Original Article

The Combined Effects of Swimming Exercise and Garlic Extract on some Mediator Factors on the Cardiac Angiogenesis and Fibrosis in aged Rats

Habibian Masoumeh[1*], Asadi Mohammad Ali

Received:11/21/2015 Revised:2/28/2016 Accepted:3/7/2016

1.Dept of Physical Education and Sports Sciences, Islamic Azad University, Qaemshahar Branch, Qaemshahar, Iran
2.Dept of Physical Education and Sports Sciences, Islamic Azad University, Sari Branch, Sari, Iran

Pars Journal of Medical Sciences, Vol. 13, No.4, Winter 2016

Pars J Med Sci 2016;13(4):39-46

Abstract

Introduction:
Aging is a major risk factor for gradual structural and functional damage to the heart and is associated with different changes in angiogenesis and cardiac fibrosis. The present study was conducted to determine the combined effect of swimming exercise and garlic extract on some of the factors mediating angiogenesis and cardiac fibrosis in old rats.

Materials and Methods:
A total of 35 old Wistar rats were randomly divided into a control, a saline, an aerobic exercise, a garlic and an aerobic exercise plus garlic group. The rats were given an exercise regimen of swimming 60 minutes per day and three days per week for eight weeks. The aerobic exercise and the aerobic exercise plus garlic groups were treated with 2.5 g/kg of body weight garlic extract. The pre- and post-intervention levels of Vascular Endothelial Growth Factor (VEGF) and Transforming Growth Factor beta 1 (TGF-β1) were measured in all the groups. The data obtained were analyzed using the one-way ANOVA at a significance level of $P<0.05$.

Results:
The results obtained showed a significant increase in VEGF and a significant reduction in TGF-β1 in the old rats following eight weeks of swimming, garlic supplementation and their combination ($P<0.05$). However, these interventions were not significantly different from each other in the effects exerted on VEGF and TGF-β1 in the old rats ($P>0.05$).

Conclusion:
Each of the non-medicinal interventions of either regular exercise, garlic supplementation or their combination is likely to reduce age-related changes in angiogenesis and cardiac fibrosis through the positive regulation of VEGF and the reduction of TGF-β1.

Keywords: Aging, Angiogenesis, Heart Tissue, Fibrosis, Exercise

Introduction

Aging is associated with increased incidence of cardiovascular diseases, including heart attacks (1) and reduced ischemia tolerance (2). Disruption of angiogenesis frequently occurs in old cells or tissues, such that reduced angiogenesis in older adults is associated with increased incidence of myocardial infarction and other cardiovascular diseases (3), impairment in structural changes of vascular networks with ischemic tissues and reduced recovery in the damaged

* Corresponding author, Address: Dept of Physical Education and Sports Sciences, Islamic Azad University, Qaemshahar Branch, Qaemshahar, Iran
Tel: 09111285726 Email: habibian_m@yahoo.com

[DOI: 10.29252/jmj.13.4.39]

tissue (4, 5). Vascular endothelial growth factor (VEGF) is the most potent stimulator for angiogenesis (3) that stimulates endothelial cell proliferation and differentiation and increases vascular permeability. It also mediates endothelium-dependent vasodilation and supports vascular survival by inhibiting vascular endothelial apoptosis (6). Downregulation of VEGF is considered one of the important factors in age-induced angiogenesis damage (4, 5). In cardiac myocytes, VEGF is decreased with aging (7). On the other hand, aging of the heart is associated with structural and morphological changes which can lead to decreased cardiac function, left ventricular hypertrophy (LVH), resulting in heart failure (8); thus aging plays an important role in cardiac fibrosis (9). In cardiovascular system age-dependent sedimentation and increased collagen in vessel walls, interstitial space and cardiac perivascular lead to decreased cardiac and arterial compliance (8). Transforming growth factor beta-1 (TGF-β1) is a potent stimulus for collagen synthesis (10) and plays an important role as a multifunctional cytokine in cell migration, proliferation, differentiation, apoptosis and extracellular matrix protein production (11). TGF-β signaling pathway is a central regulator in cardiac fibrogenesis and may also positively regulate pro-angiogenesis pathway (9). In addition, TGF-β1 has hypertrophic effects on the heart and modulation of inflammatory cell function (8). Previous studies reported overexpression of TGF-β1 in the heart of transgenic rats associated with hypertrophy and cardiac fibrosis (12) and also considerable TGF-β1 values and atrial fibrosis in older rats compared to younger ones (13), thus, it is essential to find new ways for reducing fibrosis and increasing angiogenesis in the cardiac muscle tissue of older adults (3).

