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Original Article

Effects of hydroalcoholic extract from aerial parts of the sterile stems of *Stachys inflata* on myocardial infarct size in rats

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Abstract

Recently a potent anti-inflammatory effect of hydroalcoholic extract of the aerial parts of the sterile stems of *Stachys inflata* has been reported. This study examined whether hydroalcoholic extract isolated from aerial parts of non-flowering stems of *Stachys inflata* (standardized to contain 4.5% caffeic acid derivatives) reduce myocardial infarct size arising from coronary artery occlusion (30min) and reperfusion (2 h) in anaesthetized rats. In addition, the extract was also tested on the incidence and severity of ischaemic arrhythmias. Infusion of the extract (1.35 μg/kg/min) 5 min before coronary artery ligation and for the duration of the ischaemic (30 min) and reperfusion (2 h) periods resulted in a marked ($p<0.001$) decrease in infarct size (from 48.2 1% in control to 29.3 2.7% in treated rats). However, an infarct size of 46.7 3.4% was seen with the higher dose of the extract (2.70 μg/kg/min). The extract had no effect either on the severity and incidence of ischaemic arrhythmias or on the blood pressure.

These results suggest that the hydroalcoholic extract of aerial parts of *Stachys inflata* attenuates the infarct size following ischaemia and reperfusion without any effect on the cardiovascular system. Anti-inflammatory actions of the extract may play a major role in reducing the infarct size.

Keywords: *Stachys inflata*; Myocardial infarct size; *Ischaemia*; Inflammation.

Introduction

Stachys inflata Benth is a native plant widely distributed in Iran (1), being popularly named "Poulk" or "Ghol-e-Argavan". Aerial parts from sterile stems of *Stachys inflata* have been used as a folk medicine and the people in the north of Iran believed it to cure infective, asthmatic, rheumatic and other inflammatory diseases.

Recently we have reported a potent anti-inflammatory action of hydroalcoholic extract of aerial parts of non-flowering stems of *Stachys inflata* in carrageenan-induced model of paw inflammation (2). Aerial parts of flowering stems of the plant had no anti-inflammatory effects (unpublished). The inflammatory mechanisms can be partly responsible for myocardial damage during ischaemia and reperfusion. To our knowledge the effect of *Stachys inflata* on cardiovascular system and on infarct size following ischaemia and reperfusion has not been elucidated to date. However, investigators reported that extracts or constituents of plants belonging to the genus stachys exert significant anti-inflammatory, antitoxic (3), antihepatitis (4), antibacterial (5), anti-anoxia (6) and anti-nephritic (7,8) effects. As active principals phenylethanoid glycosides (9, 10), triterpenoids, steroids (11, 12) and flavonoids (13, 14) were identified in the genus stachys. The effects of hydroalcoholic extract of aerial parts from sterile stems of *Stachys inflata* on infarct size following ischaemia and reperfusion and on the incidence and severity of ischaemic ventricular arrhythmias were studied.

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Experimental

Extract preparation

Stachys inflata is a woody based plant, annual or biennial herb, with 30 cm height. Whole plant is densely covered with long white silky hairs, giving it a whitish appearance. Leaf-blades are 10-40 mm long, lanceolate to oblong, entire. Flowers are pink with 20-24 mm long, calyx ventricose, in remote, verticillate spike. The flowered stems are erect. *Stachys inflata* belongs to family of Lamiaceae and genus of Stachys. It spreads from Greece to Transcaucasus and is a native plant wiedely distributed in Iran (2).

Aerial parts from nonflowereing stems of *Stachys inflata* (400 g) were minced and extracted four times with 600 ml 70% methanol-water while being macerated at room temperature for 48 hours each time. Methanol was evaporated by using a rotary evaporator under low pressure at 50 C. The extract was kept at 4 C until used. The dried extract was standardised to contain 4.5% caffeic acid derivatives using modefied Nichiforesco and Coucou (15) method.

