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Research Article

AMELIORATION OF MYOCARDIAL ISCHEMIA REPERFUSION INJURY BY SIMVASTATIN IN RATS

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ABSTRACT
The present study has been designed to investigate the effect of Simvastatin, a 3-hydroxymethyl-glutaryl coenzyme A (HMG-CoA) reductase inhibitor, on ischemia-reperfusion (I/R)-induced myocardial injury. The isolated Langendorff-perfused rat hearts were subjected to global ischemia for 30 min followed by reperfusion for 120 min. Myocardial injury was assessed by measuring myocardial infarct size alongwith release of lactate dehydrogenase (LDH) and creatine kinase (CK) in the coronary effluent. Additionally, the oxidative stress parameters were analyzed in the heart which was assessed by measuring lipid peroxidation, superoxide anion generation and reduced glutathione. I/R was noted to produce myocardial injury, as assessed in terms of increase in myocardial infarct size, LDH and CK in coronary effluent. Moreover, oxidative stress was noted to be increased due to I/R injury as assessed in terms of decreased TBARS (thiobarbituric acid-reactive substance) and superoxide anion generation levels alongwith increase in reduced glutathione levels in the heart. Treatment with Simvatstain at different concenterations (3 µMol, 10 µMol and 30 µMol) afforded cardioprotection against I/R-induced myocardial injury in rat hearts as assessed in terms of reductions in myocardial infarct size, LDH and CK levels in coronary effluent. Moreover, the high degree of oxidative stress produced as a result of I/R injury was noted to be reduced by Simvastatin treatment. It may be concluded that reductions in myocardial infarct size and oxidative stress may be responsible for the observed cardioprotective potential of Simvastatin against I/R-induced myocardial injury.
KEY WORDS: Simvastatin, HMG-C0A, Ischemia-reperfusion injury, Oxidative stress

INTRODUCTION

The reperfusion to previously ischemic myocardium induces detrimental changes in the myocardium resulting in inflammation, oxidative damage and cardiac dysfunction known as ischemia-reperfusion (I/R)-injury[1,2]. Oxidative stress, intracellular calcium overload, neutrophil and leukocyte activation, excessive intracellular osmotic load, apoptotic and necrotic myocytes death have been implicated in the pathogenesis of I/R-induced myocardial injury[2,3]. It has been widely accepted that oxidative stress plays an important role in producing lethal injury associated with myocardial I/R[4]. Surprisingly, reactive oxygen species (ROS) produced at the onset of reperfusion has been noted to enhance the oxidative stress in heart which is known to cause the detrimental changes in heart[5]. The HMG-CoA reductase inhibitors commonly known as statins, possess manifold favorable effects above and beyond that of cholesterol lowering in affording cardioprotection[6]. Simvastatin, a potent HMG-CoA reductase inhibitor, has been well reported to be a potent cardioprotective agent due to its antioxidant properties[7]. Simvastatin has been noted to prevent the leukocytic and aortic productions of reactive oxygen species (ROS) alongwith inhibition of protein and lipid oxidation products such as thiobarbituric acid reactive oxygen species (TBARS) confirming its antioxidant potential[7,8]. In addition, experimental studies have shown that treatment with simvastatin attenuated the oxidative stress and produced cardioprotection by decreasing malondialdehyde (MDA) levels and increasing the superoxide dysmutase (SOD) and nitric oxide (NO) levels[9,10]. Further, treatment with simvastatin reduced oxidative stress and infarction volume thereby ameliorating ischemic damage in rats that confirmed the cardioprotective potential of the drug. Moreover, experimental studies in rats have shown that the treatment with simvastatin decreased oxidative stress in diabetic-hypercholesterolemic rats further confirming its antioxidant potential[11]. In addition, Simvastatin has been well reported to alleviate myocardial contractile dysfunction and lethal ischemic injury in isolated Langendorff-perfused rat heart model[12-15]. Therefore, the present study was undertaken to investigate the cardioprotective effect of Atorvastatin against I/R-induced myocardial injury in rat hearts.

