Cellular and Molecular Biology

Journal homepage: www.cellmolbiol.org

Therapeutic Effects of Allopurinol on the Function of Left Ventricular and Activity of Matrix Metalloproteinase Enzymes (MMPs) in Patients with Chronic Heart Failure

Hua Deng[1], Qiaolian Li[2], Dan Zhu[1*]

[1]*Department of Cardiovascular medicine, Chenzhou No.1 People's Hospital, Chenzhou City, China 423000*
[2]*Children Department of Ophthalmology, Chenzhou No.1 People's Hospital, Chenzhou City, China 423000*

ARTICLE INFO

Original paper

Article history:
Received: February 15, 2022
Accepted: April 06, 2022
Published: May 31, 2022

Keywords:
Allopurinol, Left ventricular function, Heart failure, Metalloproteinase

ABSTRACT

Heart failure is a growing public health problem, especially in the elderly, often occurring due to ischemia and coronary artery disease. Allopurinol can protect against myocardial ischemia and improve myocardial energy utilization during ischemia. On the other hand, matrix metalloproteinase (MMP) enzymes play an essential role in causing atherosclerosis, obstruction, and myocardial infarction. Therefore, in the present study, the effect of allopurinol on the function of the left ventricular and the activity of MMP-1, MMP-2, MMP-3, and MMP-9 were evaluated in heart failure patients. In this clinical trial, 82 patients were randomly assigned to allopurinol or placebo in addition to standard treatment. Echocardiographic evaluations were performed before treatment and six months after treatment. Also, after allopurinol treatment, plasma and peripheral blood mononuclear cells were extracted from control and intervention groups. The active form of MMPs was measured by ELISA and mRNA expression by Real-time PCR. The rate of change in left ventricular ejection fraction in the allopurinol group was significantly higher than patients in the control group. There was also found more improvement in NYHA class in patients receiving allopurinol than in the control group. ELISA results showed that all plasma MMP levels in the control group were significantly higher than those in the allopurinol group ($P<0.001$). Quantitative determination of mRNA expression in MMPs by Real-time RT-PCR revealed that, except for MMP-9, there was no significant difference in the expression of evaluated MMPs between the treatment and control groups. In general, the results showed that long-term administration of allopurinol improves left ventricular function, and it has beneficial effects on the life quality of patients with heart failure.

DOI: http://dx.doi.org/10.14715/cmb/2022.68.5.13

Introduction

Heart failure is a significant and pervasive health problem in the community due to heart damage and genetic, hormonal-neuro-inflammatory, and biochemical changes in myocytes and interstitial tissue (1). Heart failure is characterized by an imbalance between left ventricular function and myocardial energy expenditure, defined by the absence of mechano-energetic pairing. Despite considerable impaired left ventricular function, the consumption of contracted myocardial oxygen remains relatively unchanged (2). As a result, it reduces the mechanical effectiveness of the contraction. In the clinical evidence of this phenomenon, it has been proven that these factors worsen the point due to increased left ventricular activity at the expense of a disproportionate increase in myocardial oxygen consumption (3).

The mechanism responsible for the lack of mechanical coupling is unclear. However, laboratory evidence suggests that reactive oxygen components play a vital role in this phenomenon (4). Oxygen-free radicals accumulate in the pericardial fluid and blood circulation in patients with heart failure, indicating that heart failure is a condition of oxidative stress (5). Although there are several strong sources of oxygen free radicals, elevated serum uric acid levels in patients with heart failure indicate that xanthine oxidase (XO) activity is involved (6). XO is a source of superoxide, which is actually a reactor in purine metabolism and also produces oxygen free radicals, and despite conflicting reports, XO activity in the human myocardium has been proven (7).

In animal models of heart failure, XO inhibition has

*Corresponding author. E-mail: zzq5555@126.com

been shown to improve myocardial efficacy and myocardial contractile response to dobutamine and its activity (8). Several clinical studies have demonstrated that inhibition of XO improves the endothelial activity in patients with diabetes, coronary artery disease, and especially in patients with chronic heart failure (9, 10).

