The Relationship of Hsp-70 with Calcineurin, SOD and Catalase Post-Acute Myocardial Infarction in Wistar Rats Model

by Johanes Nugroho

Submission date: 26-Apr-2022 01:06PM (UTC+0800)
Submission ID: 1820603424
File name: talase_Post-Acute_Myocardial_Infarction_in_Wistar_Rats_Model.pdf (677.21K)
Word count: 3502
Character count: 18214

Journal of Cardiovascular Diseases & Diagnosis

Nugroho et al., J Cardiovasc Dis Diagn 2020, 8:2

Research Article Open Access

The Relationship of Hsp-70 with Calcineurin, SOD and Catalase Post-Acute Myocardial Infarction in Wistar Rats Model

Nugroho J[1,2], **Darius C**[2], **Probohoesodo MY**[3] **and Ghea C**[4]

[1]*Department of Cardiology and Vascular Medicine, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia*

[2]*Dr. Soetomo General Hospital, Surabaya, Indonesia*

[3]*Department of Clinical Pathology, Faculty of Medicine, Airlangga University-Dr. Soetomo Hosiptal, Surabaya, Indonesia*

[4]*PILAR Research and Education, Cambridge, UK*

***Corresponding author:** Nugroho J, MD, Department of Cardiology and Vascular Medicine, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, Tel: +6282139090953; E-mail: j.nugroho.eko@fk.unair.ac.id

Received: April 01, 2020; **Accepted:** April 07, 2020; **Published:** April 15, 2020

Abstract

Background: Hsp-70 production increased following myocardial ischemia. Hsp-70 helps to reduce infarct area through unclear mechanism. It appears that Hsp-70 activates calcineurin and induce antioxidant enzymes such as superoxide dismutase (SOD) and catalase. No work has been done, however, to show correlation between Hsp-70 and calcineurin, SOD and catalase post-acute myocardial infarction. In the present study, therefore, we sought to investigate the relationship of Hsp-70 with calcineurin, SOD and catalase post-acute myocardial infarction (AMI).

Methods: An experimental study involved 24 Wistar rats as models of chronic coronary occlusion. The rats were randomly divided into 4 group: no-intervention after AMI (N), sedentary intervention after AMI (S), exercise intervention after AMI (E), and sham (C). Intervention consisted of 2 weeks of recovery then 4 weeks of sedentary for group S or exercise for group E. Hsp-70, calcineurin, SOD, and catalase expression in heart were evaluated the difference among groups. Correlation between Hsp-70 to other proteins was analysed also.

Results: Hsp-70 and calcineurin was higher in group S and E compared to group N and C. Hsp-70 (MD=0.97, 95% CI 0.60 to 1.34), calcineurin (MD=1.25, 95% CI 0.68 to 1.82, p< 0.05), catalase (MD= 0.57, 95% CI 0.25 to 0.88, p< 0.05), and SOD (0.42, 95% CI 0.14 to 0.69, p< 0.05) significantly higher in Group E compared to S. Sham group had higher SOD and catalase activity than group E. Hsp-70 was correlated with calcineurin (r=0.856, p<0.05). Hsp-70 was correlated with catalase and SOD when the analysis included only groups who had intervention.

Conclusion: Hsp-70 and calcineurin was increased, but catalase and SOD was depleted in post-AMI condition. Exercise improved Hsp-70, calcineurin, catalase, and SOD activities post-AMI. Hsp-70 expression increased directly calcineurin expression.

Keywords: Exercise; Myocard infarct; Hsp-70; Calcineurin; SOD; Catalase

Introduction

70-kDa heat shock proteins (Hsp-70) acts as a molecular chaperone that assist in the correct folding of nascent and stress-accumulated mis-folded proteins and prevents their aggregation [1]. It is well established that ischemia result in an increase in Hsp-70 production in the heart and the amount of Hsp-70 synthesis correlates with the extent of myocardial protection after ischemia/reperfusion injury [2]. Inducing super expression of Hsp-70 reduced significantly infarcted area [3,4].