Surgical procedure

Male Wistar rats (280-300 g) were anaesthetized with sodium pentobarbital (60 mg/kg intraperitoneally, i.p.) and maintained under anaesthesia by bolus injections of sodium pentobarbitone (6 mg intravenously) as required. The trachea was cannulated for artificial respiration and systemic arterial blood pressure was recorded from a catheter inserted into the left carotid artery. Right jugular vein was cannulated for the administration of anaesthetic or solutions of extract. A standard limb lead I ECG was monitored continuously throughout the experimental period. The chest was opened by a left thoracotomy at a point *2 mm to the sternum; ribs 4 and 5 were then sectioned. Artificial respiration was immediately started with room air (volume 1.5 ml / 100g, rate 54 strokes/min), which is sufficient to maintain pCO2, pO2, and pH within normal limits (16). After the pericardium was incised, the heart was exteriorized by gentle pressure on the ribcage and a 6/0 braided silk suture (attached to a 10-mm micropoint reverse cutting needle) was placed around the left coronary artery as described previously (17). The heart was replaced in the chest, and the animal was allowed to stabilize for 15 min. Any animal with mean arterial pressure (MAP) less than 70 mmHg was discarded. Hydroalcoholic extract was dissolved in saline. Saline infused animals (n=10) were used as control group for hydroalcoholic extract-treated rats (n=9-10). Infusion via the right jagular vein was commenced 5 minutes prior to occlusion of the coronary artery and maintained during the period of occlusion (30 minutes) and reperfusion (2 hours). Coronary occlusion was achieved by threading the loose ends of the ligature through a polyethylene occluder and clamping in place. Release of the clamp allowed reperfusion of the previously ischaemic tissue.

Measurement of myocardial infarct size

Following 2 hours reperfusion and prior to killing the animals, the ligature around the coronary artery was re-tied and a slow bolus intravenous injection of Evans Blue dye (0.5 ml; 3% *w/v*) was given. After adequate perfusion of myocardial tissue with the dye, the animal was sacrificed and the heart was rapidly removed. The ventricle was cut into four equal transverse slices perpendicular to the apex-base axis. These were then placed in 1% triphenyltetrazolium chloride (TTZ) solution at 37 C for 10 minutes to dye the non-infarcted region (18). This procedure resulted in the normally-perfused tissue being stained blue, non-infarcted, non-perfused tissue stained brick red and infarcted tissue remaining unstained. The tissue free of Evans Blue was dissected from the normally perfused tissue and weighed as the area at risk. The infarcted tissue (unstained by TTZ) was then dissected from the stained tissue (dark red) of the area at risk and weighed to determine the infarct size as a percentage (by weight) of the area at risk.

Parameters measured and arrhythmias analysis

Systolic and diastolic blood pressure (BP) and mean arterial blood pressure (MAP) were measured from the arterial BP trace. Heart rate

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Table 1. Effects of hydroalcoholic extract of *Stachys inflata* on ischaemic arrhythmias

Arrhythmia	Saline (control) n=10	Hydroalcoholic extract 1.35 μg/kg/min; n=9	Hydroalcoholic extract 2.7 μg/kg/min; n=10
Arrhythmia counts			
Total VEBs*	516±150	684±188	502±147
VT	293±87	267±79	217±33
Duration (sec)			
VT	61±19	50±18	65±13
Reversible VF	125±38	119±46	151±62
Incidence (%)			
VT	100	100	100
Reversible VF	100	100	100
Irreversible VF	30	33	40
Total VF	100	100	100

*Total VEBs is sum of arrhythmias o ccurring as single extrasystoles , salvos, and VT

was calculated from the ECG. Both the ECG and BP were continuously recorded on a Narko (MK-III-S) physiograph. Ventricular arrhythmias were analysed according to the guidelines of the Lambeth Conventions for the determination of experimental arrhythmias (19), which were identified as single ventricular ectopic beats (VEBs), salvos (couplets and triplets), and ventricular tachycardia (VT, defined as a run of four or more consecutive ectopic beats). The total number of ventricular arrhythmias was calculated as the sum of these three types of arrhythmia. The incidences of VT and reversible and irreversible ventricular fibrillation (VF) were determined for each group.

