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Research Article

DOI:10.2478/rrlm-2021-0036

The Effect of Resveratrol on Sphingosine-1 and Oxidative/ Nitrosative Stress in an Experimental Heart Ischemia Reperfusion Model

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Abstract

***Objectives**: Resveratrol (RSV) is a natural polyphenolic compound showing significant antioxidant effects. In this study, we aimed to investigate the effects of resveratrol on the sphingosine-1-phosphate (S1P) and oxidative stress biomarkers in hearth ischemia-reperfusion (I/R). **Materials and Methods**: The biochemical and histopathological effects of RSV on cardiac ischemia-reperfusion injury were investigated through ELISA- and light microscope. **Results**: We observed statistically significant differences between the treatment group and the control group in terms of malondialdehyde (MDA) level, catalase (CAT) and superoxide dismutase (SOD) activities ($p<0.05$). Histopathologically, we also observed decreased Polymorphonuclear Leucocyte (PMNL) infiltration, myocardial edema, miyositolysis in the treatment group compared to the I/R and sham groups. **Conclusion**: Resveratrol may play an important role in cardiac I/R injury through its anti-inflammatory and antioxidant effects which were biochemically and histopathologically confirmed in the present study.*

***Keywords**: resveratrol, ischemia/reperfusion injury, antioxidant, oxidative stress, heart*

Received: 25th May 2021; Accepted: 5th October 2021; Published: 20th October 2021

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Introduction

Cardiovascular diseases (CVD) are the major cause of the highest mortality rates globally (1). Among them, myocardial infarction, is the first sign of ischemic heart disease in many cases, which is defined as irreversible cardiac muscle necrosis caused by prolonged ischemia. Although the incidence of ischemic heart disease has decreased significantly in the past 30 years, it is still one of the main causes of mortality (2).
Ischemia is generally defined as oxygen deficiency with insufficient perfusion in the tissue or organ, leading to accumulation of toxic metabolites and depletion of cellular energy stores (3, 4). In this context, Superoxide radical (SOR) mediated lipid peroxidation and related products such as MDA, are the major cause of cell membrane injury leading to cell death
It has been already shown that coronary endothelial dysfunction associated with decreased nitric oxide formation might lead to increased oxidative stress and severe myocardial damage both during and after the coronary atherosclerosis phases (5-8).
Despite our knowledge of the pathophysiology of ischemia that we have accumulated during recent decades, reperfusion strategies aiming to restore the ischemic tissue are not devoid of side effects damage (9). Furthermore, although reperfusion strategies in the heart can alleviate mortality rates to some degree, these approaches are insufficient to prevent the myocardial dysfunction after ischemic injury (10).
Resveratrol (3,5,4'-trihydroxystilbene) is a polyphenolic natural antioxidant used for various therapeutic purposes. A good example is a recent study in French which showed that increased red wine consumption was related to a decrease in CVD (11). Further studies confirmed the beneficial role of resveratrol in CVD, diabetes, obesity, depression, and cancer, which have led to its consideration as an effective anti-apoptotic, anti-inflammatory, anti-oxidant, and anti-mitochondrial agent (11, 12). Also, recent ischemia-reperfusion (I/R) studies showed that resveratrol also exerted significant neuro- and cardio-protective effects (13, 14). Numerous studies show that resveratrol is capable of decreasing the oxidative injury by capturing superoxide and hydroxyl radicals. Resveratrol serves also as a powerful in vivo antioxidant related to its ability to increase nitric oxide levels and decrease the mitochondrial oxidative load (15-18). Since the amount of free oxygen radicals in the cardiac tissue increases significantly in the early reperfusion period, it is plausible to hypothesize that resveratrol might exert significant protective effect against cardiac ischemia by its antioxidant effect (6, 19-21). In this respect, several studies have shown that the cardio-protective effects of resveratrol are associated with increased catalase glutathione and nrf-2 levels (22-25) which are important mediators of inflammation (24-26). Sphingosine-1-phosphate (S1P) is an important sphingolipid metabolite involved in vascular system cell growth, apoptosis, adhesion, migration, and immune system modulation (27, 28). Furthermore, there is rapidly increasing evidence suggesting that S1P leads to increased cell survival (29, 30).
To the best of our knowledge, no previous study evaluated the role of resveratrol on CAT, SOD, MDA, and S1P in cardiac I/R injury. To fill this gap in the literature, we aim to examine the effects of resveratrol on cardiac I/R injury.