In ischemic myocardial cells, Hsp-70 helps prevent cell death through binding with proteins that play a role in inhibiting cell necrosis [5]. Hsp-70 activates calcineurin via a calmodulin-dependent and independent pathway [6]. Hsp-70 may induce antioxidant superoxide dismutase (SOD) through reactive oxygen species (ROS). [7] Myocardial catalase activity was induced as well as Hsp-70 after heat shock [8,9]. No work has been done, however, to show correlation between Hsp-70 and calcineurin, COD and catalase after ischemia *in vivo*. Therefore, we sought to investigate the relationship of Hsp-70 with calcineurin, COD and catalase post-acute myocardial infarction (AMI).

Methods

Male Wistar rats meeting certain criteria, such as the age of 13 ± 2 weeks and body weight of 200-300 grams were used for this study. Procedures of care and animal use were conducted in accordance with the guidelines of laboratory animal care approved by the Ethics Committee of the Faculty of Veterinary Medicine, Airlangga University.

The rats were randomly assigned into one of the following 4 groups. Group N had LAD ligation then had no further intervention and sacrificed on the subsequent day. Group S had LAD ligation then was let to recover for 2 weeks and had sedentary intervention then were sacrificed 4 weeks after. Group E had LAD ligation then had recovery time for 2 weeks and performed mild exercise consisting of swimming exercises 5 times a week for 4 weeks with the duration of exercise was

determined individually by 40% of the maximum time, then was sacrificed. Group C was sham where had thoracotomy without ligation then sacrificed after 6 weeks.

All the animals were distributed in plastic cages (each cage consisted of three rats) under dark condition for 12 hours and light condition for 12 hours. The temperature of the cages was set between 22-27 °C. Drink and pellets were freely distributed to the animals. Exercise was carried out for four weeks.

LAD ligation protocol

The rats were anesthetized with an intramuscular injection of ketamine HCl 50 mg/kg and xylazine 5 mg/kg. The animals were intubated orotracheally using 14 G IV catheter which was inserted wire inside. IV cath was connected to 3-way stop cock. One port was connected to oxygen source ½ L/minute via cannula. Ventilation was performed through open and close the other port of 3-way stop cock with frequency of 70-80 times per minute. The surgery was carried out under sterile conditions. A left parasternal thoracotomy was performed at the fourth and fifth intercostal space, and the heart was exposed by stripping the pericardium. The heart exposed to identify the coronary artery branch. A ligature was then placed around the left coronary artery using prolene 6/0 at 1-2 mm distal to left atrial appendage. After ligation, the surgical wounds were sutured closed. The animals were observed during recovery until fully conscious and then extubated.

Hsp-70, calcineurin, catalase, SOD expression evaluation

According to the prescribed time, rats were sacrificed in anesthetized conditions with Ketamine hydrochloride (50 mg/kgBB). The chest cavity is opened from the middle of the chest and the heart is excised by binding to the aortic ascendens. Then the excised tissue was put into 10% Phosphate-buffered Saline (PBS), then macroscopic morphology was examined and immunohistochemical preparations were made.

Statistical Analysis

The expressions of Hsp-70, calcineurin, SOD, and catalase were examined as much as 10 times with an area width of 125 µm x 125 µm. All data are expressed as means ± SD. Differences among groups were analysed using independent t-test. Correlation was analysed using Pearson correlation. The α-level for statistical significance was set at 0.05.

Results

Hsp-70 expression in the group E was higher than in other groups as shown in Table 1. Group C had the lowest Hsp-70 expression. There was significant difference in Hsp-70 expression between group N and S (Mean difference (MD): -0.30; 95% CI -0.47 to -0.13; p<0.05).

Group	Hsp-70	Mean Difference (95% CI)		
	Mean ± SD	Group S	Group E	Group C
Group N	0.38 ± 0.15	-0.30 (-0.47 to -0.13)*	-1.27 (-1.65 to -0.89)*	0.32 (0.16 to 0.47)*
Group S	0.68 ± 0.12		-0.97 (-1.34 to -0.60)*	0.62 (0.49 to 0.75)*
Group E	1.57 ± 0.43			1.58 (1.22 to 1.94)*
Group C	0.07 ± 0.08			
Note: *Significant result (p<0.05)				

Table 1: Hsp-70 expression in each group.

There was significant difference in calcineurin expression between each group, except between N and C (MD=0.07; 95% CI -0.12 to 0.25; p=0.41) as shown in Table 2. Calcineurin expression in group E was higher than other groups. Sham group had lower calcineurin expression compared to other groups. There was difference in calcineurin expression between group N and S (Mean difference (MD): -0.25; 95% CI -0.42 to -0.08; p<0.05).

