Furthermore, the mean of (dp/dt) min, increased after *A. wilhelmsii* injection which was statically meaningful (-1752 *vs* -1350 and -1249 mmHg s^{-1} for 40 and 80 mg/kg respectively, P<0.05) (Fig. 2B).



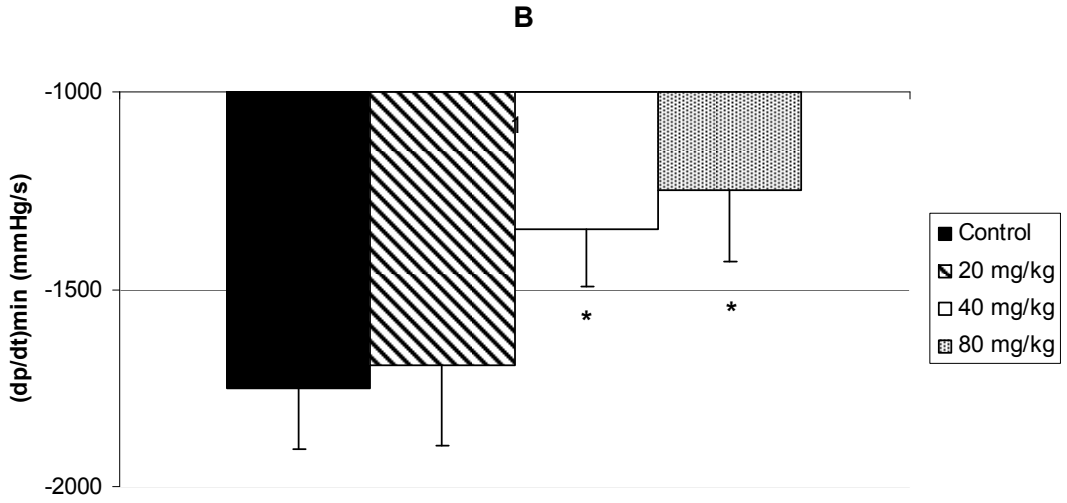


Fig 2: Effect of *Achillea wilhelmsii* extract on maximum rate of rise of left ventricular pressure during ventricular contraction [(dp/dt)max](A) and maximum rate of fall of left ventricular pressure during left ventricular relaxation [(dp/dt)min](B). (n=6, * P<0.05)

In addition, the 80 mg/kg dose of extract decreased LVDP significantly (124.14 *vs* 78.2 mmHg, P<0.05)(Fig. 3). The *A. wilhelmsii* extract did not affect on heart rate.

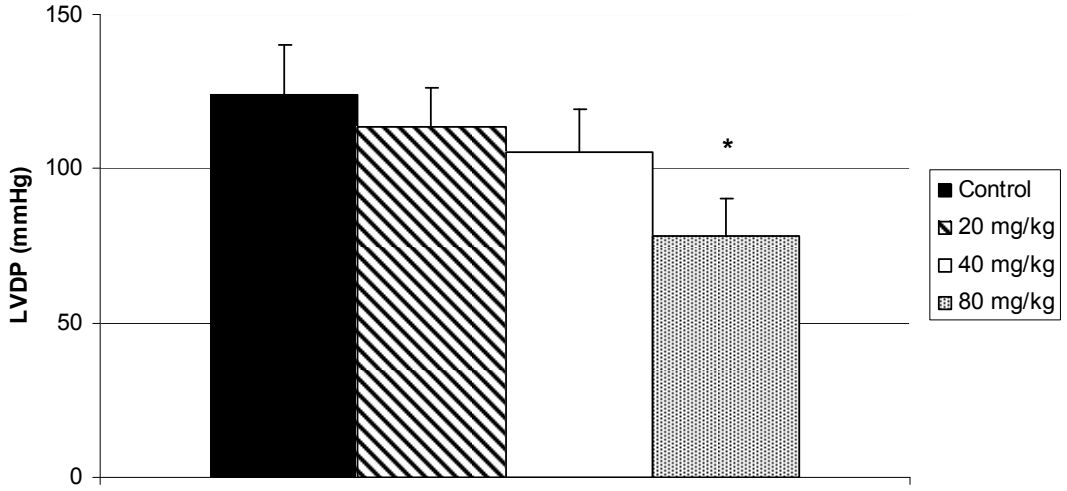


Fig 3: Effect of *Achillea wilhelmsii* extract on LVDP (left ventricular develop pressure) (n=6, * p<0.05)