Several new studies have shown that long-term treatment with allopurinol in animal models of heart failure after myocardial infarction improves contractile function and catecholamine response and changes in hypertrophy and left ventricular fibrosis (11-13). These benefits occur by suppressing increased activity, XO superoxide production, and oxidative protein changes in heart failure. Although the benefits of allopurinol appear to be secondary to XO inhibition, other mechanisms may be present, such as radical allopurinol clearance of hydroxyl, lowering uric acid levels, and suppression of its associated inflammatory activity (14). On the other hand, various studies have shown that changes in the activity and expression of matrix metalloproteinases (MMPs) in cardiovascular tissues are involved in the progression of cardiovascular diseases such as myocardial infarction, ischemia-reperfusion injury, heart failure, and the matrix deformation process (15). The activity of MMPs is regulated at the transcriptional, translational, and post-transcriptional levels (16). Therefore, in the current study, therapeutic effects of allopurinol were evaluated on the function of left ventricular and MMPs (MMP-1, MMP-2, MMP-3, and MMp-9) in patients with chronic heart failure.

Materials and methods

Studied patients and experimental evaluations

In this clinical trial, patients with chronic and persistent heart failure with class II to IV of the New York Heart Society classification had a left ventricular diastolic end diameter of at least 60 mm and a left ventricular ejection fraction of less than 40% studied.

Exclusion criteria included hyponatremia, hypokalemia, renal failure, and mitral regurgitation. The 82 patients were randomly divided into two groups. One group of patients (43 patients) was given 300 mg of allopurinol orally daily, and the control group (39 patients) was given a placebo. All patients in both groups were given digoxin, Lasix, carvedilol, captopril, Aldactone, and aspirin. This treatment lasted for six months. Patients' uric acid levels were measured before and six months after treatment. The parameters related to the left ventricular function that were measured in patients before and after allopurinol treatment using transthoracic echocardiography based on the guidelines of the American Echocardiographic Association were Left Ventricular End-Diastolic Diameter (LVDD), Left Ventricular Ejection Fraction (LVEF), Systolic and diastolic blood pressure, NYHA class (before and after six months of treatment).

Blood collection and isolation of PBMCs

In this study, peripheral blood samples were collected from patients during the first 26 hours after AMI to assess plasma levels of MMPs. These blood samples were also used to examine the genes and proteins of MMPs. Plasma was obtained by centrifugation of blood samples and stored at -85°C until use. PBMCs were isolated from blood donors by density gradient centrifugation on a follicle (Axis-Shield PoC AS, Oslo, Norway) to investigate the expression of MMPs genes and proteins.

Plasma levels of MMPs

Plasma concentrations of MMPs were obtained using ELISA kits (Amersham Pharmacia Biotech, Little Chal Font UK) previously reported (17). Measurements were performed according to the kit instructions. The test was performed three times from each sample to confirm the findings.

Real-time RT RCR

Gene expression was assessed for MMP-1, MMP-2, MMP-3, MMp-9, and GAPDH. The mRNA was isolated from PBMCs (by Sigma Aldridge kit) and converted to cDNA using the kit (Sigma) according to the kit instructions.

The cDNA production was performed according to the instructions of the kit (Invitrogen California, USA) using dT-pattern primers. The expression of MMPs mRNA was tested by Real time-RT RCR using Invitrogen expression (Taqman q-PCR). The primer and probe sequences are summarized in Table 1. Ends 3’ and 5’ of each probe were labeled with 6-carboxy fluorescein (FAM) and 6-carboxy-tetramethyl rhodamine (TAMRA) extinguisher. Probe and primer

sequences were obtained from previously published sequences (18-20).