Assessment of hemodynamic responses to the extract

To determine the effect of the hydroalcoholic extract of *Stachys inflata* on heart rate and arterial blood pressure, a separate group of rats (n=10) was prepared for the measurement of HR and arterial BP. Then a cumulative dose-response curve to bolus injection of the extract (equal to 60-360 μg/kg caffeic acid) was constructed. The doses of the extract were given at 15 minutes intervals.

Statistics

Except for the incidences of VT and VF, all results are shown as mean±SEM. We used the Mann-Whitney nonparametric U test to compare the number of VEBs and infarct size between groups and the Fisher-Irwin (Chi square with Yates correction) to compare incidences of VT and VF. We assessed MAP and HR data by one-way analysis of variance and examined significant differences by the Neuman-Keuls range test. Differences between groups were considered significant at $p<0.05$.

Results

Effects of Stachys inflata *on arrhythmias after coronary artery occlusion*

Acute occlusion of the left main coronary artery in both control and treated anaesthetized rats resulted in immediate ST-segment changes and arrhythmias began ~4-5 min after occlusion reached a peak of activity at ~9-12 min, and then declined by 15-20 min after occlusion. Table 1 summarises the effects of *Stachys inflata* extract on total arrhythmia count after coronary artery occlusion.

Compared to the control group, hydroalcoholic extract of *Stachys inflata* at both doses of 1.35 and 2.70 μg/kg/min produced no

Table 2. Haemodynamic responses in anaesthetised rats given cumulative doses of the hydroalcoholic extract of *Stachys inflata* (n=10).

	The extract cumulative concentration (μg/kg)						
	0	**60**	**120**	**180**	**240**	**300**	**360**
Sys	91±10	93.5±12	96.5±13	91±15	80±17	84±14	77±16
Dias	43±10	71±10	73±11	68±13	58±16	59±15	55±19
MAP	78±10	79±11	81±11	76±14	65±16	67±14	62±18
HR	331±16	319±18	295±21	294±25	276±26*	269±26*	255±30*

*$p<0.05$ denotes significant differences within group, from pre -injection value (i.e at concentration zero). (Sys) Systolic, (Dias) diastolic, (MAP; mmHg) mean arterial blood pressure and (HR; beats/min) heart rate.

Table 3. The effect of the hydroalcoholic extract of *Stachys inflata* on myocardial infarct size after 30 minute coronary artery occlusion and 2 hours reperfusion.

Groups	n	Area at risk (mg)	Infarcted tissue (mg)	Infarct size (%)
Control	9	559±40	280±10	48.2±1
Extract (1.35 μg/kg/min)	8	517±39	161±25*	29.3±2.7*
Extract (2.7 μg/kg/min)	10	576±20	269±30	46.7±3.4

*p<0.001 compared to control

effect on either total number of ectopic beats (VEBs) or on those occuring as VT (Table 1). The number of single extrasystoles and salvos was not affected by hydroalcoholic extract. There was no significant change in the total incidence of VF, the time spent in reversible VF or VT and mortality due to irreversible VF by any dose of the extract.

Haemodynamic responses to hydroalcoholic extract of Stachys inflata

The haemodynamic responses to hydroalcoholic extract of *Stachys inflata* are summerised in Table 2. In anaesthetised rats, I.V cumulative bolus injections of the extract of *Stachys inflata* (from 60 to 360 μg/kg) had no significant effect on either systolic or diastolic and on the mean arterial blood pressure (MAP) compared to pre-injection values. Beside of a slight but significant decrease in the heart rate at the higher doses of 240 - 360 μg/kg of the extract none of the low doses altered the heart rate significantly. Mean arterial blood pressure and heart rate were also measured before and during coronary artery occlusion and reperfusion. There was no significant difference between groups in heart rate or mean arterial blood pressure at any time point during extract infusion. Coronary artery occlusion in control and in rats receiving extracts resulted in a slight (insignificant) and transient reduction in blood pressure which continued to the end of the experiments. In spite of a slight reduction in blood pressure during infusion of the extract and coronary artery occlusion, heart rate in all groups was unchanged from the pre-occlusion value.