MATERIALS AND METHODS

Experimental Animals

The experimental protocol used in the present study was approved by the Institutional Animal Ethical Committee. Wistar albino rats of either sex weighing 180-220 g were used. They were housed in Institutional animal housing and were maintained on rat feed (Kisan Feeds Ltd., Chandigarh, India) and tap water ad libitum.

Isolated Rat Heart Preparation

Rats were heparinized (500 IU i.p.) and sacrificed by stunning. The heart was rapidly excised and immediately mounted on a Langendorff apparatus[16]. The heart was enclosed in a double walled jacket, the temperature of which was maintained at 37°C by circulating hot water. The preparation was perfused with Krebs-Henseleit (K-H) solution (NaCl 118 mM; KCl 4.7 mM; $CaCl_2$ 2.5 mM; $MgSO_4.7H_2O$ 1.2 mM; $NaHCO_3$ 25 mM; KH_2PO_4 1.2 mM; $C_6H_{12}O_6$ 1 mM) pH 7.4, maintained at 37 °C and bubbled with 95% O_2 and 5% CO_2. The coronary flow rate was maintained at around 7 mL/min, and the perfusion pressure was kept at 80 mmHg. Global ischemia was produced for 30 min by blocking the inflow of physiological solution and it was followed by perfusion for 120 min.

Laboratory Assays

Myocardial infarct size was measured macroscopically using triphenyl tetrazolium chloride (TTC) staining employing volume method[17]. The myocardial injury was assessed by measuring the release of lactate dehydrogenase (LDH) and creatine kinase-MB (CK-MB) in the coronary effluent using the commercially available enzymatic kits (Vital Diagnostics, Thane, Maharashtra, India). The level of thiobarbituric acid reactive substances (TBARS), an index of lipid peroxidation in the heart was estimated according to the method of Ohkawa et al.[18]. The superoxide anion generation was assessed by estimating the reduced nitro blue tetrazolium (NBT) using the method of Wang et al.[19]. Moreover, the reduced glutathione content in each heart was estimated using the method of Beutler et al.[20].

Experimental Protocol

Five groups of 8-10 animals each were employed in the present study. In all groups, each isolated perfused heart was allowed to stabilize for 10 min by perfusing with K-H solution.

Group I (Normal Control): Isolated normal rat heart was perfused for 150 min using K-H solution after 10 min of stabilization.

Group II (I/R): Isolated normal rat heart after 10 min of stabilization was subjected to 30 min of global ischemia followed by 120 min of reperfusion

Group III (Sim Treated I/R-3 µMol): After 10 min of stabilization, isolated normal rat heart was infused with Simvastatin (3 µMol) for 10 min. The heart was then subjected to 30 min of global ischemia followed by 120 min of reperfusion.

Group IV (Sim Treated I/R-10 µMol): After 10 min of stabilization, isolated normal rat heart was infused with Simvastatin (10 µMol) for 10 min. The heart was then subjected to 30 min of global ischemia followed by 120 min of reperfusion.

Group V (Sim Treated I/R-30 µMol): After 10 min of stabilization, isolated normal rat heart was infused with Simvastatin (30 µMol) for 10 min. The heart was then subjected to 30 min of global ischemia followed by 120 min of reperfusion.

Statistical Analysis

The results were expressed as mean ± SD. The data obtained from various groups were statistically analyzed using one-way ANOVA followed by Tukey's multiple-comparison test. A P value < 0.05 was considered to be statistically significant.

Drugs and Chemicals

The LDH and CK enzymatic estimation kits were purchased from Vital Diagnostics, Thane, Maharastra, India. DTNB and NBT were obtained from Loba Chem, Mumbai, India. Simvastatin, 1,1,3,3-tetramethoxy propane and reduced glutathione were procured from Sigma-Aldrich, USA. All other reagents used in this study were of analytical grade.

RESULTS

Effect of I/R on Myocardial Infarct size and Oxidative Stress

I/R was noted to increase the infarct size in rat hearts as assessed macroscopically using TTC (Fig. 1). Moreover, the global ischemia for 30 min followed by reperfusion for 120 min significantly increased LDH and CK release in the coronary effluent in rat hearts. Maximum release of LDH was noted immediately after reperfusion (Fig. 3), while maximum release of CK was noted at 5 min of reperfusion (Fig. 2).

