

The Effect of Regular Aerobic Exercise with Garlic Extract on Heart Apoptosis Regulatory Factors in Chronic Kidney Disease

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Received: 02 July 2017

Accepted: 05 September 2017

Published in October 2017

Abstract

Objective: Physical activity and herbal medicine can inhibit apoptosis with two different mechanisms. The aim of this study was to assess the combined effect of regular aerobic exercise with garlic extract on heart apoptosis regulatory factors in aged rats with chronic kidney disease.

Materials and Methods: In this experimental research, 42 aged male Wistar rats (48-52 weeks) were selected and divided into 6 groups: control, doxorubicin, doxorubicin-salin, doxorubicin-garlic, doxorubicin-exercise, doxorubicin-garlic-exercise (combined). Chronic kidney disease was induced by a single doxorubicin injection (8.5 mg/kg). Swimming training was programmed 3 days/week, 30 min/day for 8 weeks. Both the doxorubicin garlic and combined groups with garlic extract were administered by garlic gavage at a dose of 2.5 g/kg. The renal Bax and Bcl-2 levels were evaluated by ELIZA method. A one-way analysis of variance was used to data analysis (P -value<0.05).

Results: The results showed that, 8 weeks swimming training, garlic supplementation and the combination of exercise and garlic extract caused significant Bax increase and Bcl-2 and the ratio Bax/Bcl-2 decrease in heart tissue of aged rats with chronic kidney disease.

Conclusion: Based on the results of physical activity and garlic separately induce a protective effect on the heart tissue. But combined interventions did not accelerate the combined effects. Therefore, it is suggested to use them separately.

Keywords: Aerobic exercise, Apoptosis, Garlic, Doxorubicin

Introduction

During the last two centuries, number of people suffering from chronic kidney disease (CKD) has increased in many countries. The age pattern for this disease inclines towards old age and it grows equally all over the world (1-2). Without considering other factors, cardiovascular disease is more

prevalent among CKD patients. Cardiovascular diseases cause approximately 40 to 50 percent of all deaths in CKD patients (3). The Meta-analysis of 16 articles showed that 29 percent of 80098 heart failure patients had moderate to severe kidney failure and 63 percent had mild kidney failure(4). Progress of

heart failure causes left ventricular ejection fraction decreases and kidney hypo-perfusion which induce kidney function failure development and (1,5,6).

As people get old, the heart tissue efficiency (systolic pressure changes and regenerative potential reduction) decreases and the left ventricle gets hypertrophy that causes left ventricular internal diameter and cardiac output reduction(7). Aging is an important issue that is relevant to the efficiency of body organs, this efficiency is affected by different factors such as chemicals and especially oxidative stress with internal and external origins and it is highly probable that a large number of heart tissues get apoptosis in a way that the results of Kajstura studies show 20 percent apoptosis increase in the heart of 2 year old mice compared to 1 year old rats (8).

Doxorubicin or Adriamycin is one of the strongest anthracycline drugs which are used to cure several kinds of cancer. Doxorubicin injures mitochondrial membrane and releases cytochrome C by producing oxygen free radicals and hence causes apoptosis induction in cardio myocytes (8-9). A research about the effects of doxorubicin on Bcl2 and Bax apoptotic gene expression of the rats' hearts was done which showed that doxorubicin increases the mitochondrial fibrosis and decreases Bcl2 gene expression and increases Bax gene expression.

In response to apoptotic stimulant, a variety of internal and external signals set gene expressions that control the start of apoptosis. In the internal pathway, genes express proteins such as Bax that start apoptosis and proteins such as Bcl2 that prevent apoptosis (10-11).

It is generally probable that physical activity can slow down CDK processes in aged population. Both internal and external apoptotic pathways may be involved (12).

Nowadays herbal medicine is used to cure or prevent diseases. Traditional use of garlic has been proved. Garlic elements can cure plasma lipid concentrations, oxidative stress and inflammatory cytokines (13-14).

Therefore, no considerable research has been found on the concurrent effect of training and medicinal plants on CKD patients. The aim of this study was to evaluate, the effect of regular swimming training for 8 weeks along with consuming garlic extract on some apoptotic and anti-apoptotic indexes of heart tissues of aged mice.