Materials and Methods

Animals

For this study, female Wistar albino rats weighing from 180 to 230 g were used. Animals were kept under optimum conditions (21±1ºC, 40 to 70% humidity, 12/12 dark-light cycle) in the Laboratory Animal Unit of Kahramanmaras Sutcu Imam University and provided with free access to food and water. This study was approved by the Local Ethics Committee of Kahraman-

maras Sutcu Imam University (2020/06-01). Operative procedure, anesthesia, and animal care methods applied during the tests were suitable with the principles set out in the Guide for the Care and Use of Laboratory Animals issued by the National Institute of Health.

Study Groups

Rats were randomly divided into 4 groups to form study groups and were anesthetized with ketamine (50mg/kg) by intramuscular injection.
Control group (n = 8): Nothing was administered to the rats in this group.
Sham group (n = 8): This group began with intraperitoneal administration of 1 mL saline (0.9% NaCl) two days before the procedure, with 10 min of ischemia and 10 min of reperfusion performed. Following the reperfusion, 1 mL of saline (single dose) was administered again.
Resveratrol treatment group (n = 8): This group began with intraperitoneal administration of 1 mL saline (0.9% NaCl) two days before the procedure, with 10 min of ischemia and 10 min of reperfusion performed. Following the reperfusion, 1 mL of saline (single dose) was administered again. One mililiter of Resveratrol (50 mg/kg/day) was intraperitoneally administered two days before the procedure and then surgery was performed. The heart was subjected to 10 minutes of ischemia and 10 minutes of reperfusion after the surgery. Following the reperfusion, 1 mL (single dose) of Resveratrol was administered again.
Only ischemia-reperfusion group (n = 8): Following the intervention and surgery, the heart was subjected to 10 minutes of ischemia and 10 minutes of reperfusion.
The rats were brought to the laboratory and were individually weighed and anesthetized by administering 50 mg/kg of ketamine hydrochloride (Ketalar vial, Eczacıbası Turkey) intraperitoneally to each of them. Tracheal intubation was performed in rats under anesthesia (anesthesia with Ketamine). The thorax of the rats was opened by making a left thoracotomy between the 4^{th} and 5^{th} thoracic levels, approximately 3 mm. By removing the pericardium and pressing the abdomen, the heart was gently removed from the thorax, and after passing a 6/0 silk threaded atraumatic round needle close to the origin of the left anterior descending coronary artery, the heart was released back into the thorax. Both ends of the silk thread were passed through a short polyethylene pipe (inner diameter 1mm, length 15mm). After a 15-minute stabilization period, the thread placed around the coronary artery was clamped with the help of a clip and temporary regional ischemia was performed for 20 minutes. Reperfusion was performed by loosening the threads and removing the tube.
At the end of the tests, animals were sacrificed and cardiac tissues were removed. Cardiac tissues were subjected to biochemical and histopathological analysis.

Biochemical Analysis

The tissues were homogenized with three volumes of ice-cold 1.15% KCI. Activities of antioxidant enzymes, MDA and S1P levels were measured in the supernatant obtained after centrifugation at 14.000 rpm.
SOD activity in the tissue samples was measured by the method described by Fridovich (31). This method uses xanthine and xanthine oxidase to produce superoxide radicals. The assay medium consisted of 0.01 M of phosphate buffer, CAPS (3-cyclohexilamino-1- propanesulfonicacid) buffer solution with pH 10.2, substrate solution and 80 µL of xanthine oxidase. SOD activity was expressed as U/mg protein (31).
CAT activity was determined by measuring the reduction in hydrogen peroxide concentration at 230 nm by the method of Beutler. Assay medium that consisted of 1 M of Tris HCI, 5 mM of Na_2EDTA buffer solution (pH 8.0), 10 mM of H_2O_2 and tissue sample in a final volume of 1.0 ml. CAT activity was expressed as U/mg protein (32).

The MDA level, which should be observed in tissue samples, was measured with the Thiobarbituric Acid (TBA) test (Ohkawa method). The reaction mixture consisted of 0.1 ml of sample, 0.2 ml of 8.1% sodium dodecyl sulphate (SDS), 1.5 ml of 20% acetic acid, and 1.5 ml of 0.8% aqueous solution of TBA. The pH of the mixture was regulated to 3.5. The volume was then brought to 4.0 ml using distilled water. Then 5.0 ml of a mixture of n-butanol and pyridine (15:1, v/v) was added and shaken. After the mixture was centrifuged at 4000 rpm for 10 minutes, the absorbance of the organic layer was obtained as 532 nm (33).