PCR was performed as follows: Initial denaturation stage: 95°C for 15 minutes (one cycle); Denaturation step: 95°C for 30 seconds (40 cycles); Annealing stage: 60°C for 60 seconds (40 cycles). All samples were compared with GAPDH. At the end of PCR, baseline and threshold values were published using ABI 7500 prime software, and ct values were transferred to Microsoft Excel for evaluation.

Table 1. Primers and probes used in mRNA expression by Real-time RT PCR

Gene		Sequence (5'-3')
MMP-1	Forward	GAGGGTCAAGCAGACATCATGA
	Reverse	CAAGATTTCCTCCAGGTCCATC
	Probe	FAM-TGTCAGGGGAGATCATCGGGACAA-TAMRA
MMP-2	Forward	TTCCTGGGCAACAAATATGAGA
	Reverse	TGGTCGCACACCACATCTTT
	Probe	FAM-AGCGCCGGCCGCAGTGA-TAMRA
MMP-3	Forward	TTCCTGATGTTGGTCACTTCAGA
	Reverse	CCTGTATGTAAGGTGGGTTTTCCT
	Probe	FAM-CCTTTCCTGGCATCCCGAAGTGG -TAMRA
MMP-9	Forward	CCCTGGAGACCTGAGAACCA
	Reverse	AACCATAGCGGTACAGGTATTCCT
	Probe	FAM-TCTCACCGACAGGCAGCTGGCA-TAMRA
GAPDH	Forward	GAAGGTGAAGGTCGGAGTC
	Reverse	GAAGATGGTGATGGGATTTC
	Probe	FAM-CCGACTCTTGCCCTTCGAAC-TAMRA

Data analysis

Qualitative data analysis obtained from the experimental groups was performed using descriptive statistical methods, χ^2. Quantitative data analysis was performed using the t-test statistical method. In all cases, P <0.05 was considered the significant difference between the groups.

Results and discussion

The results showed that there was no difference in demographic characteristics between the two groups (Table 2).

Table 2. Demographic information of patients in the study groups at the time of intervention

Characteristics	Control Group (n=39)	Allopurinol Group (n=43)	P-value
Age (year)	65 ± 10.5	62.6 ± 12.1	0.29
Gender (Male)	25	27	0.76
Blood Sugar (mg/dl)	117.5 ± 58.6	130.7 ± 49.4	0.23
Blood Triglyceride (mg/dl)	161.4 ± 44.8	157.7 ± 57.3	0.71
Blood Cholesterol (mg/dl)	191.2 ± 40.7	188 ± 36.5	0.67
Blood HDL (mg/dl)	42.4 ± 6.5	43.3 ± 7.9	0.51
Disease Duration (year)	5.1 ± 4.4	4.6 ± 3.8	0.44

The control group's mean left ventricular ejection fraction (LVEF) was 29.9 ± 5 percent before treatment and 29.6 ± 5 percent after treatment. Statistical analysis did not show a significant difference between the mean left ventricular ejection fraction in the control group before and after treatment. There was no difference between the mean left ventricular diastolic end diameter (LVEDD) and the mean left ventricular end-systolic diameter (LVESD) in the pre- and post-treatment control group. Statistical analysis of data on the mean rate of the premature ventricular filling during the diastole phase (Peak E), mean rate of the late ventricular filling during the diastole phase (Peak A), the ratio of premature to late ventricular filling during the diastole phase (E/A Ratio), and the mean time of E wave descent to baseline (DcT) in the control group did not show any difference before and after treatment (Table 3).