Materials and Methods

This is an experimental study on old Wistar male rats. In this study, 42 aged male rats (age= 40 to 50 weeks; initial weight= 250-300 gr) were divided into 6 groups: 1) control group, 2) saline group, 3) sham group, 4) aerobic exercise group, 5) garlic group and 6) garlic-exercise group. During the study phases, the studied rats were kept in transparent polycarbonate cages (size=30*15*15) made by Razi Rad Co. in the following condition: light/dark cycle ratio (LDR): 12h: 12h, temperature=22oc \pm 2 oc, humidity= 50% \pm 5% and ventilation condition= acceptable. The food of trials was produced by Karaj Dam Behparvar Food Co. and was put in every cage based on the weight of rats that were measured once three days with standard weighing machine and considering the natural share of 10 gr per 100 gr of body weight. In all stages, water was supplied freely to rats within animal-specialized bottles with a volume of 500 ml.

Old garlic was kept for 3 hours in room temperature and humidity and extraction process was performed through maceration method. To start extraction process, a total of 50 gr ground garlic was poured in a 1 liter balloon and methanol was added up with a ratio of 1:3. The solution, then, was filtered by Buchner funnel and methanol was added up to the remained slag. The slag was filtered again after 24 hours and was added to the first extract. The obtained solution was distilled in a vacuum distillation device at a temperature of 50oc and at a speed of 70 rpm until the solution volume was decreased to 1/5 of its initial volume. At this point, the extract tank was separated from the device. When the

solution was cooled it was decanted for three times with 50 ml chloroform. The remained volume was poured in a Petri dish with a given weight. When the extract was dried, it was weighted and every ¼ gram of the extract powder was mixed with 56 ml distilled water. The supplement group and supplement-exercise group received 1 ml garlic extract for every kilogram of their body weight on daily basis for 8 weeks through oral gavage. Saline group received the same volume of saline supplement in the same way (29). Before the commencement of the main protocol, the trials of exercise group were trained for one week (five days per week and each time for five minutes) in order to learn swimming. The main exercise plan was as follows: swimming for 8 weeks (three days per week and 30 minutes for every session (13)) in water (temp=32oc ±2 oc). A five minutes interval was considered before and after the main exercise in order to let the trials warm and cool. Blood sample collection and biopsy operations were conducted 48 hours after the last exercise session and after 10-12 hours of fasting. The rats were anesthetized by intra peritoneal injection of Ketamin (30-50 mg/kg) and Xylazine (3-5 mg/kg). Then, their blood and heart were immediately removed and placed in a freezer at -70 oc for measuring Bax and Bcl-2 levels purposes. To avoid the influence of circadian heart rhythm rate, sampling operation was started at 8:00 a.m. and was completed at 11:30 a.m.

A total of 0.5 gr of the collected tissue heart was placed in liquid nitrogen. The tissue, then, was homogenized in 1 ml phosphate buffered saline 1x with 1mM PMSF in a homogenizer device at 0oc. The obtained solution was centrifuged for 10 minutes at 4oc (speed=4000 rpm). In the next step, the outer layer of solution was filtered and used in ELISA test. The concentration of Bax and Bcl-2 heart levels were measured through ELISA method using Cusabio Kit, Sunlong Biotch, China with a sensitivity of 1 pg/ml.

Levene test and ANOVA were used to assess the equality of variables variance and the

change between groups, respectively. Tukey test was used to determine differences between groups. In this assessment, significant level of P -value ≤ 0.05 was considered as the measure for rejecting the null hypothesis. All statistical calculations were conducted in SPSS 16 and all graphs were drawn in Excel.

Results

Doxorubicin induction caused considerable decrease in Bcl2 (P -value:0.001, %46.58) cardiac level in aged mice. However, compared to doxorubicin (Respectively %61/04, P -value:0.005; %52.32, P -value:0.023; %64.53, P -value:0.002) and doxorubicin + saline groups (%55.62; P -value:0.009; %47.52, P -value:0.041; %58/99, P -value:0.005), Bcl2 cardiac level in mice with CKD increased considerably after aerobic trainings for 8 weeks using garlic complements and a combination of both. No other difference of the effect of the mentioned factors was observed in the Bcl2 cardiac level increase (figure 1).