S1P levels in tissue samples were obtained using commercially available enzyme-linked immunosorbent assay (ELISA) kits (MyBioSource Company, USA) according to the manufacturer's protocol and repeating each measurement in duplicate.

Histopathological Evaluation

Tissues were fixed in 10% neutral buffered formaldehyde solution for 24 hours for histopathological examination. All samples were regularly followed up in the tissue tracker to prepare paraffin blocks. Using these paraffin blocks, 5 μm serial sections were prepared for each tissue sample with a microtome and stained with Hematoxylin-Eosin (H&E) stain. The study was performed by one pathologist without knowing which tissue sample was included and by random selection from tissue samples. Preparations were subjected to histopathological examination using a light microscope. Groups include myocardial edema, myositolysis, focal hemorrhage and PMNL infiltration. Pathological scoring; 0-no, 1-mild, 2-moderate, and 3-severe.

Statistical analysis

Jamovi (ver 1.6.23.0) was used for statistical analysis. One-Way Anova test was performed on data with normal distribution, and Kruskal-Wallis test on data with non-normal distribution. Welch's One-Way Anova test was used for the groups with $p < .05$ in the group to be made One-way Anowa, and the Games-Howell Post-Hoc Test was used for the comparison between the paired groups. Fisher's One-Way Anova test was used for homogeneity $p > .05$ and Tukey Post-Hoc Test was used for comparison between paired groups.

Results

Biochemical results

The results of Shapiro-Wilk normality test, the distribution of the S1P data of the sham group was found to be non-normal. Other data were normally distributed. Since the result of SOD biochemical in Levene's test was $p > .05$, Tukey test was used for SOD biochemical and Games-Howell test was used for MDA and CAT biochemicals in post-hoc comparison between groups. For non-normally distributed S1P biochemical, the groups were compared with the Dwass-Steel-Critchlow-Fligner Pairwise Comparisons test.

According to One-Way Anova test, MDA activity (Table 1; $F = 23.5$, $df2 = 13.9$, $p < .001$), CAT activity (Table 1; $F = 20.2$, $df2 = 12.8$, $p < .001$),

Table 1. One-Way Anova and Kruskal-Wallis test results

		One-Way Anova			
		F	**df1**	**df2**	**p**
MDA (nmol/mg protein)	Welch's	23.5	3	13.9	<.001
CAT (U/mg protein)	Welch's	20.2	3	12.8	<.001
SOD (U/mg protein)	Fisher's	13.5	3	28	<.001
		Kruskal-Wallis			
		χ^2	**df**	**ε^2**	**p**
S1P (ng/L)		22.2	3	0.717	<.001

and SOD activity (Table 1; F = 13.5, df2 = 28, p < .001) scores were significantly decreased while S1P activity (Table 1; Chi square = 22.2, p < .001, df = 3, ε^2 = .717) levels were significantly increased (Kruskal-Wallis test).

The MDA activity was higher in the sham group, resveratrol, and I/R compared to the control group (p<.001), while the MDA activity was higher in the I/R group than the resveratrol treatment groups (p<.001) (Fig. 1, Table 2).

The CAT activity was higher in the sham and control groups, compared with the control and I/R group, respectively (p<.001), while the CAT activity was higher in the sham and resveratrol treatment group than the I/R groups (p<.001). (Fig. 2, Table 2).

The SOD activity was higher in the sham and control groups than the control and I/R group, respectively (p<.001) while SOD activity levels was higher in the sham groups than resveratrol treatment and I/R groups (p<.001). We also found that SOD activity was higher in resveratrol treatment group than I/R groups (p<.001) (Fig. 3, Table 2).

The S1P activity was higher in the sham and I/R group compared with the control and (p<.001), while S1P activity was higher in the I/R group compared with the control and resveratrol treatment groups (p<.001) (Fig. 4, Table 2).

