The effect of angiotensin converting enzyme inhibition on gene expression of VCAM-1, VEGF and serum level of nitric oxide in normocholestrolemic and hypercholestrolemic male rats

ABSTRACT:

Background: There are growing evidences revealing the fact that the renin – angiotensin system involves in many cardiovascular diseases, including atherosclerosis. This system can be activated during hyperlipidemia and angiotensin II leads to increase in migration of monocytes, cytokine levels and gene expressions of VEGF and VCAM-1. VEGF has a role in the development of atherosclerosis and VCAM-1 is involved in monocyte recruitment and increase in atherosclerotic plaque formation. The relationship between nitric oxide and angiotensin II is controversial. In the present study, angiotensin II formation was inhibited by angiotensin converting enzyme inhibitor and gene expression of VCAM-1 and VEGF and nitric oxide levels in serum of hypercholesterolemic and normocholesterolemic rats were evaluated.

Methods: Forty male Wistar rats were divided into 4 groups including; normal diet + saline injection (control), hypercholesterol diet + saline injection, normal diet + captopril injection and hypercholesterol diet + captopril injection. The hypercholesterolemia diet (rat chow supplemented with 4% cholesterol and 1% cholic acid) were used for 8 weeks. After confirming the development of hypercholesterolemia, captopril (50 mg/kg) and saline were intraperitoneally injected for 6 weeks. Before and after starting the diet and after the i.p. injections, blood was taken from the retro-orbital sinus of the animals' eyes and serum levels of cholesterol, triglycerides, LDL, HDL, and nitric oxide were measured. At the end of the diet period, the animals were anesthetized with ether and the thoracic aorta was isolated. After tissue homogenization, RNA was extracted and gene expressions of VCAM-1 and VEGF were determined by Real Time PCR method.

Results: After cholesteroldiet, the serum levels of cholesterol, triglycerides and LDL were increased and HDL was decreased compared to the control group. After captopril injection, a reduction in the serum levels of cholesterol, triglycerides, and LDL as well as a raise in HDL levels were observed compared to the control group. VCAM-1 and VEGF gene expressions increased after hypercholesterolemia diet and showed a decrease after captopril injection. Serum nitric oxide levels decreased after hypercholesterolemia diet but no significant change was observed after captopril injection.

Conclusion: Angiotensin converting enzyme inhibitors cause a dramatic reduction of atherosclerosis progression process that may take effect through inhibiting expression of genes associated with inflammation and angiogenesis.

Keywords: Atherosclerosis, hypercholesterolemia, renin – angiotensin system, VCAM-1, VEGF, nitric oxide

THE EFFECTS OF HYDROALCHOHOLIC EXTRACT OF ACHILLEA WILHELMSII ON FUNCTIONAL ACTIVITIES AND OXIDATIVE STRESS INJURY DURING ISCHEMIC-REPERFUSION IN ISOLATED RAT HEART

ABSTRACT:

Background: Damaging effects of oxidative stress on myocardial ischemia - reperfusion has been shown in previous studies. Because that Achillea also has antioxidant properties and protective function of the heart, The aim of this study was to investigate the effect of aqueous - alcoholic extracts of *Achillea wilhemsii* activities in both functional and oxidative stress injuries induced by ischemia-reperfusion injury in the isolated rat heart .

Method: In this study, male Wistar rats (250- 280gr) who were randomly divided into 6 groups (n= 10. In each group). According to the study design, animals received normal saline, vitamin C or Achillea wilhemsii extract orally by gavage for 4 weeks. 1) Control group: saline, 2) group of -control- ischemia (CI) 3) vitamin C (10mg/kg), 4-6) Extract groups (E 100, E 200 and E 400 mg/kg). At the end of the treatment after anesthesia, animals were euthanized and the hearts were removed and mounted on a Langendorff system being perfused by oxygenated Krebs. After 20 min of stabilization, hearts were subjected to 20 min of global ischemia followed by 40 min of reperfusion. Evaluated parameters were measured using a pressure transducer connected to a data acquisition system (Power Lab). At the end of the record, the heart tissue samples were stored at -80 ° C for evaluating oxidative stress markers; malondialdehyde (MDA), the total thiol groups (-SH), superoxide dismutase (SOD) and catalase (CAT). Also coronary flow was collected from heart tip, before ischemia and during the first 10 minutes of reperfusion and then was maintained at -80 ° C for measuring the cardiac enzymes of lactate dehydrogenase (LDH) and Ceratin kinase (CK) were used .