Doxorubicin induction caused considerable increase in Bax cardiac level in aged mice (P -value: 0.001, %47.95). However, compared to doxorubicin (Respectively %24.20, P -value:0.001; %19.07, P -value:0.006; %26.67, P -value:0.001) and doxorubicin + saline (Respectively %23.20, P -value:0.001; %18.00, P -value:0.012; %25.70, P -value:0.001) groups, Bax cardiac level in mice having chronic kidney disease decreased considerably after aerobic trainings for 8 weeks using garlic complements and a combination of both (figute2).

Doxorubicin induction caused considerable increase in ratio Bax/Bcl-2 (P -value:0.001, %176.54) cardiac level in aged mice. However, compared to doxorubicin (Respectively %50.21, P -value:0.001; %41.21, P -value:0.001; %55.01, P -value:0.001) and doxorubicin + saline (Respectively %48.35, P -value:0.001; %42.13, P -value:0.003; %53.33, P -value:0.001) groups, Bax cardiac level in mice having CKD decreased considerably after aerobic trainings for 8 weeks using garlic

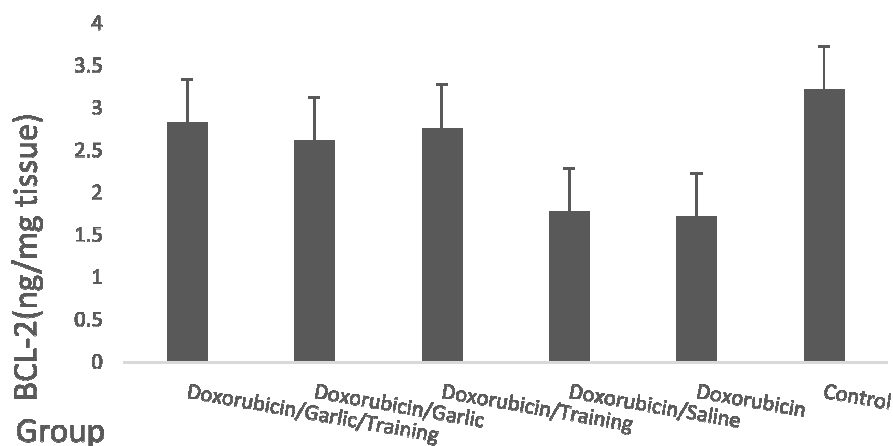


Figure 1. BCL-2 in Rat heart tissue homogenate (Mean±Std).

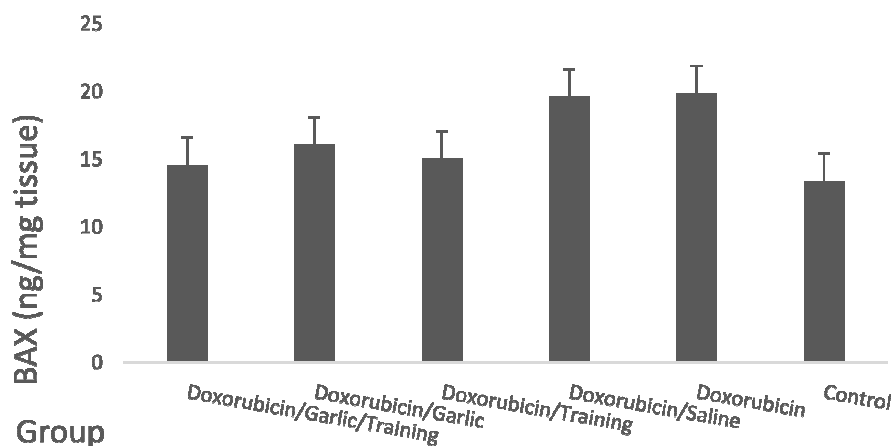


Figure 2. BAX in Rat heart tissue homogenate (Mean±Std).

complements and a combination of both. No other difference of the effect of the mentioned factors was observed in the ratio Bax/Bcl-2 cardiac level decrease (figure3).