Continuous aerobic exercises are considered the first treatment line to reduce the risk of coronary artery disease along with aging, such that reduced stiffness in large elastic arteries is observed in middle-aged and older adults who perform regular aerobic exercise compared to sedentary people (14, 15). According to researchers, exercise improves downregulation of angiogenic signaling cascade in the heart and reduced capillary density due to aging (4), such that increased expression of protein and VEGF mRNA in young exercised mice (16) and decreased expression of TGF-β1, carotid artery stiffness in old mice (17) are observed. However, the effect of swimming is very limited on factors affecting the levels of fibrosis and angiogenesis (TGF-β1 and VEGF) in old hearts. On the other hand, garlic and its products have been generally known as a non-pharmacological method to prevent and treat cardiovascular diseases (18, 19). The results of both experimental and clinical studies suggest that garlic has favorable effects on cardiovascular system (20). It has been observed that using garlic extract is associated with reduced diastolic dysfunction and cardiac fibrosis (21). Thus, due to changes of cardiac fibrosis and angiogenesis processes caused by aging (4, 5, 8) and supportive role of exercise (14, 15) and garlic (20, 21) against cardiovascular diseases and also reduction of the atherosclerosis risk, the present study was conducted to examine the combined effect of regular swimming and garlic extract on the values of some growth factors involved in angiogenesis and cardiac fibrogenesis in old rats.

Materials and Methods

The present experimental study was conducted with post-test research design with a control group on 35 adult Wistar male rats aged 48 to 50 weeks old (over a year) weighing 250-300 g. Rats were reared, and after they were moved to the new environment and were first classified with 10-gram differences in weight in different cages. Then, a rat was randomly selected as a sample and was placed in one

[DOI: 10.29252/jmj.13.4.39]

of the control, saline, aerobic exercise, garlic and aerobic exercise + garlic groups (with nearly same weight values) to determine the experimental and control groups from the rats in each cage (seven rats per group). During the study, the rats were housed in cages made of transparent polycarbonate with dimensions of 42×26.5×15 cm, at the temperature of 22± 2 °C, with humidity 55±5% and on a 12 h light-dark cycle with well ventilation and freely access to water and food pellets (produced by Behparvar Karaj). All stages were conducted with respect to organizational instructions regarding the care and use of animals and with the approval of the Ethics Committee with License No. 20821404932013 in the Islamic Azad University, Sari.

Training Protocol

The rats were familiarized with water in the workout groups a week before the start of the main protocol for five minutes a day, five days a week. The main exercise program was 60 minutes swimming in a tank specific for rodents, from 10-12 am three days per week for eight weeks (22). Five minutes was considered for warming and cooling the rats before and after exercise. The water temperature was also 32±2 °C. After swimming on each session, the rats were dried by a hot air flow using a heater specific for rodents.

Preparation of Garlic Extract

After cleaning and crushing old garlic, it was kept at normal temperature and humidity for three months and was extracted by maceration. For extraction, 50 grams of crushed garlic was mixed with methanol at a ratio of 1 to 3, and the solution was filtrated after 24 hours by filter paper and a Buchner funnel. Then the filtered solution was distilled in a vacuum distillation at a temperature of 50 °C until the residual volume reached a fifth of its initial volume. The resulting solution was decantated three times with chloroform (50 ml) and was dried in an oven at 50 °C. Powdered extract was mixed with distilled water and was gavaged at a rate of 2.5 g/kg bw to supplement and supplement training groups (23). Saline group was gavaged by saline with the same values as the supplement group.