Table 3. Evaluation of patients in the control group before and after treatment

Control Group			
Variable	Before Treatment	After Treatment	P-value
Systolic Blood Pressure (mmHg)	121 ± 24	113 ± 17	<0.0001
Diastolic Blood Pressure (mmHg)	74 ± 15	70 ± 13	0.03
Blood Uric Acid Level (mg/dl)	5.8 ± 1.8	5.9 ± 1.8	0.84
Blood Creatinine Level (mg/dl)	1.1 ± 0.2	1.2 ± 0.1	0.66
Blood Sodium Level (mg/dl)	142 ± 2	141 ± 2	0.77
Blood Potassium Level (mg/dl)	4.5 ± 0.2	4.4 ± 0.2	0.78
NYHA Class	3 ± 0.6	2.8 ± 0.7	0.42
LVEF (%)	29.9 ± 5	29.6 ± 5	0.89
LVEDD (cm)	6.1 ± 0.6	6.2 ± 0.6	0.32
LVESD (cm)	5.2 ± 0.7	5.1 ± 0.8	0.71
Peak E (m/s)	0.89 ± 0.2	0.84 ± 0.3	0.16
Peak A (m/s)	0.82 ± 0.2	0.81 ± 0.2	0.82
E/A ratio	1.1 ± 0.3	1.2 ± 0.5	0.36
DcT (ms)	223 ± 77	227 ± 82	0.44

The mean LVEF in the allopurinol group was 27.5 ± 6% before treatment and 30.1 ± 7% after treatment. The statistical analysis results showed a significant difference in patients' mean left ventricular ejection fraction. The mean left ventricular ejection fraction was higher than treatment after treatment (P<0.0001). The statistical analysis results showed a significant difference in the mean LVEDD of patients. The mean diameter of the left ventricular end-diastolic end after treatment was significantly (p = 0.019) lower than the mean of the lower extremity. Statistical analysis of mean LVESD, mean Peak E velocity, mean Peak-A speed, E/A ratio, and mean DcT time in the allopurinol group showed no difference before and

after treatment (Table 4).

ELISA results showed that all plasma MMP levels after allopurinol treatment in the control group were significantly higher than those in the allopurinol group (P<0.001) (Table 5).

Table 4. Evaluation of patients in the allopurinol group before and after treatment

Allopurinol Group			
Variable	Before Treatment	After Treatment	P-value
Systolic Blood Pressure (mmHg)	120 ± 20	109 ± 13	<0.0001
Diastolic Blood Pressure (mmHg)	73 ± 11	68 ± 7	0.002
Blood Uric Acid Level (mg/dl)	6.5 ± 1.7	5.3 ± 1.4	<0.0001
Blood Creatinine Level (mg/dl)	1.2 ± 0.2	1.1 ± 0.1	0.78
Blood Sodium Level (mg/dl)	141 ± 2	142 ± 3	0.67
Blood Potassium Level (mg/dl)	4.5 ± 0.2	4.4 ± 0.3	0.87
NYHA Class	2.9 ± 0.5	2.4 ± 0.6	<0.0001
LVEF (%)	27.5 ± 6	30.1 ± 7	<0.0001
LVEDD (cm)	6.1 ± 0.5	6.0 ± 0.5	0.019
LVESD (cm)	5.2 ± 0.6	5.1 ± 0.7	0.09
Peak E (m/s)	0.87 ± 0.3	0.90 ± 0.2	0.24
Peak A (m/s)	0.92 ± 0.2	0.89 ± 0.2	0.25
E/A ratio	1.1 ± 0.5	1.0 ± 0.4	0.91
DcT (ms)	193 ± 67	189 ± 59	0.58

Table 5. The amount of MMPs proteins in the ELISA method after allopurinol treatment in the study groups

	MMP-1 (ng/ml)	MMP-2 (ng/ml)	MMP-3 (ng/ml)	MMP-9 (ng/ml)
Allopurinol Group	1.11 ± 0.17	1.32 ± 0.13	1.00 ± 0.17	1.66 ± 0.55
Control Group	7.00 ± 1.84	3.04 ± 1.11	4.18 ± 1.03	15.45 ± 3.19
P-value	<0.001	<0.001	<0.001	<0.001

Quantitative determination of mRNA expression in MMPs by Real-time RT-PCR revealed that, except for MMP-9, there was no significant difference in the expression of evaluated MMPs between the treatment and control groups after treating with allopurinol (Figure 1).