Results:. 2 minutes after reperfusion in the group of E 400 heart rate was significant increased compared to the CI group and other groups ($p<0.05$). Maximum left ventricular pressuer (Max LVP) showed a significant increase in E 100 group compared to the CI group ($p<0.01$). Significant increasing trends were observed in left ventricular developed pressure (LVDP) and Max LVP parameters in group E 100 compared to CI group ($p<0.05$). Max dp / dt, Min dp / dt and rate-pressure product (RPP) had increasing trends in E 100 group compared to CI group. Coronary flow was increased in E 200 group than other groups, but this increase was not significant. The MDA levels of heart tissues in all three groups of extract were reduced significantly compared to CI group ($p<0.001$). An Increases was observed in tissue thiol (-SH) level of E 400 group in comparison to CI group ($p<0.05$). The SOD and CAT levels were significantly increased in E 200 and E 400 groups compared to CI group ($p<0.01$ and $p<0.001$ respectively). 2 minutes after reperfusion LDH enzyme level in group of E 400 was significantly reduced in comparison to CI group ($p<0.01$). In the same time, CK enzyme levels showed significant decrease in all three group of extract ($p<0.05$ to $p<0.001$).

Conclusion: The results of this study showed that the extract of *Achillea wilhemsii* has protective effects against ischemia-reperfusion injuries in isolated rat heart. As well as improves cardiac dysfunction induced by ischemia - reperfusion that according to the findings of this research, improvement could be associated with antioxidant properties of *Achillea wilhemsii*.

Keywords: *Achillea wilhemsii*, ischemia-reperfusion, oxidative stress, rats

THE EFFECTS OF HYDROALCHOHOLIC EXTRACT OF TEUCRIUM POLIUM L.ON APOPTOSIS INDUCED BY ISCHEMIA-REPERFUSION IN ISOLATED RAT HEART

ABSTRACT:

Objectives:

Considering the recent studies, Ischemic Heart Disease (IHD) is the most important cause of mortality in most countries around the world. As reduction of coronary blood flow leads to IHD, restitution of blood flow (reperfusion) of the affected area is the only logical treatment for this condition. However, reperfusion itself has been shown to exacerbate myocardial cellular injury, popularly known as ischemic-reperfusion (I/R) injury which itself is one of the underlying factors facilitating and accelerating the apoptosis in the myocardium. The aim of this study was to investigate the effects of *Teucrium polium* hydro-alcoholic extract on I/R induced apoptosis in the isolated heart in the Wistar rats.

Methodology:

60 Wistar rats divided into 6 groups (n=10 in each group);1. Control group perfused for 80 minutes 2. Control- ischemia group: 20 minutes Krebs, 20 minutes of ischemia and 40 minutes reperfusion with Krebs & 3-6: treated groups: 20 min perfusion with different doses of the extract (0.5, 1, 2 mg/kg) and vitamin C, 20 min ischemia and 40 min reperfusion with different doses of the extract and vitamin C. Cardiodynamic parameters were measuredby Power Lab and recorded by Lab Chart software. .then the heart samples were stored at -80 till using them for evaluating the expression of related involved genes of apoptosis (Bax and BCl2) by Real Time PCR.

Findings:

Teucrium polium extract caused the increase of HR, Max LVP, LVDP, RPP, +dp/dt, and decrease of Min LVP, -dp/dt. The gene expression level of Bax that was elevated in control-ischemia group showed a significant decrease in extract groups. While that of Bcl2 gene and the Apoptosis index increased. The findings showed that the extract of 0.5 mg / kg have the greatest effect in improving cardiodynamic parameters as well as in gene expression pattern.

Conclusion:

Finally, based on previous studies and current study it can be stated that hydro-alcoholic extract of *Teucrium polium* with positive inotropic and -to some extents- chronotropic effect having been able to improve heart function in ischemia-reperfusion lesion process. In addition, the present study demonstrated that *Teucrium polium* extract reduced the gene expression of pro-apoptotic gene , Bax and augmented the gene expression levels of Bcl2, as an anti-apoptotic gene and therefore the index of apoptosis relative gene expression (Bcl2 / Bax) was increased by this extract. So perhaps improvment of the cardiodynamic parameters in this model could be attributed to anti-apoptotic effect of *Teucrium polium*.

Keywords:

Ischemia-reperfusion, Apoptosis, *Teucrium polium*, Isolated heart.