Biopsy and Homogenization

The rats were anesthetized by intraperitoneal injection of ketamine (90 mg/kg) and xylazine (10 mg/kg) 72 hours after the last interventions. After the thoracic cavity was incised, the cardiac muscle tissue was carefully separated and stored at 70 °C until laboratory studies. After the cardiac muscle tissue was homogenized in phosphate-buffered saline, the levels of VEGF and TGF-ß1 were measured by ELISA and special kits with a sensitivity of less than 5.01 ng/l and 2.1 pg/ml.

Statistical Analysis

Shapiro-Wilk and Levene tests were used to examine the normality of data distribution and homogeneity of variances. The mean data were compared by one-way analysis of variance and in the case of significant difference between groups, Tukey's test was used to determine the difference. Statistical analysis was performed by SPSS-20 at a significance level of $P<0.05$.

Results

The results of Shapiro-Wilk and Levene tests ($P=0.485$ for VEGF and $P=0.417$ for TGF-ß1) implied normal distribution of data and homogeneity of variances, respectively. Given calculated F value (8.955 and 7.539, respectively) from one-way ANOVA, a significant difference was observed between the mean values of cardiac VEGF and TGF-ß1 of old rats after eight weeks. The results of Tukey's test also showed that after eight weeks, the levels of cardiac VEGF were significantly increased in rats of workout group, garlic and garlic + exercise (577.14 ± 48.98, 556.00 ± 40.28 and 596.14 ± 36.73 ng/g of protein, respectively) compared to the control group (482.43 ± 55.94 ng/g of protein) (19.60%, P=0.005, 15.24%, P=0.041, and 23.54%, P=0.001, respectively; Figure 1).

But, eight weeks of swimming, using garlic supplementation and the combination of both interventions significantly reduced the values of TGF-ß1 (323.28±48.82, 336.71±38.95 and 305.00± 58.11 pg/mg of protein, respectively) compared to the control group (406.43± 30.46 pg/mg of protein) in old rats (20.46%, P=0.011, 17.15%, P=0.043, and 24.95%, P=0.001, respectively; Figure 2). In addition, no significant difference was observed between the effects of exercise, garlic supplementation and the combination of both interventions on increased VEGF or decreased TGF-ß1 in old rats (P>0.05).

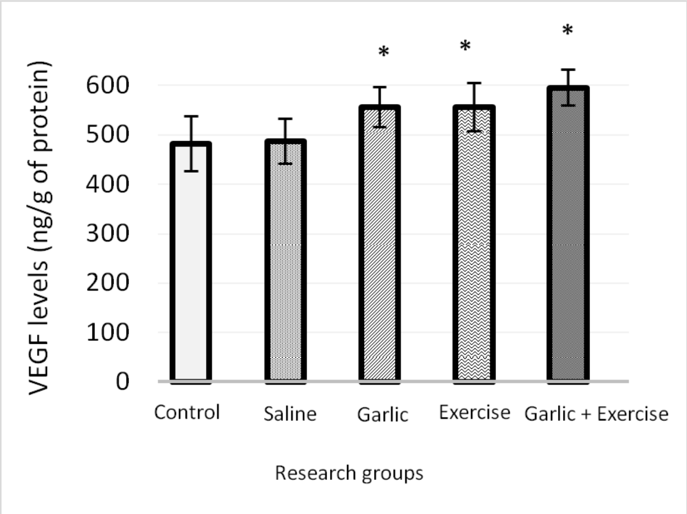

Figure 1: Comparison of cardiac VEGF in different research groups
*: Significant difference between control and saline groups. The data is based on the mean and standard deviation.