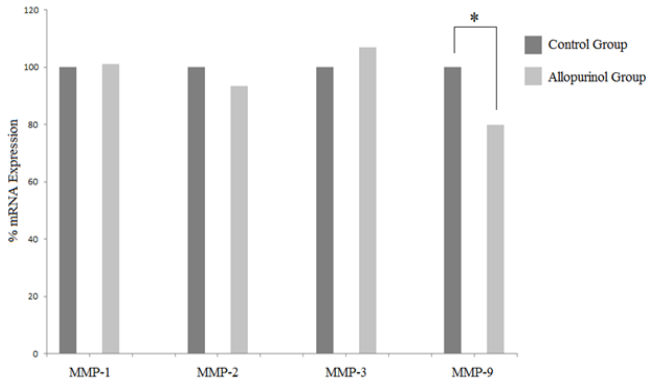

Figure 1. The mRNA expression of MMP-1, MMp-2, MMp-3, and MMp-9 by Real-time RT-PCR after allopurinol treatment in the study groups (The control group was considered 100%); * means P <0.05 compared to the control group

Recent studies have shown the role of xanthine oxidase (XO) and its associated oxidative components in the pathogenesis of heart failure (21, 22). Studies in animal and laboratory models of heart failure have shown that inhibition of XO can be beneficial in treating patients with debilitating heart failure. *In vitro* studies performed on isolated hearts by Ferdinandy *et al*. (23) have demonstrated that the progressive spread of heart failure is associated with increased myocardial XO levels, contributing to increased cardiac oxidative stress. In another model of heart failure in dogs, a fourfold increase in myocardial activity or XO levels was observed, which was associated with an increase in oxidative stress in the heart (24). De Jong *et al*. (25) also observed a 50% increase in chronic heart failure in rats. In a study conducted by Ndrepepa *et al*. (26) and Piepoli *et al*. (27), an increase in uric acid levels and an increase in myocardial XO activity in patients with chronic heart failure were reported. These observations led to the initiation of interventional studies with allopurinol as an XO inhibitor.

In the present study, oral administration of allopurinol significantly reduced patients' serum uric acid levels compared to the controlled group. Allopurinol administration significantly increased left ventricular ejection fraction (approximately 3% increase) and decreased left ventricular diastolic end diameter. These observations indicate the beneficial effects of allopurinol on left ventricular function in patients with heart failure. Even the score of patients based on NYHA class in patients in this group showed a significant decrease after allopurinol treatment. In a model of chronic heart failure in dogs, the Indhu *et al*. (24) found that 200 mg of allopurinol administration reduced myocardial oxygen consumption and increased contractile strength, and was effective. The results of a study by Ukai *et al*. (28) also performed on a model of chronic canine heart failure in dogs showed that allopurinol improved left ventricular systolic function at rest and stimulated stimulation in the active state. A study by Cicero *et al*. (29) also showed that 100 mg of oral allopurinol daily reduced retention and increased myocardial contractility in heart failure. In a study by Suzuki *et al.* (30), allopurinol administration increased survival, decreased XO activity, improved contractility, and responded to isoprene. Researchers have also shown a

reduction in oxygen-free radicals and myocardial dysfunction following allopurinol therapy (31).