Group	Calcineurin	Mean Difference (95%CI)		
	Mean ± SD	Group S	Group E	Group C
Group N	0.40 ± 0.06	-0.25 (-0.42 to -0.08)*	-1.50 (-2.13 to -0.87)*	0.07 (-0.12 to 0.25)
Group S	0.65 ± 0.18		-1.25 (-1.82 to -0.68)*	0.32 (0.09 to 0.54)*
Group E	1.90 ± 0.95			1.57 (1.00 to 2.14)*
Group C	0.33 ± 0.18			
Note: *Significant result (p<0.05)				

Table 2: Calcineurin expression in each group.

There was no significant difference in catalase expression between each group, except between N and S; S and E as shown in Table 3. Group E had higher catalase expression compared to group S (MD= 0.57; 95% CI 0.27 to 0.87; p<0.05). There was significant difference in catalase expression between N and S (Mean difference (MD): 0.27; 95% CI 0.02 to 0.52; p<0.05).

Group	Catalase	Mean Difference 95% CI)		
	Mean ± SD	Group S	Group E	Group C
Group N	0.62 ± 0.24	0.27 (0.01 to 0.52)*	-0.30 (-0.65 to 0.05)	-0.05 (-0.43 to 0.33)
Group S	0.35 ± 0.14		-0.57 (-0.88 to -0.25)*	-0.32 (-0.65 to 0.02)
Group E	0.92 ± 0.30			0.25 (-0.17 to 0.67)
Group C	0.67 ± 0.34			
Note: *Significant result (p<0.05)				

Table 3: Catalase expression in each group.

There was significant difference in SOD expression between each group, except between N and S (MD= -0.07; 95% CI -0.23 to 0.09; p=0.37) as shown in Table 4. SOD expression in group E was higher than group N and S, but not group C. Sham group had higher SOD expression compared to other groups.

Group	SOD	Mean Difference 95% CI)		
	Mean ± SD	Group S	Group E	Group C
Group N	0.40 ± 0.13	-0.07 (-0.23 to 0.09)	-0.48 (-0.76 to -0.20)*	-1.10 (-1.37 to -0.83)*
Group S	0.47 ± 0.12		-0.42 (-0.69 to -0.14)*	-1.03 (-1.30 to -0.77)*
Group E	0.88 ± 0.28			-0.62 (-0.97 to -0.26)*
Group C	1.50 ± 0.27			
Note: *Significant result (p<0.05)				

Table 4: SOD expression in each group.

Correlation analysis showed that HSP had strong positive correlation with calcineurin (r=0.856, p=0.000). As shown in Table 5, there was no significant correlation between Hsp-70 with catalase and SOD. However, Hsp-70 was correlated with catalase and SOD when the analysis included only group who had intervention.

Variables	Calcineurin	Catalase	SOD
HSP 70a	r=0.856 (p=0.000)	r=0.344 (p=0.099)	r= –0.193 (p=0.366)
HSP 70b	r=0.781 (p=0.003)	r=0.801 (p=0.002)	r=0.646 (p=0.023)
Note: a: Analysis in 4 groups b: Analysis in group who had intervention (E and S)			

Table 5: Correlation Hsp-70 with calcineurin, catalase and SOD.

Discussion

Hsp-70 post-AMI

In our study we found that Hsp-70 in group E was higher than other groups. In reverse, sham group had lower Hsp-70 expression compared to other groups. This indicated that Hsp-70 will be expressed in cardiomyocytes experiencing acute myocardial infarction, and then will increase more after having mild exercises. This result was consistent with previous studies that exercise increased Hsp-70 expression in heart [10-12].

Hsp-70 expression may increase after exercise because of influence of extracellular Hsp-70 caused by exocytosis of skeletal muscle cells and production of intracellular Hsp-70 in the myocardial cells [13,14]. Extracellular Hsp-70 will bind to TLR 4 which is widely found in the cell wall of cardiomyocytes [15]. The Hsp-70-TLR 4 bonding process will help Hsp-70 enter through the help of endolysosomes [16,17]. Increased intracellular Hsp-70 will also be influenced by exercises that directly affect changes in myocardial cells such as increased intracellular calcium, changes in acidity that will activate HSF 1 in the intracellular and release bonds with Hsp-70 [18,19].