THE EFFECTS OF HYDROALCHOHOLIC EXTRACT OF TEUCRIUM POLIUM ON FUNCTIONAL ACTIVITIES AND OXIDATIVE STRESS INJURY DURING ISCHEMIC-REPERFUSION IN ISOLATED RAT HEART

ABSTRACT:

Background: The deleterious effect of oxidative stress on myocardial ischemia-reperfusion has already been shown in previous studies. Since *Teucrium polium L. (TP)* has anti-oxidative and cardio-protective properties, the aim of this study was to investigate the effects of aqueous-alcoholic extract of *TP* on ischemia-reperfusion induced oxidative stress and functional injuries in the isolated rat heart.

Method: Eighty male Wistar rats were randomly divided into 8 groups as follows:
Control, 2) Control-ischemia, 3) Prevention extract 0/5mg/ml, 4) Prevention extract 1mg/ml, 5) Prevention extract 2mg/ml, 6) Treatment extract 0/5mg/ml, 7) Treatment extract 1mg/ml, 8) Treatment extract 2mg/ml. On the experiment day, the animals were anaesthetized and their hearts were removed and put on the Langendroff apparatus and were perfused by oxygenated Krebs solution. After 20 minutes of stabilization, the hearts were subjected to 20 minutes of global ischemia followed by 40 minutes of reperfusion. In prevention groups, after 20 minutes of stabilization, there was a 20-minute perfusion of the extract followed by 20 minutes of global ischemia and after those 40 minutes of perfusion of Krebs solution. In treatment groups, after 20 minutes of stabilization, the hearts were subjected to 20 minutes of global ischemia followed by 40 minutes of reperfusion by the extract. Evaluated parameters were measured using a pressure transducer connected to a data acquisition system (Power Lab). At the end of the record, the heart tissue samples were stored at -80 ° C for evaluating oxidative stress markers including; malondialdehyde (MDA), total thiol groups (-SH), superoxide dismutase (SOD) and catalase (CAT). Also coronary flow was collected from heart tip, before ischemia and during the first 10 minutes of reperfusion and maintained at -80 ° C for measuring the cardiac enzymes of lactate dehydrogenase (LDH) and ceratin kinase (CK).

Results: 2 minutes after the reperfusion in both prevention and treatment groups of the extract 2mg/ml, there was a significant decline in the heart rate compared to that of control-ischemia group's, ($p<0/001$ and $p<0/01$ respectively). Maximum left ventricular pressure (Max LVP) in prevention and treatment groups of extract 0/5 mg/ml were significantly increased compared to control-ischemia group, ($p<0/01$).
left ventricular developed pressure (LVDP) and Max LV in both prevention and treatment groups of extract 0/5 mg/ml showed significant increasing trends compared to control-ischemia group, ($p<0/05$, $p<0/01$ respectively). Max dp.dt and Min dp.dt and Rate Pressure Product in both prevention and treatment groups of extract 0/5 showed significant increasing trends compared to control-ischemia group.
The coronary flow in all prevention and treatment extract groups were increased compared to that of control-ischemia group.
The MDA level of the heart tissues in prevention extract group 2 mg/ml and treatment extract group 1,2 mg/ml were significantly lower than that of control-ischemia group, ($p<0/01$, $p<0/05$ and 0/001 respectively). Significant increases were observed in tissue thiol level (-SH) of prevention and treatment extract groups 1, 2 mg/ml compared to control-ischemia group, ($p<0/05$, $p<0/01$ respectively).
The SOD level in prevention extract group 1,2 mg/ml and treatment extract group 2mg/ml were significantly increased compared to those of control-ischemia group, ($p<0/01$, $p<0/05$ respectively).
The catalase level in prevention and treatment extract groups 0/5 and 2 mg/ml were higher than that of control-ischemia group, ($p< 0/05$, $p<0/05$ and $p<0/001$ respectively).
The LDH level in prevention extract group 0/5 and 2, (4 minutes and 8 minutes after the reperfusion respectively) and in treatment group 0.5, 2 minutes after the reperfusion were significantly lower than that of control-ischemia group, ($p<0/05$ and $p<0/01$, $P<0/05$ respectively).
The CK-MB level in prevention 1,2 and all treatment groups, 2 minutes after the reperfusion, were remarkably lower than control-ischemia group, ($p<0/01$, $p<0/001$).

Conclusion: The results of this study showed that the extract of *Teucrium polium* has protective effects on ischemia-reperfusion injuries in isolated rat heart and can improve the ischemia-reperfusion induced functional impairments which might be due to anti-oxidative properties of the *Teucrium polium* based on this study.

Keywords:
Teucrium polium, ischemia-reperfusion, oxidative stress, rats