The Chronotropic and Inotropic Effects of Aqueous-Ethanolic Extract of *Achillea Millefolium* on Rat's Isolated Heart

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Summary

Achillea is widely used in traditional medicine. There are some reports of *Achillea* effects such as antihypertensive and blood lipid lowering, antiulcer, antibacterial, and antispasmotic. The aim of this study was to investigate the effects of aqueous-ethanol extract of *Achillea millefolium* on rat's isolated heart.

24 male Wistar rats were randomly divided into three groups: in group 1 the heart was perfused by Krebs Henseleit (K.H) solution, in group 2 the heart was perfused by K.H calcium free solution and in group 3 the heart was perfused by K.H plus diltiazem (10 µm/l) solution. In all groups the three concentrations of *A. millefolium* (0.01, 0.0125, 0.02 mg/ml) were infused to the heart for 30 s and the heart contractility and heart rate (HR) were measured by an isotonic transducer.
The extract reduced contractility during the infusion significantly in all three groups, but 2 minutes after the stopping infusion contractility increased which was not significant. Comparison of the changes in contractility between the three groups showed that there is a significant difference between groups 1 and 2 and also between groups 2 and 3 but the difference between groups 1 and 3 was not significant. The extract showed a significant reduction of the heart rate in all groups by three concentration of the extract ($P<0.01$). The depressant effect of the extract on heart rate was lasted after two minutes of stopping the extract infusion and the hear rate significantly reduced by three concentration of the extract in all groups ($P<0.05$).
The results show the negative cardiac choronotropic and inotropic effects of *A. millefolium* extract which are to some extent exerted independent of the inward calcium current throw the L-type calcium channels.

Key Words: *Achillea millefolium*, Isolated heart, Heart rate, Heart contractility

Introduction

Medicinal plants have been used for over 2000 years and an increasing attention has been paid to herbal medicine products because of their effectiveness and lower cost in recent years. *Achillea*, is one of the most important genera of the compositae family and comprises more than 140 spices. The biological activity of *Achillea* was recently reviewed (1). Several effects such as anti-inflammatory (2), antibacterial (3-5), antitumor (6,7), antispasmotic (8,9), choleretic (10), antiulcer (11), and hepatoprotective (9) were reported. *A. millefolium* has chemical components including flavonoids (apigenin, luteolin and quercetin), alkaloids (achilleine), cineol, lignans, dicaffeoylquinic acids, rutin, β-pinen, sabinen, thujone, borneol, sesquiterpenoids and monoterpenoids (12-17). There are some reports on cardiovascular effect of *Achillea* like electrocardiogram and cardiac enzymes (18), hypotensive (19) antihypertensive and antihyperlipidemia (20) but the cardiac effect of *Achillea* was not shown. Thus we evaluate the effect of *A. millefolium* extract on isolated rat heart and the possible role of L-type calcium channels in its effect.

Material and Methods

Animals:
Twenty four male Wistar rats (weighed 200-250 g) were divided randomly into 3 groups: in group 1 the heart was perfused by K.H solution, in group 2 the heart was perfused by K.H calcium free solution and in group 3 the heart was perfused by K.H plus diltiazem (10 μm/l, Sigma) solution (21). In all groups the three concentrations of *A. millefolium* (0.01, 0.0125, 0.02 mg/ml) were infused to the heart for 30 seconds.