Discussion

The results of this study showed that doxorubicin induction increased the Bax level of cardiac tissues in aged mice and decreased Bcl2 cardiac level. Therefore, the Bax/Bcl2 rate in cardiac tissues of aged mice after doxorubicin induction increased considerably. Our findings showed that doing regular aerobic training along with using garlic extract prevents opposite indexes that come from old age and doxorubicin induction that increases

Bax protein and decreases Bcl2 that increases cell death.

As one gets older, the cardiac cell death rate increases. Aging is a complicated morphological and physiological process. The changes in coronary artery system and extracellular matrix are all involved in the appearance of two important cell death patterns such as apoptosis and necrosis. Lakata et al. (1987), Orbank et al. (2003) and Lerry et al. (2003) found that in the old cells of cardiac tissue, P16/INK4A and P53 gene expression increases and because of changes in telomerase enzyme, the cell gets involved in an aging process and the cell death pattern comes back as apoptosis (15-17). Pandia et al. (2006) and Nag et al. (1986) stated that apoptosis induction occurs due to the

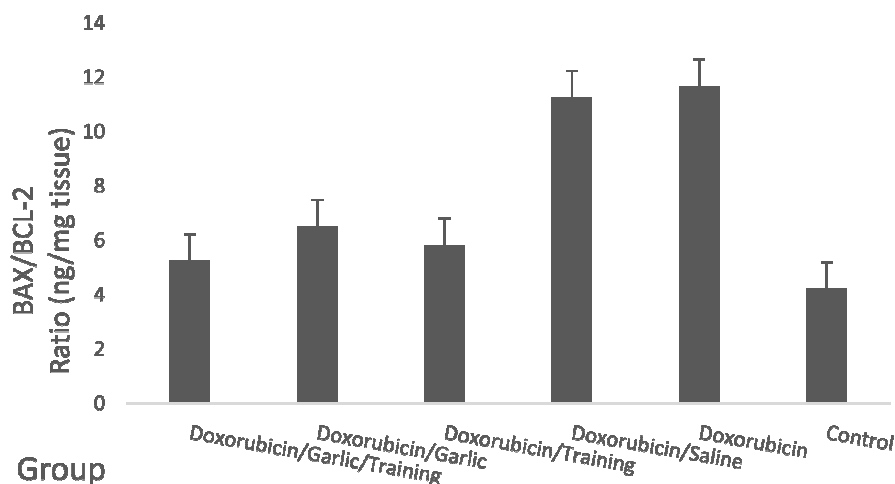


Figure 3. BAX/Bcl-2 Ratio in Rat heart tissue homogenate (Mean±Std).

reduction of expression and function of Sarcoplasmic ATPase Reticulum Ca^{2+} enzyme and Bcl2 and Survivin expression reduction (18,19).

On the other hand, present information showed that doxorubicin causes tissue injuries by disturbing important mitochondria functions. Mohammadi showed that as Bax gene expression increased and Bcl2 gene expression decreased after doxorubicin induction, mitochondria membrane instability increased and hence its permeability to cytochrome C increased. Therefore, it can be deduced that doxorubicin injection causes mitochondria membrane injury and creates apoptosis using the internal pathway in rat's myocardial cell (11).

Zolaqzadeh Dabidiroshan (2013) reported that doing regular aerobic training before doxorubicin injection, makes the oxidant and anti-oxidant imbalance coming from drug toxicity reverse in the liver tissue and suggested that regular aerobic training can be used as a medical approach to reduce liver toxicity coming from doxorubicin(20).

Chen et al. recently showed that CDK coming from doxorubicin increased the Bax/Bcl2 rate and the cytochrome C in the mitochondria. But training made this rate reverse and also reduce the reactive oxygen species (21).