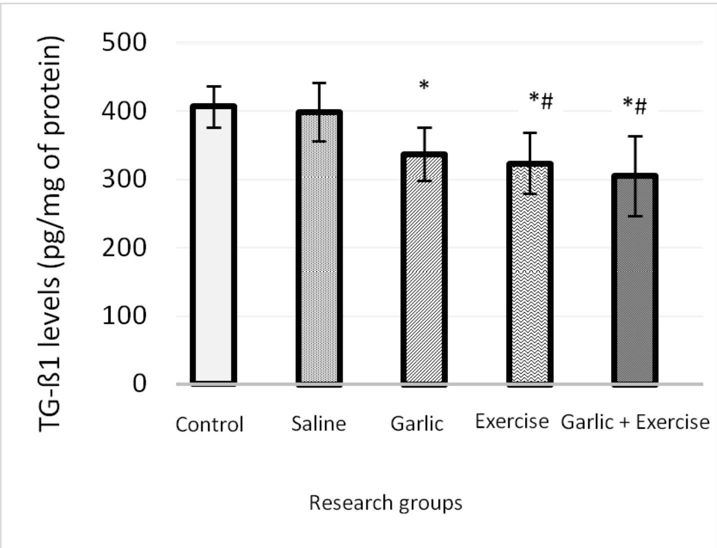

Figure 2: Comparison of TG-ß1 values in different research groups
*: Significant difference between control and saline groups; #: significant difference compared to the saline group.
The data is based on the mean and standard deviation.

[DOI: 10.29252/jmj.13.4.39]

Discussion

Today, access to information seems necessary for management of age-induced structural changes and cardiac dysfunction (24). In this study, the effect of swimming aerobic training on the values of VEGF and TGF-ß1 was examined in old rats' cardiac muscle tissue. According to the results, eight weeks of aerobic exercise, using garlic supplementation and the combination of both interventions resulted in a significant decrease in TGF-ß1 and increase in cardiac VEGF in old rats. The evidence shows that cardiac fibrosis and heart muscle stiffness in mice with cardiac defect of TGF-β1 was reduced suggesting the effect of TGF-β intervention on the synthesis of extracellular matrix in the heart (25). In addition, Ahluwalia et al. reported that aging is associated with 3.7-fold reduction in angiogenesis, 3-fold reduction in VEGF, 24-month old Fischer rats F-344 (26). Thus, it seems that interventions of the present study can have protective effects on the cardiac muscle tissue of old rats by reducing cardiac fibrosis and suppressing possible side effects, and also increased angiogenesis. In this regard, Wagatsuma et al. reported increased levels of VEGF with increasing hypoxia-inducible factor 1-alpha in the cardiac muscle tissue of rats trained to swim after a 9-day period of swimming (50 minutes daily) (27). Similarly, other researchers reported a significant increase in molecular expression and cardiac VEGF in old rats (23 months old) which was significantly lower than sedentary young rats after swimming for eight weeks (4) or reduction of increased TGF-ß1 in left ventricle tissue in old rats (31 months old), after twelve weeks training for 45 minutes per session, five days a week (28). In addition, Feliner et al. showed that 10 to 14 weeks of moderate-intensity physical activity on rotating wheels resulted in reversing the increased stiffness of carotid artery in old rats leading to decreased collagen I and III in the expression of TGF-β1, while it did not affect young rats (17), but no change was reported by other researchers in myocardial tissue of protein expression of TGF-β1 in young male Wistar rats eight weeks after running on a treadmill (29); a possible cause of this contradiction may be due to younger subjects and normal distribution of myocardial TGF-β1 values. Although, in the present study, the values of VEGF and myocardial tissue of TGF-ß1 in old rats were not compared with young rats, which is one of the limitations of this research, it seems that some of the desired effects caused by research interventions on the cardiac muscle tissue of old rats can be moderated through myocardial VEGF and TGF-ß1 values which can lead to improved angiogenesis and reduced myocardial fibrosis.