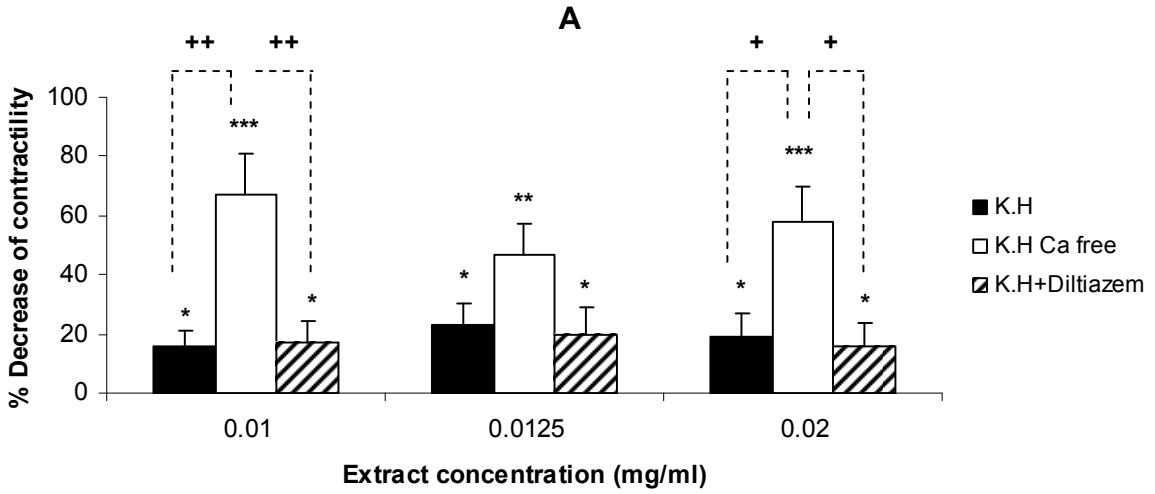
Preparation of plant extract:
Aerial parts of *A. millefolium* that was identified by Ferdowsi University Herbarium (voucher No. 1357-2216-6) were collected from Nishabour city (Khorasan province, Iran) and was dried at room temperature. Dried powder (200 g) was macerated with 750 ml ethanol (50%) at 30°C for 24h and was shaken intermittently. The solution was then filtered and dried on 40°C oven. The dried extract was dissolved in the three different Krebs Henseleit (K.H) (K.H, K.H calcium free and K.H+diltiazem) solutions to make 0.01, 0.0125 and 0.02 mg/ml concentrations.

***Isolated heart preparation*:**
The animals were anesthetized with sodium thiopental (50 mg/kg, i.p) and then heparin was administered (5000 U/kg, i.p). While maintaining artificial ventilation, the chest was opened at the median line and the pericardium was opened widely. A perfusion cannula was immediately inserted into the ascending aorta to perfuse the coronary arteries with K.H solution as the following concentrations (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11.7 gassed with 95% O_2 and 5% CO_2 (pH=7.4) at a constant temperature (37°C). The heart was removed from the chest and connected to the Langendorrff setup under a constant pressure (60 mmHg) (22). After 30 minutes period to stable the heart, the heart contractility and heart rate (HR) were measured by an isotonic transducer and data was recorded by computer. The percent changes of heart contractility and HR calculated as: [(B-A)/A] X 100
Where A was the HR or heart contractility before the infusion of the extract and B was the HR or heart contractility after the infusion of the extract.

Statistical analysis:
The data was analyzed by paired t-test in each group and by ANOVA between groups. All data were presented as mean±SEM and $P<0.05$ was considered statistically significant.

Results

Inotropic effects: A. millefolium extract showed a biphasic effect on heart contractility. The extract reduced contractility during the infusion, but 2 minutes after the stopping infusion, contractility increased. Comparison of the heart contractility before and after the end of infusion (30 seconds) of the three concentrations of *A. millefolium* extract showed that the heart contractility significantly decreased in all groups (Fig 1A). Comparison of the changes in contractility between the three groups showed that there is a significant difference between groups 1 and 2 and also between groups 2 and 3 ($P<0.01$) in 0.01 and 0.02 mg/ml concentrations, but the difference between groups 1 and 3 was not significant (Fig 1A).
After two minutes of stopping the extract infusion the contractility of the heart increased in all three groups in comparison with before infusion however it was not significant (Fig 1B).