Discussion

In the present study the impacts of *A. wilhelmsii* extracts on cardiac parameters such as (dp/dt)max, (dp/dt)min, and LVDP were investigated. In control groups the injection of normal saline with the same volume of *A. wilhelmsii* extract did not affect on cardiovascular function, thus the results of test groups could be attributed to *A. wilhelmsii* extract. The data demonstrated that (dp/dt)max was significantly decreased around 22.3% and 31.3% in 40 and 80 mg/kg dosages of *A. Wilhelmsii,* respectively. These results indicate a negative inotropic effect of the herb extract. The (dp/dt)max is an indicator for maximum rate of rise of LVP during ventricular contraction and a valid criterion for inotropic effect of a substance.

Furthermore, *A. wilhelmsii* extract significantly increased (dp/dt)min (23% and 28% in 40 and 80 mg/kg dosages, respectively) . This parameter is an indicator for maximum rate of fall of LVP during left ventricular relaxation and rising of this parameter indicates reduction in rapidity of ventricular relaxation and therefore a negative inotropic effect. Taken together, the reduction of (dp/dt)max and rising of (dp/dt)min after treatment indicate a significant suppression of cardiac performance. Furthermore, LVDP is significantly decreased after administration of 80 mg/kg of *A. wilhelmsii* extract (47.1%) which confirmed the negative inotropoic effect of the plant. Taking together our findings in the present study, reveal that *A. wilhelmsii* extract can inhibit cardiac performance by a negative inotropic effect. This negative inotropic effect may due to decrease of calcium influx from extracellular or decrease of calcium releasing from intracellular storage. Lignans which are found in *A. wilhelmsii* have negative inotropic and vasorelaxant effect (20). In another study in our laboratory, *A. millefolium* extract showed negative inotropic and chronotropic effect on isolated rat heart which was mainly mediated by decreasing of calcium releasing from intarcellular storage (unpublished data). Thus it is more possible the cardiac depressant effect of *A. wilhelmsii* mediated mainly by inhibiting of calcium releasing from intarcellular storage. However, to more clarify the exat mechanism of cardiac effect of *A. wilhelmsii* further studies are needed.

In addition, this study demonstrated that *A. wilhelmsii* extract in 80 mg/kg was able to reduce MAP (17.5%) in normotensive condition which may indicate a relaxation effect of the extract on vascular smooth muscles. Previous studies have demonstrated that *A. wilhelmsii* extract had antihypertensive effects (18). Some other studies showed antispasmodic effects of other species of *Achillea* on ileum and duodenum smooth muscles (8,9,21,22). Moreover, *A. wilhelmsii* contains important ingredients such as carvacrol, luteolin, apigenin and 1,8-cineole which can influence vascular smooth muscle tone. In many studies the antispasmodic and vasorelaxant effects of carvacrol (23-25), luteolin (26,27), apigenin (28) and 1,8-cineole (29,30) have been demonstrated. luteolin has vasolexant effect by inhibiting of sarcolemmal Ca^{2+} channels, release from intracellular Ca^{2+} stores and activation of K^{+} channels (25).

Taken together, it seems that the *A. wilhelmsii* ingredients can induce hypotensive effects in rabbits. Blood pressure is not determined by vascular factors alone and cardiac interaction is also important for determining the final arterial blood pressure value. *A. wilhelmsii* showed a negative cardiac inotropic effect and it is reasonable to suppose that reduction of MAP may partly due to this negative inotropic effect.

Conclusion

The results of the present study show that *A. wilhelmsii* extract has hypotensive and negative cardiac inotropic effects in rabbit. These positive pharmacological properties should be investigated more to find the responsible ingredients and the more reliable function of the herb on blood pressure lowering.

Acknowledgments

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