The Positive Inotropic and Chronotropic Effects of *Teucrium Polium L.* Extract on Guinea Pig Isolated Heart

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Summary

There are several reports about the antidiabetic, antispasmodic and antihypertensive properties of *Teucrium podium L*. (TP). The aim of this study is to investigate the inotropic and chronotropic effects of aqueous-ethanol extract from TP on guinea pig isolated heart.
24 Dunkin Hartley guinea pigs weighed 350-450 g were re randomly divided into three groups: group 1 in which the heart was perfused by Krebs solution, group 2 in which the heart was perfused by Krebs calcium free solution and group 3 in which the heart was perfused by Krebs +diltiazem solution. Three concentrations of aqueous-ethanol extract from TP were infused to the heart for 1 minute. The heart contractions were recorded by an isotonic transducer and data was saved by computer.
The extract of TP increased the contractility and HR in all three groups. Comparing mean percentile changes of contractility and HR between the three groups showed a significant difference between groups 1 and 3 and also between groups 2 and 3, but the difference between groups 1 and 2 was insignificant.
The TP extract has positive chronotropic and inotropic effects on isolated heart. These effects are probably due to the agonistic action of the extract on L-type calcium channels.

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Introduction

The use of plants for medical purposes dates back to the ancient times. For example Lamiaceae or Labiatae, also known as the Mint family, is a family of plants comprises about 210 genera and 3,500 species. One of the most popular species of this family native to the Mediterranean region and the Middle East is TP which has been used for over 2000 years in traditional medicine mainly for its antidiabetic, antipyretic, anti-inflammatory and antispasmodic properties (1,2). In some part of Iran it is traditionally used for treatment of heart failure (1). Phytochemical analyses of TP have identified several constituents comprising mostly flavonoids, sesquiterpenoids, neoclerodane diterpenoids (3,4), tannin and alkaloids (5,6). Previous studies have demonstrated some of the pharmacological effects of TP such as antibacterial (7), anti-inflammatory [8], antioxidant (9,10), antiulcerogenic (11), antinociceptive ([12,13), antidiabetic (14,15), antispasmodic (16,17,18) and hepatotoxicity (19). Despite some reports that have revealed many precise effects of TP on cardiovascular system including decrease of blood lipid (20,21), induction of vascular relaxation (17) and lowering blood pressure (22), there is still no specific study to show its effect/s on heart itself and thus the goal of this study was to investigate this effect and therefore we sought to find out any inotropic and chronotropic effects of aqueous-ethanol extract of TP.

Material and Methods

Preparation of plant extract

Aerial parts of TP that was identified by university botanists were collected from hills around Ferdows city (south of Khorasan province, Iran) and was dried at room temperature. Dried powder (170 g) was macerated with 500 ml ethanol (50%) at 30°C for 24h and was shaken intermittently. The solution was then filtered and dried on 40°C ban marry. The average w/w yield was 12.5%. The dried extract was dissolved in the three different Krebs solutions to make 0.5, 1, 2 mg/ml concentrations.

Isolated heart preparation

Twenty four Dunkin Hartly guinea pigs (weighed 300-400 g) were used. The animals were divided randomly into 3 groups: in group 1 the heart was perfused by a Krebs solution, in group 2 the heart was perfused by Krebs calcium free solution and in group 3 the heart was perfused by Krebs plus diltiazem (10 μm/l) solution. In all groups the three concentrations of TP (0.5, 1, 2 mg/ml) were infused to the heart for 1 min. 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 Krebs Henseleit, KH (concentrations in mmol/l: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11.7) equilibrated 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 apparatus under a constant pressure (60 mmHg) (23). The heart contractility and heart rate (HR) were measured by an isotonic transducer and data was recorded by computer.

Statistical analysis

The data was analyzed by paired t-test and one-way ANOVA. All data are presented as mean±SEM and P<0.05 was considered statistically significant.

Results

Chronotropic effects: Comparison of the changes in heart rate before and after infusion of TP extract showed the following results: two concentrations (1 and 2 mg/ml) in group 1 and all three concentrations (0.5, 1, 2 mg/ml) in groups 2 and 3 increased HR significantly (Fig 1).