Cappola *et al*. (32) found that intra-coronary administration of allopurinol had a straightforward and rapid improvement in myocardial infarction. A retrospective study of 1760 patients by Struthers *et al*. (33) found that low doses of allopurinol increased mortality in patients with chronic heart failure. In contrast, doses higher than 300 mg per day of allopurinol have led to a significant increase in the survival of these patients. In a study on patients with normal uric acid levels and patients with elevated uric acid levels, the capacity of the peripheral arteries to dilate also improves local and systemic blood flow. Farquharson *et al*. (34) also studied the administration of 300 mg of allopurinol daily to 11 patients with chronic heart failure. They found that allopurinol improved endothelial dysfunction and improved patients' work capacity. In patients with heart failure, George *et al*. (35) found that allopurinol improves endothelial function because of its ability to reduce oxidative stress in arteries. In the present study, an increase in left ventricular function in patients receiving allopurinol co-occurred with a decrease in serum uric acid levels. In the study of George *et al*. (35), Probenecid was used to reduce the serum level of uric acid without affecting XO activity. In comparison with allopurinol, it was shown to reduce the uric acid level and inhibit the inhibitory effect of XO. The patient is not in good condition, and the beneficial effects of allopurinol are exerted by inhibiting XO and not by lowering uric acid levels.

This study evaluated different plasma concentrations of MMPs in the control and allopurinol treatment groups, and significant differences in plasma concentrations between MMPs were obtained. All plasma MMP concentrations increased significantly in patients with different conditions. It suggests that different MMPs may play different roles in patients with chronic heart failure. Some studies have shown increased expression and activity of MMPs in the hearts of humans, mice, and pigs during post-MI deformation processes (36). Despite the variety of information about the definitive timing of MMP activity after myocardial infarction, it has become clear that MMP activity begins rapidly and less than one day after MI (36-37).

Cardiac MMPs are produced by fibroblast-like cells, inflammatory cells, and cardiomyocytes. Although the expression of most MMPs increases during specific physiological and pathological deformation processes (15). MMP-9 mRNA expression suggests the presence of additional regulatory mechanisms in myocardial insufficiency. The slight increase in the mRNA expression of MMPs is contrasted with the large number of active MMPs measured by ELISA. Thus, although transcriptional regulation is essential for MMP production, matrix degradation requires latent enzymes to be activated by proteolytic cleavage. The circadian synthesis of MMP with inactive pre-enzymes indicates a temporal imbalance between synthesis and MMP activity (16).

This study showed that long-term administration of allopurinol and standard treatment of patients with chronic heart failure could improve left ventricular function and improve their quality of life. Although there are various mechanisms in justifying the beneficial effects of allopurinol on left ventricular function, inhibition of xanthine oxidase and reducing uric acid levels are among the main mechanisms in this field. So far, few studies have been done on the effects of allopurinol on heart function in patients with heart failure. More extensive and more central studies can effectively recognize the results of allopurinol.

Acknowledgments

The authors are thankful to the higher authorities for the facilities provided.

Interest conflict

The authors declare that they have no conflict of interest.

Funding

No funding received for this study.

Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Statements and Declarations

The author declares that no conflict of interest is associated with this study.

Authors' contribution

This study was done by the authors named in this article, and the authors accept all liabilities resulting from claims which relate to this article and its contents.