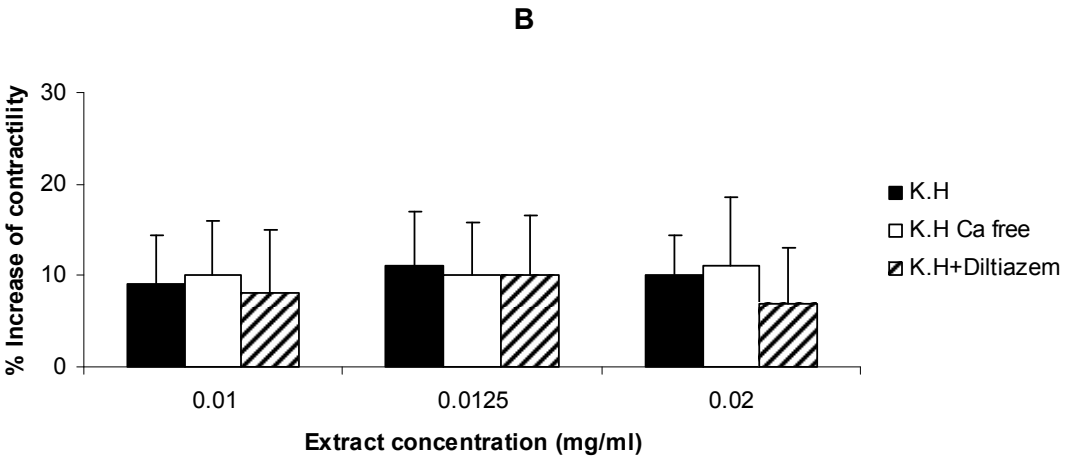
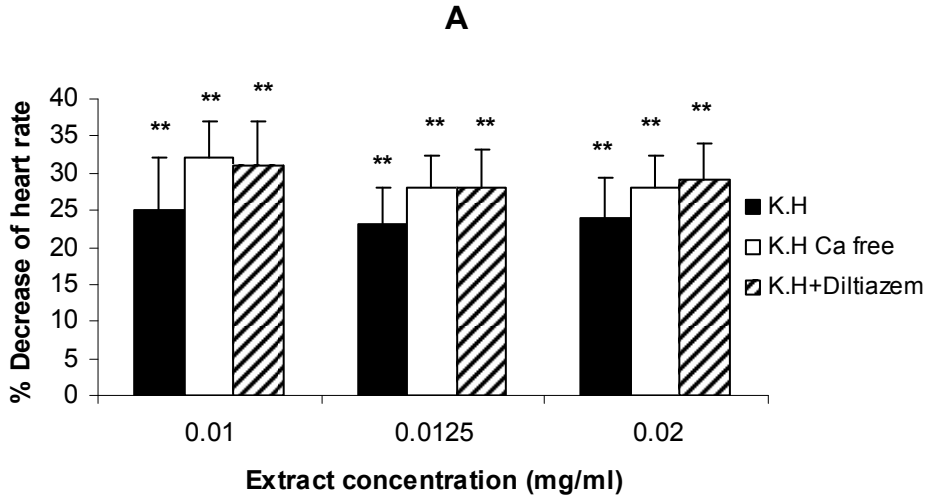


Fig 1: Effects of *A. millefolium* extract on isolated heart contractility A: after 30 seconds of extract infusion and B: after 2 minutes of stopping extract infusion. (n=8 in each group) **P*<0.05, ***P*<01, ****P*<0.001 compare with before extract infusion; +*P*<0.05, ++*P*<0.01 compare between groups.

Chronotropic effects: *A. millefolium* extract showed a depressant effect on hear rate. Comparison of the changes in heart rate before and after the end of infusion (30 seconds) of the extract showed significant reduction of the heart rate in all groups by three concentration of the extract (*P*<0.01)(Fig 2A). Comparison of the heart rate changes between the three groups revealed no significant differences between groups. The depressant effect of the extract on heart rate was lasted after two minutes of stopping the extract infusion and the heat rate significantly reduced by three concentration of the extract in all groups (Fig 2B). Comparison of the heart rate changes between the three groups two minutes after stopping infusion of the extract revealed no significant differences between groups (Fig 2B).