Although the exact mechanisms of exercise on regulating myocardial TGF-β1 and VEGF values are not well determined, the activation of VEGF is mediated by hypoxia-inducible factor 1-alpha. On the other hand, it was observed that the reduction of nuclear transfer in hypoxia-inducible factor 1-alpha is likely lead to a reduction in the activation of VEGF (most potent stimulation of vascular endothelial in myocardial microvascular endothelial cells) and impaired angiogenesis in the old heart (26). Angiogenesis response to VEGF is also mediated to some extent by endothelial nitric oxide synthase, such that angiogenesis induced by VEGF was impaired in rats with eNOS _ / _ (30). VEGF signaling pathway is the largest stimulating factor for physiological and pathological angiogenesis and physical activity leads to an increase in capillary density, the ratio of capillaries to myocytes, and increased myocardial VEGF and its receptor. Increased VEGF binding to the angiogenic receptor leads to increased phosphorylation of endothelial nitric oxide synthase through the activation of AKT and ultimately increased angiogenic process in trained old rats (4). In addition, VEGF also stimulates the

[DOI: 10.29252/jmj.13.4.39]

activation of matrix metalloproteinase-2, which has an important role in angiogenesis and can reduce collagen IV in the basement membrane and abnormal collagen type I and III (the main components of cardiac extracellular matrix) (31). TGF-β may also play an important role in cardiac fibrosis associated with aging through induced differentiation of myofibroblasts and increased matrix protein synthesis through cardiac fibroblasts (32). On the other hand, TGF-β also leads to inhibition and stimulation of their inhibitors and myofibroblasts. Metalloproteinase matrix (MMPs) leads to the destruction of collagen, while their inhibitors and myofibroblasts inhibit destruction resulting in increased collagen synthesis (24). On the other hand, reactive oxygen species (ROS) and angiotensin II may lead to the activation of TGF-β1 signaling pathways and positive regulation of downstream fibrogenic effectors in the old heart (33). Physical activities with increasing shear stress increase the expression and activity of endothelial nitric oxide synthase and moderate the production of nitric oxide. Exercise may also lead to the moderation of TGF-β1 and VEGF in cardiac muscle tissue of old rats by increasing antioxidant activity, reducing oxidative stress and inflammation.

Another important finding of this research was reduced TGF-β1 and increased VEGF values after using garlic extract that these effects were significantly higher in the combined intervention with swimming. In this regard, Hara et al. showed that the effect of garlic extract during 18 weeks leads to improved performance and less increased left ventricular mass and reduced interstitial fibrosis in rats sensitive to salt with a salt-rich diet (21). In reviewing other additives, Wang et al. reported that the oral consumption of curcumin is associated with downregulation of TGF-β1 expression in myocardial infarction (34). Risi et al. also observed a reduction in increased TGF-β1 values and cardiac oxidative stress after eight weeks of treatment with tempol in hypertensive rats (35). Although no study was found on the effect of garlic extract on the parameters in the present study, especially in terms of aging, which is another limitation of this study, it was well confirmed that garlic due to phytochemicals, such as S-allyl mercaptocysteine (SAMC), alliin and diallyl disulfide is an important source of antioxidant (36, 37). Cardioprotective effects of garlic were confirmed in numerous studies conducted in vitro on primary myocytes, fibroblasts and cardiac endothelial cells cultured by reducing the production of reactive oxygen species and blocking reactive oxygen species extracellular signal-regulated kinase dependent on nuclear factor kappa B (NF-kB) (37, 38), therefore, it seems that using garlic extract similar to swimming leads to increased VEGF and downregulation of myocardial TGF-ß1 in old rats by reducing oxidative stress, inflammation or increasing the antioxidant capacity.

Conclusion

The findings suggest the favorable role of swimming and using garlic extract in reduced VEGF and increased TGF-ß1 values and also higher, but insignificant changes of these variables with a combination of the two interventions, therefore, the mentioned non-pharmaceutical interventions may be useful to prevent increased fibrosis and reduced cardiac angiogenesis caused by aging due to increased VEGF and downregulation of TGF-ß1.

Acknowledgement

Hereby, we greatly appreciate the cooperation and assistance provided by all colleagues for conducting this study.

Conflict of Interests

The Authors declare that there is no conflict of interest regarding the authorship or publication of this paper.