The effects of hydroalcoholic extract of Stachys inflata on myocardial infarct size

Table 3 shows the measurements obtained from post-mortem analysis of heart tissue in the control and rats given the hydroalcoholic extract of *Stachys inflata* (1.35 and 2.70 μg/kg/min) and subjected to 30 minutes coronary artery occlusion followed by 2 hours reperfusion. Area at risk in all groups was similar. The extract at a low dose of 1.35 μg/kg/min reduced markedly ($p<0.001$) either the absolute weight of the infarcted tissue, or the infarct size expressed as a percentage (by weight) of the area at risk. The higher dose of the extract had no effect on the infarct size.

Discussion

Hydroalcoholic extract of *Stachys inflata* has been shown to exert potent anti-inflammatory activities in peripheral inflammation induced by carrageenan or formalin in rat paw (1). In the current study we evaluated the effects of two doses of hydroalcoholic extract of *Stachys inflata* in the rat model of regional ischaemia on infarct size, measured histochemically 2 hours after reperfusion.

Low dose intravenous extract administered during coronary occlusion resulted in a striking reduction ($p<0.001$) in myocardial infarct size arising from a subsequent period of myocardial ischaemia (30 min) followed by reperfusion (120 min). As can be seen from the data, the area at risk in both control and treated groups was similar, thus excluding the possibility of any marked difference between the groups. The other main determinants of infarct size, heart rate and blood pressure, were not also significantly different between control and the group which received low dose of the extract. Furthermore, infusion of the extract during a period of acute myocardial ischaemia had no effect on the incidence and severity of the consequent ventricular arrhythmias, implying that the protective action of extract against ischemia/reperfusion injury was unlikely to be a direct effect on myocardium.

There is now good evidence that ischemia/reperfusion injury is associated with an increased surface expression of adhesion

molecules, which leads to leukocyte recruitment into the cardiac tissue and inflammation (20, 21, 22). Our previous work (1) indicated that anti-inflammatory activities of hydroalcoholic extract of *Stachys inflata* in carrageenan-induced model of local inflammation were partly due to the inhibition of neutrophil infiltration, supporting the hypothesis that extract may have led to a smaller infarct size by decreasing the inflammatory response and inhibitory effect on neutrophil accumulation. Phenylethanoid glycosides, triterpenoids and flavonoids were considered to be the active components responsible for the biological actions of genus Stachys (10, 12, 13, 14) and it has been demonstrated that acteoside, a phenylethanoid glycoside of Stachys sieboldii, has suppressive effect on the accumulation of leukocytes in the nephritic glomeruli through the prevention of the up-regulation of adhesion molecules (8).

In the present study, the extract was capable of reducing infarct size only with low dose. The high dose of extract did not alter infarct size. This would agree with the findings of Maleki and co-workers (1), who showed that the higher dose of the extract had no anti-inflammatory effects. The discrepancy between the inhibitory effect upon low doses and the ineffectiveness of the higher dose of the extract might be explained by this hypothesis that some of the active constituent(s) of *Stachys inflata* at high concentration may exhibit pro-infract properties. It is also likely that the extract may have components with different anti- and pro- infarct effects.

In conclusion this study demonstrates that the treatment of rats with hydroalcoholic extract of aerial parts of *Stachys inflata* caused a pronounced reduction in myocardial infarct size arising from regional ischaemia (30 min) and reperfusion (120 min). The extract had no effect on the severity and incidence of ischaemic arrhythmias or on the heart rate and blood pressure. Therefore, the cardioprotective effects of the extract are likely due to the inhibition of inflammatory component at the site of reperfused area. Considering the higher dose of the extract was unable to reduce the infarct size, it can be concluded that the component(s) of the extract may have pro-infarct effects at high doses or the extract contains constitute(s) with different protective and non-protective action against ischaemia/reperfusion induced damage.

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