Calcineurin post-AMI and its relation to Hsp-70

No calcineurin expression difference between group N and C while it was increased in groups who had intervention indicated that calcineurin was not expressed shortly after infarction. Exercise increased calcineurin expression as shown in higher calcineurin expression in group E than Group S. It consistent with previous study that calcineurin expression peaked during ischemia [20]. There was a strong positive correlation between Hsp-70 and calcineurin. This result confirmed the conclusion from previous study that Hsp-70 directly interacted with calcineurin and stimulated an increase in calcineurin activity [21,22].

Anti-oxidant enzyme post-AMI and its relation to Hsp-70

Higher catalase and SOD expression in sham group than group who had intervention suggested that endogenous catalase and SOD was depleted post-AMI and exercise cannot return the anti-oxidant enzymes back to normal level. However, there was statistically significant increase of catalase and SOD expression in group E compared to group S. Prolonged exercise training results in improvements in catalase and SOD activities [23-25]. However, they appear to conflict with another study [12]. Age of subjects and duration of exercise appeared to be possible explanation regarding conflicting results. Old subjects may have decline antioxidant enzymes because of an age-related decline in the Hsp-70. There was adaptation response in increasing anti-oxidant enzymes after prolonged exercise [11]. Changes in antioxidant enzymes occurred during the early phase of the exercise but returned to sedentary values over time as the animals adapted to the exercise [10].

There was strong correlation between Hsp-70 and catalase and SOD in group who had intervention. It indicated that Hsp-70 increased anti-oxidant activity in ischemic condition. It was shown that Hsp-70 resulted in reduced ROS accumulation in ischemic hearts [26]. Through its chaperone activity, HPS-70 facilitates the translocation of newly synthesized SOD into the mitochondria and subsequently inhibits the generation of ROS within the mitochondria [26,27].

Conclusion

This study aimed to explain the relationship of Hsp-70 on calcineurin, SOD, and catalase post-AMI. The result showed that Hsp-70 and calcineurin was increased, but catalase and SOD was depleted in post-AMI condition. Exercise improved Hsp-70, calcineurin, catalase, and SOD activities post-AMI. Hsp-70 expression increased directly calcineurin expression.

Author Contributions

Johanes Nugroho concepted, conducted the research work, performed the investigations, and reviewed the manuscript. Christo Darius conducted literature search, data acquisition and performed the investigations. Maria Probohoesodo conducted the research work, designed and supervised the study. Cornelia Ghea analyzed the data, performed statistical analysis, and drafted the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest

Authors have no conflict of interest to declare.

References

1. Chen S, Brown IR (2007) Translocation of constitutively expressed heat shock protein Hsc70 to synapse - enriched areas of the cerebral cortex after hyperthermic stress. J Neurosci Res 85: 402-409.