Lipid peroxidations, measured in terms of TBARS, and superoxide anion generation, assessed in terms of reduced NBT, were significantly increased in rat hearts subjected to I/R. Moreover, the levels of reduced GSH were found to be decreased in the rat hearts subjected to I/R that may be attributed to the enhanced oxidative stress in I/R-induced myocardial injury (Figs. 4-6).

Effect of Simvastatin on I/R-Induced Infarct size and Oxidative Stress

Treatments with Simvastatin in different concenterations (3 µMol, 10 µMol and 30 µMol) afforded cardioprotection by significantly attenuating I/R-induced myocardial injury in rat hearts as assessed in terms of reductions in myocardial infarct size and decreased release of LDH and CK in coronary effluent (Fig. 1-3). However, maximum cardioprotection was noted at a concentration of 10 µMol.

In addition, Simvastatin treatments (3 µMol, 10 µMol and 30 µMol) markedly attenuated the I/R-induced oxidative stress in normal rat hearts, as assessed in terms of reduction in TBARS and superoxide anion generation, and the consequent increase in GSH (Fig. 4-6). However, maximum reduction of I/R-induced oxidative stress was noted at a concentration of 10 µMol.

DISCUSSION

Ischemic heart disease (IHD) represents the leading cause of morbidity and mortality worldwide whose prevalence is continuously increasing worldwide[21]. Myocardial ischemia is a condition in which the coronary blood flow to the heart is reduced, which results in deficient oxygen and nutrients supply to the heart[1,2]. Myocardial reperfusion is the restoration of blood flow to an ischemic heart. Reperfusion to an ischemic myocardium often results in lethal myocardial injury known as I/R injury[2]. The increase in infarct size and the release of LDH and CK are documented to be an index of I/R-induced myocardial injury[22,23]. In the present study, 30 min of ischemia followed by 120 min of reperfusion was noted to produce myocardial injury, as assessed in terms of increased infarct size in the heart and elevated release of LDH and CK in the coronary effluent. The maximal release of LDH was noted immediately after reperfusion, whereas peak release of CK was observed after 5 min of reperfusion - both findings in accordance with our earlier studies[24,25]. Increases in lipid peroxidation and superoxide anion generation have been suggested as indicators of oxidative stress[26,27]. The lipid peroxidations, measured in terms of TBARS, and superoxide anion generation, assessed in terms of reduced NBT, were noted to be increased as a result of I/R. In addition, the GSH level was decreased in rat hearts subjected to I/R. These indicators suggest the development of I/R-induced oxidative stress, which may be responsible for the noted I/R-induced myocardial injury in the present study. When oxygen is reintroduced during reperfusion, conversion of excess hypoxanthine to xanthine by xanthine oxidase results in the formation of ROS, including superoxide anions (O^{2-}), hydroxyl radicals (OH^{-}), hydrogen peroxide (H_2O_2) and peroxinitrite ($ONOO^{-}$)[2,3,5]. Thus, the observed marked increase in myocardial injury in the rat heart may be due to the high degree of oxidative stress as a result of I/R.

Statins, commonly known as HMG-CoA reductase inhibitors, have been widely accepted to possess various pleiotropic effects in a way to afford cardioprotection[6]. Simvastatin, a member of the statins, is a synthetic derivate of a fermentation product of *Aspergillus terreus*, initially marketed by Merck and Company under the trade name Zocor, that has been well reported to inhibit HMG-CoA reductase enzyme found in liver and show cardioprotection[7]. In addition, numerous studies have demonstrated Simvastatin to possess cardioprotective effects due to its potent antioxidant properties[7]. Simvastatin reduced the activity of NADPH-CoQ reductase, an enzyme required in generation of free radicals that evidenced its potent role as an antioxidant[28]. Moreover, treatment with Simvastatin prevented the leukocytic and aortic productions of ROS alongwith inhibition of lipid oxidation products such as TBARS confirming its antioxidant potentia[l8]. Experimental studies have shown that treatment with simvastatin attenuated the oxidative stress and produced cardioprotection by decreasing MDA levels and increasing the SOD activity[9,10]. Moreover, experimental studies in rats have shown that the treatment with simvastatin decreased oxidative stress in diabetic-hypercholesterolemic rats further confirming its antioxidant potential[11]. This contention is supported by the results obtained in the present study that treatment with Simvastatin in different concenterations (3 µMol, 10 µMol and 30 µMol), has markedly reduced the oxidative stress in rat hearts subjected to I/R, as assessed in terms of reductions in TBARS and superoxide anion generation, and consequent increase in reduced glutathione levels, with maximum reductions at a concentration of 10 µMol.