[DOI: 10.29252/jmj.13.4.39]

References:

1. Majeed F, Kelemen MD. Acute coronary syndromes in the elderly. Clin Geriatr Med 2007; 23(2): 425-440.
2.Besse S, Bulteau AL, Boucher F, et al. Antioxidant treatment prevents cardiac protein oxidation after ischemia-reperfusion and improves myocardial function and coronary perfusion in senescent hearts. J Physiol Pharmacol 2006; 57(4): 541-552.
3. Ahluwalia A, Tarnawski AS. Activation of the metabolic sensor-AMP activated protein kinase reverses impairment of angiogenesis in aging myocardial microvascular endothelial cells. Implications for the aging heart. J Physiol Pharmacol 2011;62(5):583-7.
4. Iemitsu M, Maeda S, Jesmin S, et al. Exercise training improves aging-induced downregulation of VEGF angiogenic signaling cascade in hearts. Am J Physiol Heart Circ Physiol 2006;291(3):H1290-H1298.
5. Rivard A, Fabre JE, Silver M, et al. Age-dependent impairment of angiogenesis. Circulation 1999; (1): 111–120.
6. Ferrara N, Gerber HP. The role of vascular endothelial growth factor in angiogenesis. Acta Haematol 2001;106:148–156.
7. Edelberg JM, Lee SH, Kaur M, et al. Platelet-derived growth factor-AB limits the extent of myocardial infarction in a rat model: feasibility of restoring impaired angiogenic capacity in the aging heart. Circulation 2002;105(5):608-13.
8. Biernacka A, Frangogiannis NG. Aging and Cardiac Fibrosis. Aging Dis. 2011;2(2):158-173.
9. Gramley F, Lorenzen J, Knackstedt C, et al. Age-related atrial fibrosis. Age (Dordr) 2009;31(1):27-38.
10. Siwik DA, Colucci WS. Regulation of Matrix metalloproteinases by cytokines and reactive oxygen/nitrogen species in the myocardium. Heart Fail Rev 2004;9(1):43-51.
11.Hinck AP. Structural studies of the TGF-βs and their receptors - insights into evolution of the TGF-β superfamily. FEBS Lett 2012;586(14):1860-1870.
12. Rosenkranz S, Flesch M, Amann K, Haeuseler C, Kilter H, Seeland U, et al. Alterations of β-adrenergic signaling and cardiac hypertrophy in transgenic mice overexpression of TGF-β1. Am J Physiol Heart Circ Physiol 2002;283(3): H1253-62.
13. Gramley F, Lorenzen J, Koellensperger E, Kettering K, Weiss C, Munzel T. Atrial fibrosis and atrial fibrillation: the role of the TGF-β1 signaling pathway. Int J Cardiol 2010;143(3):405-13.
14. Seals DR, Desouza CA, Donato AJ, Tanaka H. Habitual exercise and arterial aging. J Appl Physiol (1985). 2008;105(4):1323-32.
15. Seals DR, Walker AE, Pierce GL, Lesniewski LA. Habitual exercise and vascular ageing. J Physiol 2009;587(Pt 23):5541-9.
16. Husain K. Physical conditioning modulates rat cardiac vascular endothelial growth factor gene expression in nitric oxide-deficient hypertension. Biochem Biophys Res Commun 2004;320(4):1169-74.
17. Fleenor BS, Marshall KD, Durrant JR, et al. Arterial stiffening with ageing is associated with transforming growth factor-β1-related changes in adventitial collagen: reversal by aerobic exercise. J Physiol 2010;588(Pt 20):3971-82.
18. Banerjee SK, Maulik SK. Effect of garlic on cardiovascular disorders: a review. Nutr J 2002 ;1:4.
19.Chan JY, Yuen AC, Chan RY, et al. A review of the cardiovascular benefits and antioxidant properties of allicin. Phytother Res 2013;27(5): 637-646.
20.Ried K, Frank OR, Stocks NP. Aged garlic extract reduces blood pressure in hypertensives: a dose-response trial. Eur J Clin Nutr 2013;67(1):64-70.