Exercise, actually improves the apoptosis of aged mice heart tissue under doxorubicin

induction using mitochondria pathway. Apoptosis mitochondria pathway is mediated to a high degree by proteins from Bcl2 family that control mitochondria cytochrome C release (22). So it seems that exercise is beneficial in cardiac cell apoptosis reduction by reducing reactive oxygen species and preventing cytochrome C inside mitochondria. Besides, it has been reported that garlic is useful for different risk factors related to cardiovascular diseases such as lipid profile, high blood pressure, producing inflammatory cytokines and platelet activation (23). One of the active and main constituents of garlic extract is S-allyl cysteine (24) which is reported to have anti-oxidant (25), anti-cancer (26) and anti-liver toxicity activities (27). Another main garlic constituent is allicin that can have apoptotic or anti-apoptotic effects depending on the used dose. It is also proved that allicin prevents pro apoptosis expression, i.e. Bax, and decreases the cytochrome C level spread from mitochondria (28).

Conclusions

The present study findings showed pathological effects of doxorubicin on the cardiac tissue of aged mice by reducing Bcl2 levels and increasing Bax levels that increases the cell death rate. In addition regular swimming training, garlic extract complements and a combination of both for 8

weeks support cardiac tissue against cell death resulting from doxorubicin induction. This cardiac tissue support, which can be mediated by increasing Bcl2 levels or decreasing Bax level, was also seen in healthy aged mice. Therefore, training, garlic complement and a

combination of both of these methods can be considered as an effective non-pharmacological method to decrease cardiac injuries from oxidative and inflammatory stress injuries because of old age.

References

- Chen RC1, Xu XD1, Zhi Liu X2, Sun GB1, Zhu YD1, Dong X3, et al. Total Flavonoids from *Clinopodium chinense* (Benth.) O. Ktze Protect against Doxorubicin-Induced Cardiotoxicity In Vitro and In Vivo. *Evid Based Complement Alternat Med*. 2015;2015:472565.
- Carvalho FS1, Burgeiro A, Garcia R, Moreno AJ, Carvalho RA, Oliveira PJ. Doxorubicin-induced cardiotoxicity: from bioenergetic failure and cell death to cardiomyopathy. *Med Res Rev*. 2014;34(1):106-35.
- Mari-Carmen M, Gomez-Cabrera, Elena D, Jose V. Moderate exercise is an antioxidant: Upregulation of antioxidant genes by training. *Free Radic Biol Med* 2008;44:126-31.
- Poirier B, Lannaud-Bournoville M, Conti M, Bazin R, Michel O, Bariety J, et al. Oxidative stress occurs in absence of hyperglycemia and inflammation in the onset of kidney lesions in normotensive obese rats. *Nephrol Dial Transplant* 2000;15:467-76.
- Dae Yun Seo, Sung Ryul Lee, Arturo Figueroa, Yi Sub Kwak, Nari Kim, Byoung Doo Rhee, et al. Aged garlic extract enhances exercise-mediated improvement of metabolic parameters in high fat diet-induced obese rats *Nutrition Research and Practice (Nutr Res Pract)* 2012;6(6):513-9.
- Haines DD, Juhasz B, Tosaki A. Management of multicellular senescence and oxidative stress. *Journal of Cellular and Molecular Medicine* 2013;17:936-57.
- Rodríguez-Rodero S, Fernández-Morera JL, Menéndez-Torre E. Aging genetics and aging. *Aging and Disease* 2011;2:186-95.
- Kajstura J, Cheng W, Sarangarajan R, Lim P, Li B, Nitahara JA, et al. Necrotic and apoptotic myocyte cell death in the aging heart of Fischer 344 rats. *Am J Physiol* 1996;271:1215-28.
- Frias MA, Somers S, Gerber-Wicht C, Opie LH, Lecour S, Lang U. The PGE2-Stat3 interaction in doxorubicin-induced myocardial apoptosis. *Cardiovasc Res*. 2008;80(1):69-77.
- Chandran K, Aggarwal D, Migrino RQ, Joseph J, McAllister D, Konorev EA, et al. Doxorubicin inactivates myocardial cytochrome c oxidase in rats: cardio protection by Mito-Q. *Biophys J*. 2009;96(4):1388-98.
- Mohammadi Gorji S, Karimpour Malekshah AA. Effect of doxorubicin on Bcl2 and Bax expression in Rat heart. *Journal of Gorgan University of Medical Sciences*, 2013;15(1).
- Mustata S, Groeneveld S, Davidson W, Ford G, Kiland K, Manns B. Effects of exercise training on physical impairment, arterial stiffness and health-related quality of life in patients with chronic kidney disease: a pilot study. *International Urology and Nephrology*, 2011;43(4):1133-41.
- Yeh YY, Yeh SM. Garlic reduces plasma lipids by inhibiting hepatic cholesterol and triacylglycerol synthesis. *Lipids* 1994;29:189-93.
- Zare A, Farzaneh P, Pourpak Z, Zahedi F, Moin M, Shahabi S, et al. Purified aged garlic extract modulates allergic airway inflammation in BALB/c mice. *Iran J Allergy Asthma Immunol* 2008;7:133-41.
- Domenighetti AA, Wang Q, Egger M, Richards SM, Pedrazzini T, Delbridge LM. Angiotensin II mediated phenotypic cardiomyocyte remodeling leads to age-dependent cardiac dysfunction and failure. *Hypertension* 2005;46:426-32.
- Li SY, Du M, Dolence EK, Fang CX, Mayer GE, Ceylan-Isik AF, et al. Aging induces cardiac diastolic dysfunction, oxidative stress, accumulation of advanced glycation end products and protein modification. *Aging Cell* 2005;4:57-64.
- Leeuwenburgh C, Hollander J, Leichtweis S, Griffiths M, Gore M, Ji LL. Adaptations of glutathione antioxidant system to endurance training are tissue and muscle fibre specific. *Am J Physiol* 1997;272:363-69.
- Bodyak N, Kang PM, Hiromura M, Suljoadikusumo I, Horikoshi N, Khrapko K, et al. Gene expression profiling of the aging mouse cardiac myocytes. *Nucl Acids Res* 2002;30:3788-94.
- Park SK, Prolla TA. Gene expression profiling studies of aging in cardiac and skeletal muscles. *Cardiovasc Res* 2005;66:205-12.
- Zolfagharzadeh F, Dabidi Roshan V. Pretreatment hepatoprotective effect of regular aerobic training against hepatic toxicity induced by doxorubicin in rats. *Asian Pacific J Cancer Prev* 2013;14(5):5227-32.
- Kuan – Chou Chen, Chiung – Chi peng, Chi – lan Hsieh, Robert Y. Peng. Exercise Ameliorates Renal