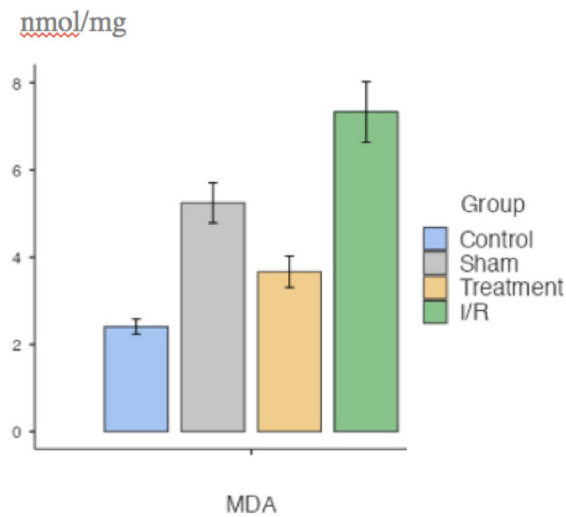

Fig. 1. MDA (nmol/mg protein) levels of the study groups

Histopathologic results

Myocardial edema, myositolysis, and polymorphonuclear leukocytes (PMNL) infiltration were shown in the I/R and sham groups. A statistically significant difference was found only in PMNL infiltration among groups (p < .05). When the RVS treatment group was compared with the I/R and sham groups for myocardial edema, myocytolysis, hemorrhage, and PMNL infiltration, statistically significant differences were found in terms of myocardial edema (p = .287), myocytolysis (p = .470), and PMNL infiltration (p = .031), except hemorrhage (p = .566). The histopathological results of groups are shown in Table 3 and Fig. 5.

Discussion

In our present study we have shown that resveratrol treatment decreased PMNL count and oxidative injury markers-in the I/R group. It was rather interesting that MDA levels, MDA being a strong oxidant molecule, were significantly lower in the treatment group compared with the sham and I/R groups. This is also consistent with previous rat cardiac I/R studies which revealed significantly increased MDA levels (34). In line with this, several trauma models for ischemia in different organs (35-37) have suggested that resveratrol not only decreased the MDA levels, but also improved the functional outcomes (14). We also observed that resveratrol significantly reversed the decreased S1P levels in the I/R group. This is in line with the synergistic role of S1P with the HDL molecule which is a strong anti-atherosclerotic and anti-oxidant molecule which reduces myocardial injury (16, 38). As such, several studies have shown that S1P ex-

Table 2. Post-Hoc test of groups

Biochemical and test type	Variable	Mean difference	t- value	df	p-value
MDA (Games-Howell Post-Hoc Test) (nmol/mg protein)	Control-Sham	-2.84	-5.77	8.95	0.001
	Control-Treatment	-1.26	-3.15	10.1	0.043
	Control-I/R	-4.92	-6.88	7.87	<0.001
	Sham-Treatment	1.58	2.7	13.2	0.075
	Sham-I/R	-2.08	-2.5	12.16	0.11
	Treatment -I/R	-3.66	-4.68	10.52	0.004
CAT (Games-Howell Post-Hoc Test) (U/mg protein)	Control-Sham	-0.17	-5.05	7.3	0.006
	Control-Treatment	-0.0297	-1.98	8.66	0.264
	Control-I/R	0.0247	4.66	9.25	0.005
	Sham-Treatment	0.1406	3.88	9.44	0.015
	Sham-I/R	0.195	5.83	7.05	0.003
	Treatment -I/R	0.0544	3.81	7.28	0.025
SOD (Tukey Post-Hoc Test) (U/mg protein)	Control-Sham	-1.69	-2.84	28	0.039
	Control-Treatment	0.0775	0.13	28	0.999
	Control-I/R	2.09	3.51	28	0.008
	Sham-Treatment	1.7713	2.974	28	0.029
	Sham-I/R	3.79	6.36	28	<0.001
	Treatment -I/R	2.01	3.38	28	0.011
		W	**p-value**		
S1P (Dwass-Steel-Critchlow-Fligner Pairwise Comparisons) (ng/L)	Control-Sham	-4.31	0.012		
	Control-Treatment	-2.52	0.28		
	Control-I/R	-4.75	0.004		
	Sham-Treatment	3.56	0.057		
	Sham-I/R	-2.82	0.19		
	Treatment -I/R	-4.75	0.004		