21. Hara Y, Noda A, Miyata S, et al. Effects of aged garlic extract on left ventricular diastolic function and fibrosis in a rat hypertension model. Exp Anim 2013;62(4):305-10.
22. Peng CC, Chen KC, Hsieh CLan, et al. Swimming Exercise Prevents Fibrogenesis in Chronic Kidney Disease by Inhibiting the Myofibroblast Transdifferentiation. PLoS One 2012; 7 (6): e37388.
23. Seo DY, Kwak HB, Lee SR, et al. Effects of aged garlic extract and endurance exercise on skeletal muscle FNDC-5 and circulating irisin in high-fat-diet rat models. Nutr Res Pract 2014;8(2):177-82.
24. Kwak H B. Aging, exercise, and extracellular matrix in the heart. J Exerc Rehabil 2013; 9(3): 338-347.
25. Brooks WW, Conrad CH. Myocardial fibrosis in transforming growth factor beta(1)heterozygous mice. J Mol Cell Cardiol 2000; 32(2):187-195
26. Ahluwalia A, Narula J, Jones MK, et al. Impaired angiogenesis in aging myocardial microvascular endothelial cells is associated with reduced importin alpha and decreased nuclear transport of HIF1 alpha: mechanistic implications. J Physiol Pharmacol 2010;61(2):133-9.
27.Wagatsuma A, Tamaki H and Ogita F. Expression of VEGF and its receptor in the heart tissue following short- term swimming training. Int J of Sport and Health Sci 2005; 3: 91-99.
28. Kwak HB, Kim JH, Joshi K, et al. Exercise training reduces fibrosis and matrix metalloproteinase dysregulation in the aging rat heart. FASEB J 2011; 25(3):1106-17.
29. Czarkowska-Paczek B, Zendzian-Piotrowska M, Bartlomiejczyk I, et al. The effect of acute and prolonged endurance exercise on transforming growth factor-beta1 generation in rat skeletal and heart muscle. J Physiol Pharmacol 2009;60(4):157-62.
30. Fukumura D, Gohongi T, Kadambi A, et al. Predominant role of endothelial nitric oxide synthase in vascular endothelial growth factor-induced angiogenesis and vascular permeability. Proc Natl Acad Sci USA 98(5): 2604-2609.
31. Friehs I, Barillas R, Vasilyev NV, et al. Vascular endothelial growth factor prevents apoptosis and preserves contractile function in hypertrophied infant heart. Circulation 2006;114(1 Suppl):I290-5.
32. Bujak M, Frangogiannis NG. The role of TGF-beta signaling in myocardial infarction and cardiac remodeling. Cardiovasc Res 2007;74(2):184-95.
33. Biernacka A, Frangogiannis NG. Aging and Cardiac Fibrosis 2011;2(2): 158-173.
34. Wang NP, Wang ZF, Tootle S, et al.Curcumin promotes cardiac repair and ameliorates cardiac

dysfunction following myocardial infarction. Br J Pharmacol. 2012;167(7):1550-62.
35. Rizzi E, Castro MM, Ceron CS,et al.Tempol inhibits TGF-β and MMPs upregulation and prevents cardiac hypertensive changes. Int J Cardiol;165(1):165-73.
36. Sener G, Sehirli O, Ipçi Y, et al. Aqueous garlic extract alleviates isehaemia-reperfusion-induced oxidative hepatic injury in rats. Journal of Pharmacy and Pharmacology; 57(1): 145-150.
37. Badr GM, Al-Mulhim JA. The protective effect of aged garlic extract on nonsteroidal anti-inflammatory drug-induced gastric inflammations in male albino rats. Evidence-Based Complementary and Alternative Medicine 2014(2014); Article ID 759642;1-9.
38. Kim SR, Jung YR, An HJ, Kim DH, Jang EJ, Choi YJ, et al. Anti-wrinkle and anti-inflammatory effects of active garlic components and the inhibition of MMPs via NF-κB signaling. PLoS One2013;8(9):e73877.

[DOI: 10.29252/jmj.13.4.39]