- Cell Apoptosis in CKD by Intervening in the Intrinsic and the Extrinsic Apoptotic pathways in a Rat Model. Evidence –Based complementary and Alternative Medicine. 2013.
22. Cory S, Huang DCS, Adams JM. The Bcl-2 family: roles in cell survival and oncogenesis, *Oncogene*, 2003;22(53):8590-607.
 23. Ginter E, Simko V. Garlic (*Allium sativum* L.) and cardiovascular diseases. *Bratisl Lek Listy*. 2010;111:452-6.
 24. Allison GL, Lowe GM, Rahman K. Aged garlic extract and its constituents inhibit platelet aggregation through multiple mechanisms. *J Nutr*. 2006;136:782-8.
 25. Herrera-Mundo MN, Silva-Adaya D, Maldonado PD, Galvan-Arzate S, Andres-Martinez L, Pérez-De La Cruz V, et al. S-allylcysteine prevents the rat from 3-nitropropionic acid-induced hyperactivity, early markers of oxidative stress and mitochondrial dysfunction. *Neurosci Res*. 2006;56:39-44.
 26. Chu Q, Lee DT, Tsao SW, Wang X, Wong YC. S-allylcysteine, a water-soluble garlic derivative, suppresses the growth of a human androgen-independent prostate cancer xenograft, CWR22R, under in vivo conditions. *BJU Int*. 2007;99:925-32.
 27. Ngo SN, Williams DB, Cobiac L, Head RJ. Does garlic reduce risk of colorectal cancer? A systematic review. *J Nutr*. 2007;137:2264-9.
 28. Seong-Jun Cho, Dong-Kwon Rhee, Suhkneung Pyo. Allicin, a major component of garlic, inhibits apoptosis of macrophage in a depleted nutritional state *Nutrition* 2006;22(11):1177-84.
 29. Seo DY, Kwak HB, Lee SR, Cho YS, Song IS, Kim N, 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.