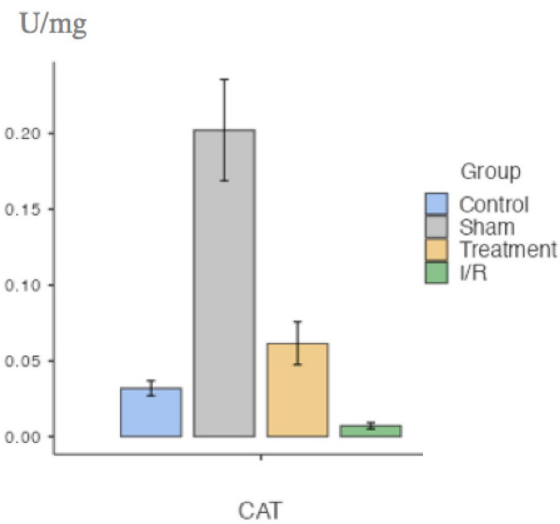

Fig. 2. CAT (U/mg protein) levels of the study groups

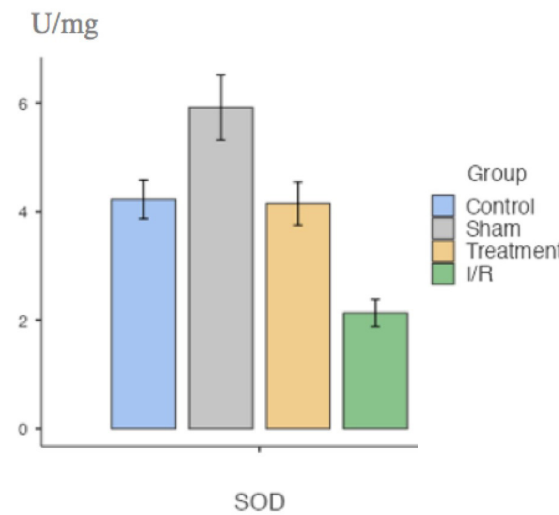

Fig. 3. SOD (U/mg protein) levels of the study groups

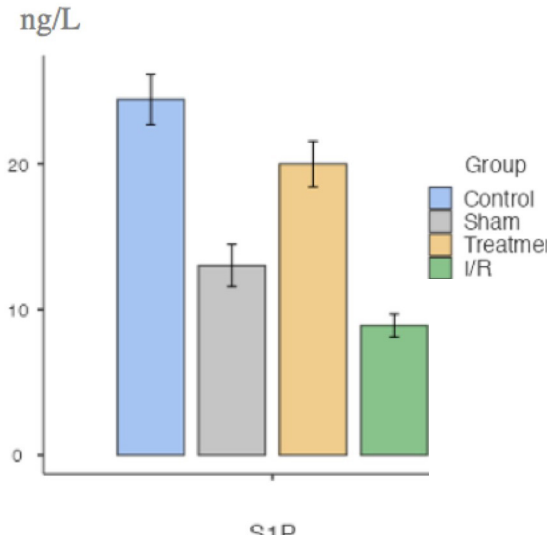

Fig. 4. S1P (ng/L) levels of the study groups

erted significant cardio-protective effects (16), suggesting again that our increased levels of in

Table 3`. Histopathological grade of the groups

	Myocardial edema	Myocytolysis	Haemorrhage	PMNL infiltration
Control	-	-	-	-
I/R	1	1	1	3
Sham	1	1	1	1
Treatment	0	0	1	0

S1P might be associated with decreased inflammation and oxidative injury related to the protective effects of resveratrol.

In our study we have chosen female rats. Although this could appear as an inconsistency, subjects in the referenced studies were only male mice, and it is still unclear whether their positive