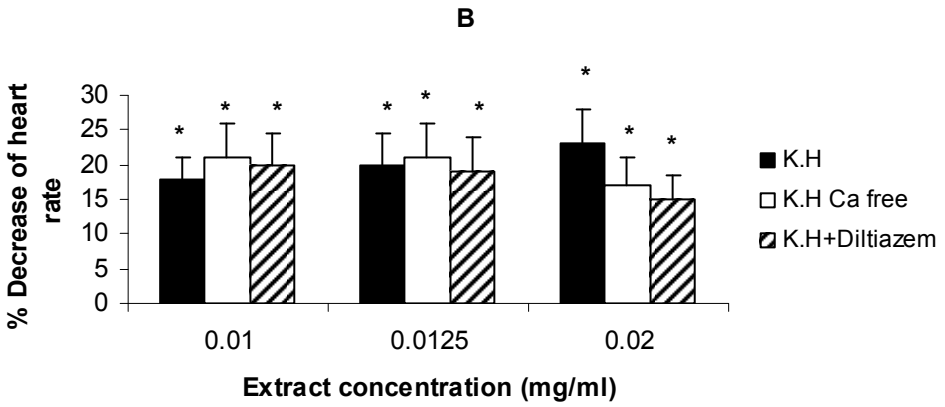


Fig 2: Effects of *A. millefolium* extract on isolated heart rate A: after 30 seconds of extract infusion and B: after 2 minutes of stopping extract infusion. (n=8 in each group) **P*<0.05, ***P*<01 compare with before

Discussion

A. millefolium showed negative inotropic and chronotropic effects on the heart during the extract infusion. There are no significant difference of heart contractility between groups 1 and 3, thus the L-type calcium channel blocking didn't affect the extract effect on heart contractility. The negative inotropic effect in group 2 was significantly more than groups 1 and 3 which indicate that the extract exert its negative inotropic effect independent of the inward calcium current throw the L-type calcium channels (Fig 1A). The heart contractility increased after 2 minutes of stopping extract infusion which was not statistically significant, thus the negative inotropic effect of *A. millefolium* had short duration (Fig 1B). Thus it may conclude that the *A. millefolium* extract has chemical compositions with fast action and short duration negative inotropic effect and also compositions with late action and positive inotropic effect. In the previous study *A. millefolium* showed a positive inotropic effect after 2 hours of intravenous injection of *A. millefolium* extract in sheep (18). Other studies showed that *A. millefolium* had antispasmodic effect on smooth muscles (8,9). There are reports that some chemical compositions which are found in *A. millefolium* such as apigenin (23), luteolin (8,24, 25) and lignans (26) have vasorelaxant effects. Lignans have negative inotropic effect (26) but louteolin has positive inotropic effect (25). Quercetin is one of the falvenoides of *A. millefolium* which has antihypertensive effect (27).

There are no significant differences of heart contractility between three groups after 2 minutes of stopping extract infusion which indicate this effect independent of the inward calcium current throw the L-type calcium channels. In the previous studies *A. millefolium* showed antispasmodic effect on intestinal smooth muscle by blockade of the calcium inward current (8,9). This difference could be attributed to the differences of smooth and cardiac muscles.

The extract showed a profound negative chronotrpic effect in all groups which was not diminishing 2 minutes after stopping the extract infusion. Comparison of the heart rate changes between three groups shows no differences which indicate the extract act throw the mechanism independent of the inward calcium current throw the L-type calcium channels (Fig 2A and B). This negative chronotropic effect lasting 2 minutes after stopping the extract infusion however it was reduced in compare to period of extract infusion. Thus the negative chronotropic effect of *A. millefolium* was lasting more than its negative inotropic effect. In the pervious stud cineole a terpenoid which is found in *A. millefolium* showed chronotropic effect (28) In another study *A. millefolium* showed any significant chronotropic effect *in vivo* condition in sheep (18). This difference may indicate the heart rate controlled by several mechanisms *in vivo* condition which they could not affect hear rate in isolated heart. The antihypertensive effect of *Achillea* (20) may partly due to this negative inotropic and chronotropi effects.
There were not significant differences between the extract concentrations on contractility and heart rate.
To better clarified the effect of *A. millefolium* on isolated heart more studies are needed. The extract may interact with beta adrenergic receptors or intracellular calcium release.

Conclusion

In general the results show that the negative cardiac choronotropic and inotropic effects of *A. millefolium* extract which are to some extent exerted independent of the inward calcium current throw the L-type calcium channels. The negative choronotropic of *A. millefolium* is stronger than its negative inotropic effect.

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