References

1. Rossignol P, Hernandez AF, Solomon SD, Zannad F. Heart failure drug treatment. Lancet 2019; 393(10175): 1034-1044.
2. Adamo L, Rocha-Resende C, Prabhu SD, Mann DL. Reappraising the role of inflammation in heart failure. Natr Rev Cardiol 2020; 17(5): 269-285.
3. Teran F, Paradis NA, Dean AJ et al. Quantitative characterization of left ventricular function during pulseless electrical activity using echocardiography during out-of-hospital cardiac arrest. Resuscitation 2021; 167: 233-241.
4. Anderson KL, Evans JC, Castaneda MG, Boudreau SM, Maddry JK, Morgan JD. Effects of Left Ventricular Versus Traditional Chest Compressions in a Traumatic Pulseless Electrical Activity Model. Mil Med 2022; 187(3-4): 351-359.
5. Khaper N, Bailey CD, Ghugre NR et al. Implications of disturbances in circadian rhythms for cardiovascular health: A new frontier in free radical biology. Free Radic Biol Med 2018; 119: 85-92.
6. Sakuma M, Toyoda S, Arikawa T et al. The effects of xanthine oxidase inhibitor in patients with chronic heart failure complicated with hyperuricemia: a prospective randomized controlled clinical trial of topiroxostat vs allopurinol—study protocol. Clin Exp Nephrol 2018; 22(6): 1379-1386.
7. Bredemeier M, Lopes LM, Eisenreich MA et al. Xanthine oxidase inhibitors for prevention of cardiovascular events: a systematic review and meta-analysis of randomized controlled trials. BMC Cardiovasc Disord 2018; 18(1): 1-11.
8. Duncan JG, Ravi R, Stull LB, Murphy AM. Chronic xanthine oxidase inhibition prevents myofibrillar protein oxidation and preserves cardiac function in a transgenic mouse model of cardiomyopathy. Am J Physiol Heart Circ 2005; 289(4): H1512-H1518.
9. Ju C, Lai RWC, Li KHC et al. Comparative cardiovascular risk in users versus non-users of xanthine oxidase inhibitors and febuxostat versus allopurinol users. Rheumatol 2020; 59(9): 2340-2349.
10. Alem MM, Alshehri AM, Cahusac PM, Walters MR. Effect of xanthine oxidase inhibition on arterial stiffness in patients with chronic heart failure. Clin Med Insights Cardiol 2018; 12: 1179546818779584.
11. Stull LB, Leppo MK, Szweda L, Gao WD, Marbán E. Chronic treatment with allopurinol boosts survival and cardiac contractility in murine postischemic cardiomyopathy. Circ Res 2004; 95(10): 1005-1011.
12. Thanassoulis G, Brophy JM, Richard H, Pilote L. Gout, allopurinol use, and heart failure outcomes. Arch Intern Med 2010; 170(15): 1358-1364.
13. Nadwa EH, Morcos GN, Salama NM, Shafik AN. Comparing the Effects of Febuxostat and Allopurinol in an Animal Model of Metabolic Syndrome. Pharmacology 2021; 106(9-10): 564-572.
14. Yang Y, Zhao J, Qiu J et al. Xanthine oxidase inhibitor allopurinol prevents oxidative stress-mediated atrial remodeling in alloxan-induced diabetes mellitus rabbits. J Am Heart Assoc 2018; 7(10): e008807.
15. Olejarz W, Łacheta D, Kubiak-Tomaszewska G. Matrix metalloproteinases as biomarkers of atherosclerotic plaque instability. Int J Mol Sci 2020; 21(11): 3946.
16. Carrick-Ranson G, Spinale FG, Bhella PS et al. Plasma matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs and aging and lifelong exercise adaptations in ventricular and arterial stiffness. Exp Gerontol 2019; 123: 36-44.
17. Kim HE, Dalal SS, Young E, Legato MJ, Weisfeldt ML, D'Armiento J. Disruption of the myocardial extracellular matrix leads to cardiac dysfunction. J Clin Investig 2000; 106(7): 857-866.
18. Jordan RC, Macabeo-Ong M, Shiboski CH et al. Overexpression of matrix metalloproteinase-1 and-9 mRNA is associated with progression of oral dysplasia to cancer. Clin Cancer Res 2004; 10(19): 6460-6465.
19. Medley TL, Kingwell BA, Gatzka CD, Pillay P, Cole TJ. Matrix metalloproteinase-3 genotype contributes to age-related aortic stiffening through modulation of gene and protein expression. Circ Res 2003; 92(11): 1254-1261.