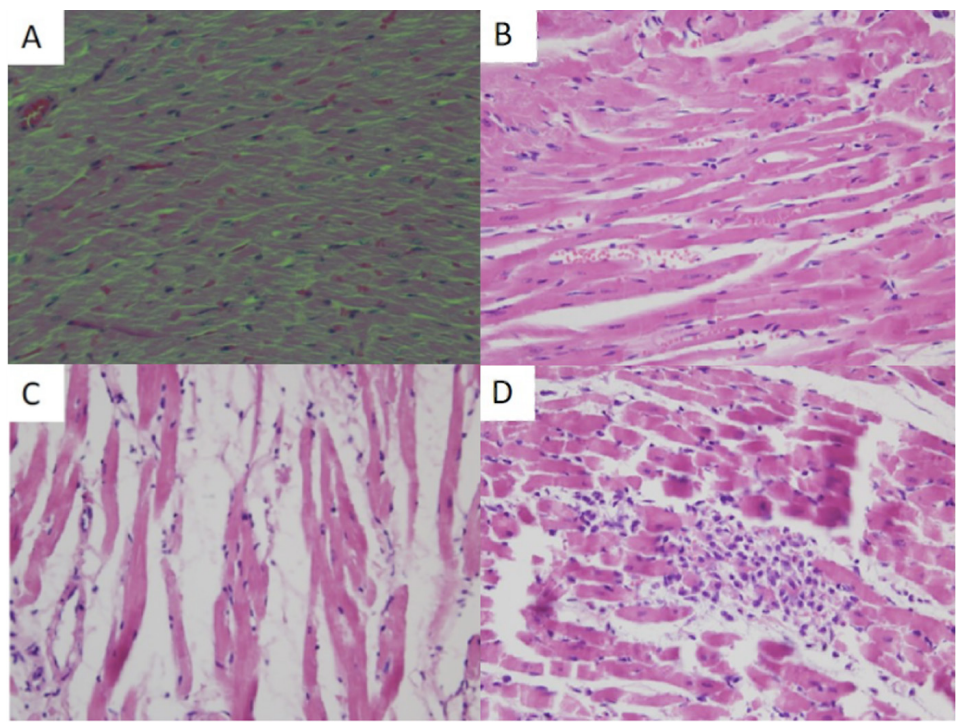

Fig. 5. The histopathological results on the effect of Resveratrol in experimental heart ischemia A: Control group: Normal heart muscle. B: Treatment group (Myocardial Hemorrhage, Inflammation decreased). C: Sham group (Myocardial Edema, Degeneration in muscle bundles). D: I / R group (Polymorphonuclear Leukocyte (PMNL) Infiltration)

results would have applied to female mice. Furthermore, we think that having a model that is reproducible in the same gender is not enough to investigate modifications to disease pathophysiology and an effective multifaceted approach requires a model that must also have the capacity to allow the detection of the effects of potential treatments or procedures in different genders. Also, the Food and Drug Administration declared that there is a requirement for the use of females in human clinical studies, which should be related to increased inclusion of female animals in studies.

As a conclusion, beyond suggesting that dietary plant polyphenols have a regulatory effect on the expression of enzymes involved in the antioxidant defense mechanisms of the cell, our present data provide strong preclinical evidence for the antioxidants used in the treatment of oxidative stress in cardiovascular diseases in female rats. Further studies evaluating the detailed oxidative and anti-oxidative stress signaling cascades may shed light on new therapeutic approaches.

Authors' contributions

SA, MG, EK and ID researched literature and conceived the study. SA, MG, EK, UO, ID and SB were involved in protocol development, operational completion of the study, data interpretation, gaining ethical approval and patient recruitment. SA, BY, SC and HAV were involved in data analysis. SA, MG and BY wrote the first draft of the manuscript. All authors critically reviewed and approved the final version of the manuscript.

Conflict of interest

None to declare.