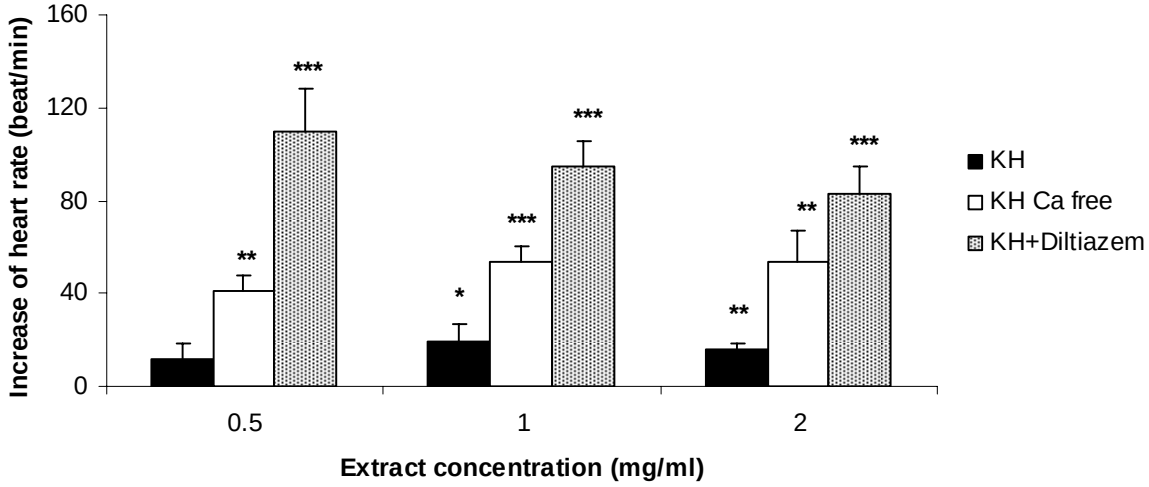

Fig 1: Comparing the heart rate changes before with after infusion of the *Teucrium podium* extract. Data are shown as mean±SEM. (n=8; paired t-test) * P<0.05, **P<0.01, ***P<0.001

Comparison of the percentile changes in the heart rate between the three groups revealed a significant difference between groups 1 and 3 and also this difference was noticed between groups 2 and 3, but the difference between groups 1 and 2 was not significant (Fig 2).

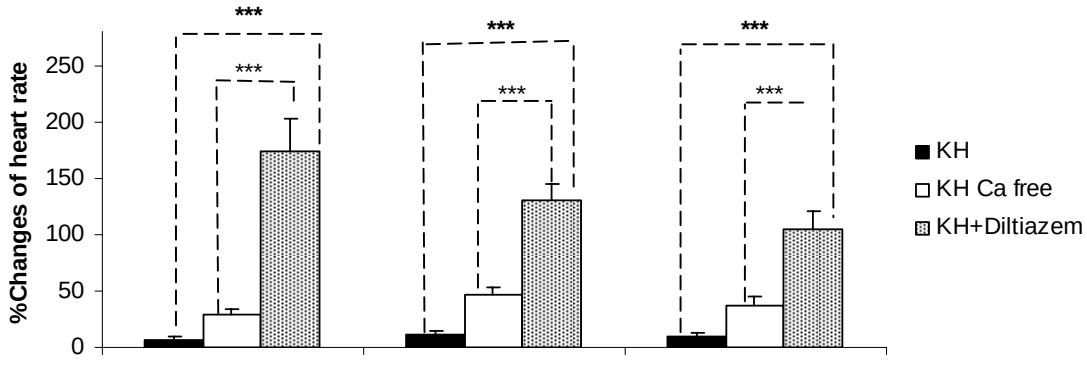

Fig 2: Comparison the effects of *Teucrium podium* extract on the heart rate of guinea pigs in three groups. Data are shown as mean±SEM. (n=8, ANOVA) ***P<0.001

Inotropic effects: Comparison of the contraction amplitude of the heart before and after infusion of the three concentrations of TP extract showed that all the three can significantly increase the heart contractility in all groups (Fig 3).

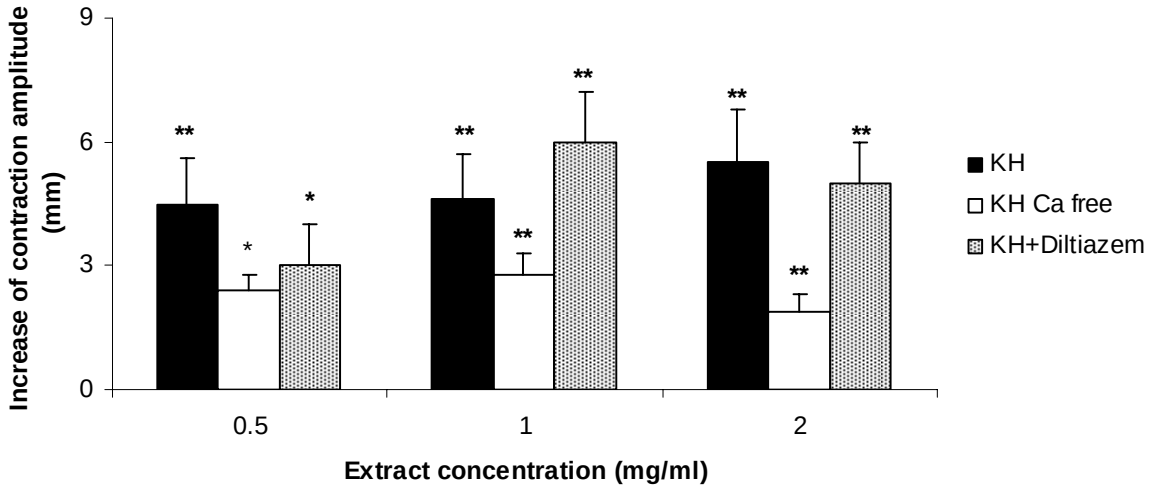

Fig 3: Comparing the heart contraction amplitude changes before with after infusion of the *Teucrium podium* extract. Data are shown as mean±SEM. (n=8; paired t-test) * P<0.05, **P<0.01

Comparison of the percentile changes in contraction amplitude between the three groups showed that there is a significant difference between groups 1 and 3 and also between groups 2 and 3, but the difference between groups 1 and 2 was not significant (Fig 4).