20. Weglarz L, Molin I, Orchel A, Parfiniewicz B, Dzierzewicz Z. Quantitative analysis of the level of p53 and p21 (WAF1) mRNA in human colon cancer HT-29 cells treated with inositol hexaphosphate. Acta Biochim Pol 2006; 53(2): 349-356.
21. Okazaki H, Shirakabe A, Matsushita M et al. Plasma xanthine oxidoreductase activity in patients with decompensated acute heart failure requiring intensive care. ESC heart failure 2019; 6(2): 336-343.
22. Watanabe K, Watanabe T, Otaki Y et al. Impact of plasma xanthine oxidoreductase activity in patients with heart failure with preserved ejection fraction. ESC heart failure 2020; 7(4): 1735-1743.
23. Ferdinandy P, Panas D, Schulz R. Peroxynitrite contributes to spontaneous loss of cardiac efficiency in isolated working rat hearts. Am J Physiol Heart Circ 1999; 276(6): H1861-H1867.
24. Indhu M, Sesh P, Loganathasamy K, Jeyaraja K, Padmanath K, Pandiyan V. Analysis of certain blood biochemical parameters in relation to oxidative stress in chronic mitral valve insufficiency of dogs with heart failure. Indian J Anim Res 2019; 53(9): 1181-1187.
25. de Jong JW, Schoemaker R, de Jonge R et al. Enhanced expression and activity of xanthine oxidoreductase in the failing heart. J Mol Cell Cardiol 2000; 32(11): 2083-2089.
26. Ndrepepa G. Uric acid and cardiovascular disease. Clinica chimica acta 2018; 484: 150-163.
27. Piepoli MF, Salvioni E, Corrà U et al. Increased serum uric acid level predicts poor prognosis in mildly severe chronic heart failure with reduced ejection fraction. An analysis from the MECKI score research group. Eur J Intern Med 2020; 72: 47-52.
28. Ukai T, Cheng C-P, Tachibana H et al. Allopurinol enhances the contractile response to dobutamine and exercise in dogs with pacing-induced heart failure. Circulation 2001; 103(5): 750-755.
29. Cicero AFG, Cosentino ER, Kuwabara M, Degli Esposti D, Borghi C. Effects of allopurinol and febuxostat on cardiovascular mortality in elderly heart failure patients. Intern Emerg Med 2019; 14(6): 949-956.
30. Suzuki S, Yoshihisa A, Yokokawa T et al. Comparison between febuxostat and allopurinol uric acid-lowering therapy in patients with chronic heart failure and hyperuricemia: a multicenter randomized controlled trial. J Int Med Res 2021; 49(12): 03000605211062770.
31. Shah AK, Bhullar SK, Elimban V, Dhalla NS. Oxidative stress as a mechanism for functional alterations in cardiac hypertrophy and heart failure. Antioxidants 2021; 10(6): 931.
32. Cappola TP, Kass DA, Nelson GS et al. Allopurinol improves myocardial efficiency in patients with idiopathic dilated cardiomyopathy. Circulation 2001; 104(20): 2407-2411.
33. Struthers A, Donnan P, Lindsay P, McNaughton D, Broomhall J, MacDonald T. Effect of allopurinol on mortality and hospitalisations in chronic heart failure: a retrospective cohort study. Heart 2002; 87(3): 229-234.
34. Farquharson CA, Butler R, Hill A, Belch JJ, Struthers AD. Allopurinol improves endothelial dysfunction in chronic heart failure. Circulation 2002; 106(2): 221-226.
35. George J, Carr E, Davies J, Belch J, Struthers A. High-dose allopurinol improves endothelial function by profoundly reducing vascular oxidative stress and not by lowering uric acid. Circulation 2006; 114(23): 2508-2516.
36. Hughes BG, Schulz R. Targeting MMP-2 to treat ischemic heart injury. Basic Res Cardiol 2014; 109(4): 1-19.
37. Ganjali S, Khajeh H, Gholami Z, Jomeh-ghasemabadi Z, Fazeli-Nasab B. Evaluation of Dormancy Failure Datura stramonium Plant Seeds under the Influence of Different Treatments. Agrotech Ind Crops 2022; 2(1): 32-41. doi: 10.22126/atic.2022.7656.1049.
38. Pan W, Yang D, Yu P, Yu H. Comparison of predictive value of NT-proBNP, sST2 and MMPs in heart failure patients with different ejection fractions. BMC Cardiovasc Disord 2020; 20(1): 1-11.