References

1. Moodie DS. The Global Burden of Cardiovascular Disease. Congenital heart disease. 2016;11(3):213. DOI: 10.1111/chd.12383
2. Andersen HR, Nielsen TT, Rasmussen K, Thuesen L, Kelbaek H, Thayssen P, et al. A comparison of coronary angioplasty with fibrinolytic therapy in acute myocardial infarction. New England J Med. 2003;349(8):733-42. DOI: 10.1056/NEJMoa025142
3. Zimmerman BJ, Granger DN. Reperfusion Injury. Surg Clin North Am. 1992;72(1):65-83. DOI: 10.1016/S0039-6109(16)45628-8
4. Paul A Lapchak JHZ. Neuroprotective therapy for stroke and ischemic disease 2017. DOI: 10.1007/978-3-319-45345-3
5. Pearson PJ, Schaff HV, Vanhoutte PM. Long-term impairment of endothelium-dependent relaxations to aggregating platelets after reperfusion injury in canine coronary arteries. Circulation. 1990;81(6):1921-7. DOI: 10.1161/01.CIR.81.6.1921
6. İkizler M DS, Erkasap N, Kaygısız Z, Sevin B, Kural T. The Hemodynamic Efficacy Of Resveratrol On Reperfusion Injury In Isolated Rat Hearts. Turkish Journal Of Thoracic And Cardiovascular Surgery. 2003;11:91-5.
7. Nazari A, Sadr SS, Faghihi M, Imani A, Moghimian M. The cardioprotective effect of different doses of vasopressin (AVP) against ischemia-reperfusion injuries in the anesthetized rat heart. Peptides. 2011;32(12):2459-66. DOI: 10.1016/j.peptides.2011.10.023
8. Robicsek F, Schaper J. Reperfusion injury: fact or myth? J Card Surg. 1997;12(3):133-7; discussion 8. DOI: 10.1111/j.1540-8191.1997.tb00112.x
9. Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Nutrition J. 2016;15(1):71. DOI: 10.1186/s12937-016-0186-5
10. Qu X, Chen X, Shi Q, Wang X, Wang D, Yang L. Resveratrol alleviates ischemia/reperfusion injury of diabetic myocardium via inducing autophagy. Exp Ther Med. 2019;18(4):2719-25. DOI: 10.3892/etm.2019.7846
11. Mao ZJ, Lin H, Hou JW, Zhou Q, Wang Q, Chen YH. A Meta-Analysis of Resveratrol Protects against Myocardial Ischemia/Reperfusion Injury: Evidence from Small Animal Studies and Insight into Molecular Mechanisms. Oxid Med Cell Longev. 2019;2019:5793867. DOI: 10.1155/2019/5793867
12. Yulug B, Hanoglu L, Khanmammadov E, Duz OA, Polat B, Hanoglu T, et al. Beyond The Therapeutic Effect of rTMS in Alzheimer's Disease: A Possible Neuroprotective Role of Hippocampal BDNF? : A Minireview. Mini Rev Med Chem. 2018;18(17):1479-85. DOI: 10.2174/1389557517666170927162537
13. Liu FC, Tsai HI, Yu HP. Organ-Protective Effects of Red Wine Extract, Resveratrol, in Oxidative Stress-Me-