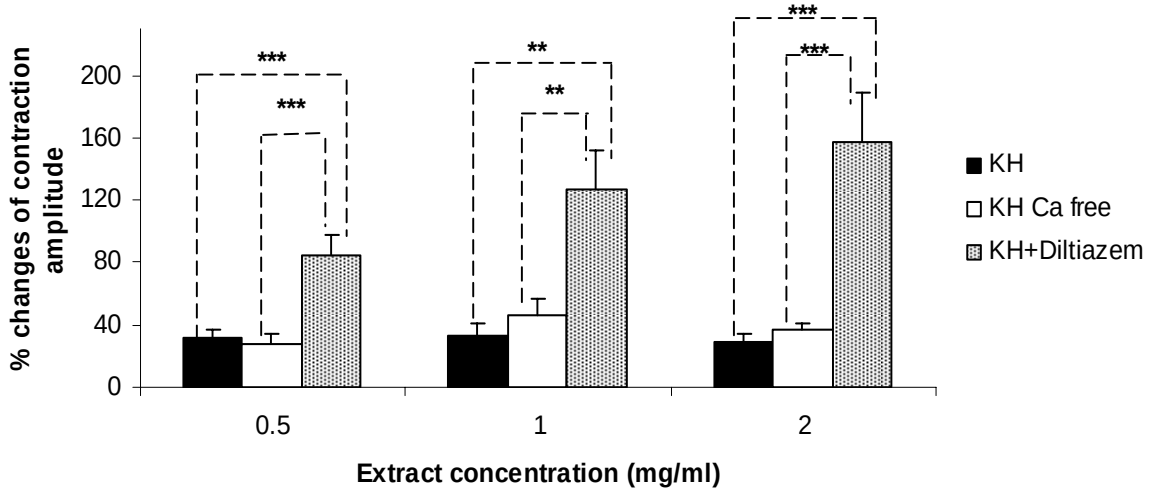

Fig 4: Comparison the effect of *Teucrium podium* extract on heart contraction amplitude between the three groups. Data are shown as mean±SEM. (n=8, ANOVA) **P<0.01, ***P<0.001

Discussion

There are many reports that show TP is widely used in traditional and herbal medicine for different medical purposes (Reviewed in introduction). Some reports have shown important effects of TP on cardiovascular system but its effect on heart had not been investigated before this study. Thus, taking into account all these effects on cardiovascular system, it should not surprise us to consider the heart as a common target affected by TP and therefore, it is feasible that many of these effects are due to cardiac effects of TP. This study was set to investigate the cardiac chronotropic and inotropic effects of TP. The results show positive choronotropic effects of aqueous-ethanol extraction of TP. Comparison of the percentile changes of the heart rate between the three groups shows the mechanism of TP extraction on HR. The significant differences between groups 1 and 3 shows that TP can markedly block the reductive effect of diltiazem (as an L-type calcium channel antagonist) on HR and increase HR significantly in group 3. The significant difference of HR between groups 2 and 3 also corroborates that TP extract can markedly block the inhibitory effect of diltiazem on L-type calcium channels and results in a marked increase in the HR compared to group 2. Therefore the TP extract can block the negative chronotropic effect of diltiazem and increase the HR significantly compared to groups 1 and 2. The results show positive inotropic effects of aqueous-ethanol extract of TP. Comparison of the percentile changes of heart contraction amplitude between the three groups clearly demonstrates the mechanism of TP extraction on heart contractility. The significant differences between groups 1 and 3 shows that TP extract can obviously block the antagonistic effect of diltiazem on L-type calcium channels and increase the heart contractility significantly. The significant difference of heart contraction amplitude between groups 2 and 3 also indicates that TP extract can evidently block diltiazem effect on L-type calcium channels and increase the heart contractility. If the TP extract has no agonistic effects on L-type calcium channels and the inotropic and chronotropic effects of the extract are exerted only by intracellular mechanisms, there must be no significant difference between groups 2 and 3. The HR and contraction amplitude increased in groups 1 and 2 are probably due to the presence of compounds with positive chronotropic and inotropic effects in TP extract, which act independently from extracellular calcium. The previous study has described the effect of TP extract on lowering blood pressure (22) exerted by an inhibitory effect of TP on vascular smooth muscle (17). The effects of TP extract on heart muscle, intestinal and vascular smooth muscles are quite different and show the dissimilar properties of these two types of muscles. The scientific findings presented in this study strongly suggest that TP is a useful herb and can be used for treatment of heart failure as it has been used in Persian traditional medicine for a long time.

Conclusion

In general these findings show that the positive cardiac choronotropic and inotropic effects of TP extract are to some extent exerted by agonistic effect on L-type calcium channels of the heart.

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