2. Marber MS, Latchman DS, Walker JM, Yellon DM (1993) Cardiac stress protein elevation 24 hours after brief ischemia or heat stress is associated with resistance to myocardial infarction. Circulation 88: 1264-1272.
3. Okubo S, Wildner O, Shah MR, Chelliah JC, Hess ML, et al. (2001) Gene transfer of heat-shock protein 70 reduces infarct size *in vivo* after ischemia/reperfusion in the rabbit heart. Circulation 103: 877-881.
4. Li Q, Shi M, Li B (2013) Anandamide enhances expression of heat shock protein 72 to protect against ischemia-reperfusion injury in rat heart. J Physiol Sci 63: 47-53.
5. Mathur S, Walley KR, Wang Y, Indrambarya T, Boyd JH (2011) Extracellular heat shock protein 70 induces cardiomyocyte inflammation and contractile dysfunction via TLR2. Circ J 75: 2445-2452.
6. Someren JS, Faber LE, Klein JD, Tumlin JA (1999) Heat shock proteins 70 and 90 increase calcineurin activity *in vitro* through calmodulin-dependent and independent mechanisms. Biochem Biophys Res Commun 260: 619-625.
7. Mustafi SB, Chakraborty PK, Dey RS, Raha S (2009) Heat stress upregulates chaperone heat shock protein 70 and antioxidant manganese superoxide dismutase through reactive oxygen species (ROS), p38MAPK, and Akt. Cell Stress Chaperon 14: 579.
8. Currie RW, Karmazyn M, Kloc M, Mailer K (1988) Heat-shock response is associated with enhanced postischemic ventricular recovery. Circ Res 63: 543-549.
9. Karmazyn MO, Mailer KA, Currie RW (1990) Acquisition and decay of heat-shock-enhanced postischemic ventricular recovery. Am J Physiol-Heart C Am J Physiol Heart Circ Physiol 259: H424-H431.
10. Harris MB, Starnes JW (2001) Effects of body temperature during exercise training on myocardial adaptations Am J Physiol Heart Circ Physiol 280: H2271-H2280.
11. Starnes JW, Taylor RP, Park Y (2003) Exercise improves postischemic function in aging hearts. Am J Physiol Heart Circ Physiol 285: H347-H351.
12. Starnes JW, Choilawala AM, Taylor RP, Nelson MJ, Delp MD (2005) Myocardial heat shock protein 70 expression in young and old rats after identical exercise programs. Gerontol A Biol Sci Med Sci 60: 963-969.
13. Yamada P, Amorim F, Moseley P, Schneider S (2008) Heat shock protein 72 response to exercise in humans. Sports Med 38: 715-733.
14. Ogawa K, Seta R, Shimizu T, Shinkai S, Calderwood SK, et al. (2011) Plasma adenosine triphosphate and heat shock protein 72 concentrations after aerobic and eccentric exercise. Exerc Immunol Rev 17: 136-149.
15. Schmitt E, Gehrmann M, Brunet M, Multhoff G, Garrido C (2007) Intracellular and extracellular functions of heat shock proteins: repercussions in cancer therapy. J Leukoc Biol 81: 15-27.
16. Guzhova IV, Arnholdt AC, Darieva ZA, Kinev AV, Lasunskaia EB, et al. (1998) Effects of exogenous stress protein 70 on the functional properties of human promonocytes through binding to cell surface and internationalization. Cell Stress Chaperone 3: 67.
17. Zou N, Ao L, Cleveland Jr JC, Yang X, Su X, et al. (2008) Critical role of extracellular heat shock cognate protein 70 in the myocardial inflammatory response and cardiac dysfunction after global ischemia-reperfusion. Am J Physiol Heart Circ Physiol 294: H2805-H2813.
18. Kiang JG, Tsokos GC (1998) Heat shock protein 70 kDa: Molecular biology, biochemistry, and physiology. Pharmacol Therapeut 80: 183-201.
19. Melling CJ, Thorp DB, Milne KJ, Krause MP, Noble EG (2007) Exercise-mediated regulation of Hsp-70 expression following aerobic exercise training. Am J Physiol Heart Circ Physiol 293: H3692-H3698.
20. Parameswaran S, Sharma RK (2015) Expression of calcineurin, calpastatin and heat shock proteins during ischemia and reperfusion. Biochem Biophys Rep 4: 207-214.
21. Norman DA, Yacoub MH, Barton PJ (1998) Nuclear factor NF-κB in myocardium: Developmental expression of subunits and activation by interleukin-1β in cardiac myocytes *in vitro*. Cardiovasc Res 39: 434-441.
22. Lakshmikuttyamma A, Selvakumar P, Sharma RK (2006) Interaction between heat shock protein 70 kDa and calcineurin in cardiovascular systems. Int J Mol Med 17: 419-423.
23. Powers SK, Demirel HA, Vincent HK, Coombes JS, Naito H, et al. (1998) Exercise training improves myocardial tolerance to *in vivo* ischemia-reperfusion in the rat. Am J Physiol Regulatory Integrative Comp Physiol 275: R1468-R1477.
24. Dai Kim J, Yu BP, McCarter RJ, Lee SY, Herlihy JT (1996) Exercise and diet modulate cardiac lipid peroxidation and antioxidant defenses. Free Radic Biol Med 20: 83-88.
25. Lawler JM, Kwak HB, Song W, Parker JL (2006) Exercise training reverses downregulation of Hsp-70 and antioxidant enzymes in porcine skeletal muscle after chronic coronary artery occlusion. Am J Physiol Regul Integr Comp Physiol 291: R1756-R1763.
26. Chen Z, Shen X, Shen F, Zhong W, Wu H, et al. (2013) TAK1 activates AMPK-dependent cell death pathway in hydrogen peroxide-treated cardiomyocytes, inhibited by heat shock protein-70. Mol Cell Biochem 377: 35-44.
27. Suzuki K, Murtuza B, Sammut IA, Latif N, Jayakumar J, et al. (2002) Heat shock protein 72 enhances manganese superoxide dismutase activity during myocardial ischemia-reperfusion injury, associated with mitochondrial protection and apoptosis reduction. Circulation 106: 270.