diated Reperfusion Injury. Oxid Med Cell Longev. 2015;2015:568634. DOI: 10.1155/2015/568634
14. Kazemirad H, Kazerani HR. Cardioprotective effects of resveratrol following myocardial ischemia and reperfusion. Molec biol reports. 2020;47(8):5843-50. DOI: 10.1007/s11033-020-05653-7
15. de la Lastra CA, Villegas I. Resveratrol as an antioxidant and pro-oxidant agent: mechanisms and clinical implications. Biochemical Society transactions. 2007;35(Pt 5):1156-60. DOI: 10.1042/BST0351156
16. Xu H, Cheng J, Wang X, Liu H, Wang S, Wu J, et al. Resveratrol pretreatment alleviates myocardial ischemia/reperfusion injury by inhibiting STIM1-mediated intracellular calcium accumulation. J Physiol Biochem. 2019;75(4):607-18. DOI: 10.1007/s13105-019-00704-5
17. Arinno A, Apaijai N, Chattipakorn SC, Chattipakorn N. The roles of resveratrol on cardiac mitochondrial function in cardiac diseases. Eur J Nut. 2021;60(1):29-44. DOI: 10.1007/s00394-020-02256-7
18. Fourny N, Lan C, Sérée E, Bernard M, Desrois M. Protective Effect of Resveratrol against Ischemia-Reperfusion Injury via Enhanced High Energy Compounds and eNOS-SIRT1 Expression in Type 2 Diabetic Female Rat Heart. Nutrients. 2019;11(1):105. DOI: 10.3390/nu11010105
19. Das DK, Maulik N. Resveratrol in cardioprotection: a therapeutic promise of alternative medicine. Molecular interventions. 2006;6(1):36-47. DOI: 10.1124/mi.6.1.7
20. Zweier JL. Measurement of superoxide-derived free radicals in the reperfused heart. Evidence for a free radical mechanism of reperfusion injury. J Biol Chem. 1988;263(3):1353-7. DOI: 10.1016/S0021-9258(19)57309-4
21. Hung LM, Su MJ, Chu WK, Chiao CW, Chan WF, Chen JK. The protective effect of resveratrols on ischaemia-reperfusion injuries of rat hearts is correlated with antioxidant efficacy. British J Pharmacol. 2002;135(7):1627-33. DOI: 10.1038/sj.bjp.0704637
22. Moskaug J, Carlsen H, Myhrstad MC, Blomhoff R. Polyphenols and glutathione synthesis regulation. American J Clin Nut. 2005;81(1 Suppl):277s-83s. DOI: 10.1093/ajcn/81.1.277S
23. Brito PM, Mariano A, Almeida LM, Dinis TC. Resveratrol affords protection against peroxynitrite-mediated endothelial cell death: A role for intracellular glutathione. Chemico-biological interactions. 2006;164(3):157-66. DOI: 10.1016/j.cbi.2006.09.007
24. Shen C, Cheng W, Yu P, Wang L, Zhou L, Zeng L, et al. Resveratrol pretreatment attenuates injury and promotes proliferation of neural stem cells following oxygen-glucose deprivation/reoxygenation by upregulating the expression of Nrf2, HO-1 and NQO1 in vitro. Mol Med Reports. 2016;14(4):3646-54. DOI: 10.3892/mmr.2016.5670
25. Cheng L, Jin Z, Zhao R, Ren K, Deng C, Yu S. Resveratrol attenuates inflammation and oxidative stress induced by myocardial ischemia-reperfusion injury: role of Nrf2/ARE pathway. Int J Clin Exp Med. 2015;8(7):10420-8.
26. Caglayan B, Kilic E, Dalay A, Altunay S, Tuzcu M, Erten F, et al. Allyl isothiocyanate attenuates oxidative stress and inflammation by modulating Nrf2/HO-1 and NF-κB pathways in traumatic brain injury in mice. Molec Biol Rep. 2019;46(1):241-50. DOI: 10.1007/s11033-018-4465-4
27. Verzijl D, Peters SL, Alewijnse AE. Sphingosine-1-phosphate receptors: zooming in on ligand-induced intracellular trafficking and its functional implications. Molecules and cells. 2010;29(2):99-104. DOI: 10.1007/s10059-010-0041-z
28. Kluk MJ, Hla T. Role of the sphingosine 1-phosphate receptor EDG-1 in vascular smooth muscle cell proliferation and migration. Circ Res. 2001;89(6):496-502. DOI: 10.1161/hh1801.096338
29. Maceyka M, Harikumar KB, Milstien S, Spiegel S. Sphingosine-1-phosphate signaling and its role in disease. Trends in cell biology. 2012;22(1):50-60. DOI: 10.1016/j.tcb.2011.09.003
30. Snider AJ. Sphingosine kinase and sphingosine-1-phosphate: regulators in autoimmune and inflammatory disease. Int J Clin Rheum. 2013;8(4). DOI: 10.2217/ijr.13.40
31. Fridovich I. Superoxide dismutases. Advances in enzymology and related areas of molecular biology. 1974;41(0):35-97. DOI: 10.1002/9780470122860.ch2
32. Ernest Beutler. Red Cell Metabolism. A Manual of Biochemical Methods 2nd ed. ed. New York: Grune and Stratton Inc 1984. 68-70 p
33. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351-8. DOI: 10.1016/0003-2697(79)90738-3
34. Annapurna A, Mudagal MP, Ansari A, Rao AS. Cardioprotective activity of chalcones in ischemia/reperfusion-induced myocardial infarction in albino rats. Exp Clin Cardiol. 2012;17(3):110-4.
35. Sener G, Tuğtepe H, Yüksel M, Cetinel S, Gedik N, Yeğen BC. Resveratrol improves ischemia/reperfusion-induced oxidative renal injury in rats. Arch Med Res. 2006;37(7):822-9. DOI: 10.1016/j.arcmed.2006.04.003
36. Aktaş HS, Ozel Y, Ahmad S, Pençe HH, Ayaz-Adakul B, Kudas I, et al. Protective effects of resveratrol on hepatic ischemia reperfusion injury in streptozotocin-induced diabetic rats. Mol Cell Biochem. 2019;460(1-2):217-24. DOI: 10.1007/s11010-019-03582-z
37. Dong W, Yang R, Yang J, Yang J, Ding J, Wu H, et al. Resveratrol pretreatment protects rat hearts from ischemia/reperfusion injury partly via a NALP3 inflammasome

pathway. Int J Clin Exp Pathol. 2015;8(8):8731-41.
38. Nusshold C, Uellen A, Bernhart E, Hammer A, Damm S, Wintersperger A, et al. Endocytosis and intracellular processing of BODIPY-sphingomyelin by murine CATH.a neurons. Biochim Biophys Acta. 2013;1831(12):1665-78. DOI: 10.1016/j.bbalip.2013.08.007