The Relationship of Hsp-70 with Calcineurin, SOD and Catalase Post-Acute Myocardial Infarction in Wistar Rats Model

ORIGINALITY REPORT

18%	15%	13%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	scidoc.org Internet Source	1%
2	Yan-Jun Song, Chong-Bin Zhong, Xian-Bao Wang. "Heat shock protein 70: A promising therapeutic target for myocardial ischemia-reperfusion injury", Journal of Cellular Physiology, 2019 Publication	1%
3	Aihua Zheng, Li Cao, Shu Qin, Yue Chen, Yong Li, Dongying Zhang. "Exenatide Regulates Substrate Preferences Through the p38γ MAPK Pathway After Ischaemia/Reperfusion Injury in a Rat Heart", Heart, Lung and Circulation, 2017 Publication	1%
4	theses.gla.ac.uk Internet Source	1%
5	Vinh Q. Chau, Fadi N. Salloum, Nicholas N. Hoke, Antonio Abbate, Rakesh C. Kukreja. "Mitigation of the progression of heart failure	1%

with sildenafil involves inhibition of RhoA/Rho-kinase pathway", American Journal of Physiology-Heart and Circulatory Physiology, 2011
Publication

6	www.frontiersin.org Internet Source	1 %
7	www.medrxiv.org Internet Source	1 %
8	repository.unair.ac.id Internet Source	1 %
9	journals.sagepub.com Internet Source	1 %
10	www.freepatentsonline.com Internet Source	1 %
11	Shukla, Arvind Kumar, Prakash Pragya, Hitesh Singh Chaouhan, Anand Krishna Tiwari, Devendra Kumar Patel, Malik Zainul Abdin, and Debapratim Kar Chowdhuri. "Heat Shock Protein-70 (Hsp-70) Suppresses Paraquat-Induced Neurodegeneration by Inhibiting JNK and Caspase-3 Activation in Drosophila Model of Parkinson's Disease", PLoS ONE, 2014. Publication	1 %
12	hdl.handle.net Internet Source	1 %

13	www.biorxiv.org Internet Source	1 %
14	Edilamar Menezes de Oliveira. "O sistema renina angiotensina no treinamento físico", Universidade de Sao Paulo, Agencia USP de Gestao da Informacao Academica (AGUIA), 2010 Publication	1 %
15	Lasers in Cardiovascular Interventions, 2015. Publication	1 %
16	journals.lww.com Internet Source	1 %
17	Someren, J.S.. "Heat Shock Proteins 70 and 90 Increase Calcineurin Activity in Vitro through Calmodulin-Dependent and Independent Mechanisms", Biochemical and Biophysical Research Communications, 19990714 Publication	<1 %
18	fjfsdata01prod.blob.core.windows.net Internet Source	<1 %
19	Systems Biology of Free Radicals and Antioxidants, 2014. Publication	<1 %
20	www.cochranelibrary.com Internet Source	<1 %
	www.oncotarget.com	

21	Internet Source	<1 %
22	physiology.org Internet Source	<1 %
23	rmdopen.bmj.com Internet Source	<1 %
24	www.bioscience.org Internet Source	<1 %
25	S. Kira. "Effects of high peak airway pressure on the expression of heat shock protein 70 in rat lungs: a preliminary study", Acta Anaesthesiologica Scandinavica, 4/2006 Publication	<1 %
26	ajpheart.physiology.org Internet Source	<1 %
27	circres.ahajournals.org Internet Source	<1 %
28	www.iacs.es Internet Source	<1 %
29	www.mdpi.com Internet Source	<1 %
30	www.orygen.org.au Internet Source	<1 %
31	repositorio-aberto.up.pt Internet Source	<1 %

32	"Heat Shock Proteins in Veterinary Medicine and Sciences", Springer Science and Business Media LLC, 2017 Publication	<1 %
33	uk.wikipedia.org Internet Source	<1 %

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		

The Relationship of Hsp-70 with Calcineurin, SOD and Catalase Post-Acute Myocardial Infarction in Wistar Rats Model

GRADEMARK REPORT

FINAL GRADE

/100

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4