In addition, a number of studies have demonstrated Simvastatin to reduce myocardial injury parameters in order to mimic cardioprotection. Treatment with simvastatin has been noted to significantly improve the endothelial function in mice[29]. Further, treatment with simvastatin reduced the infarction volume thereby ameliorating ischemic damage in rats that confirmed the cardioprotective potential of the drug. In addition, Simvastatin has been well reported to alleviate myocardial contractile dysfunction and lethal ischemic injury in isolated Langendorff-perfused rat heart model[12,13,14,15]. The present study investigated the cardioprotective potential of Simvastatin against I/R injury in rat hearts when administered at the onset of reperfusion. The data demonstrates that

administration of Simvastatin at the onset of reperfusion results in significant, dose-dependent cardioprotection, with optimal concentration ranges of 3 µMol, 10 µMol and 30 µMol with maximal protection at 30 µMol, which is in accordance with the earlier reports[14,15]. Moreover, treatments with Simvastatin afforded cardioprotection by significantly attenuating I/R-induced myocardial injury in rat hearts as assessed in terms of reductions in myocardial infarct size and decreased release of LDH and CK in coronary effluent, with maximum cardioprotection at a concentration of 10 µMol.

On the basis of the above discussion, it may be concluded that I/R-injury may formulate the heart susceptible to increased infarct size and enhanced oxidative stress. Simvastatin, due to its potent antioxidant effects, showed cardioprotection in rat hearts. Further studies are under way in our laboratory to elucidate the mechanisms involved in the attenuation of myocardial injury by statins.

REFERENCES

1. Collard CD, Gelman S. Pathophysiology, clinical manifestations, and prevention of ischemia–reperfusion injury. Anesthesiology 2001;94:1133-8.
2. Balakumar P, Rohilla A, Singh M. Preconditioning and postconditioning to limit ischemia reperfusion-induced myocardial injury: what could be the next footstep? Pharmacol Res 2008;57:403-12.
3. Yellon DM, Housenloy DJ. Myocardial reperfusion injury. N Engl J Med 2007;357:1121-35.
4. Khan NA, Chattopathyay P, Kumar D, Kishore K, Wahi AK. Ischemic reperfusion injury alters the rat heart activity. Arch Appl Sci Res 2009;1:74-80.
5. Bertuglia S, Giusti A, Del Soldato P. Antioxidant activity of a nitro derivative of aspirin against ischemia–reperfusion in hamster cheek pouch microcirculation. Am J Physiol Gastrointest Liver Physiol 2004;286:G437-43.
6. Lander JS, Coplan NL. Statin therapy in the perioperative period. Rev Cardiovasc Med 2011;12:30-7.
7. Rohilla A, Rohilla S, Singh G, Kumar A, Khan M.U. Cardioprotection with Simvastatin: An Appraisal. Int Res J Pharm 2011;2:23-7.
8. Delbosc S, Cristol JP, Descomps B, Mimran A, Jover B. Simvastatin prevents angiotensin II-induced cardiac alteration and oxidative stress. Hypertension 2002;40:142-7.
9. Bayorh MA, Ganafa AA, Eatman D, Walton M, Feuerstein GZ. Simvastatin and losartan enhance nitric oxide and reduce oxidative stress in salt-induced hypertension. Am J Hypertens 2005;18:1496-502.
10. Yin D, Liu M, Yang G, Huanga J, Gui M. Effects of simvastatin on early oxidative stress and endothelial function in apolipoprotein E-deficient mice. J Nanjing Med Uni 2007;21:359-62.
11. Kuzelová M, Adameová A, Sumbalová Z, Paulíková I, Harcárová A, Svec P, Kucharská J. The effect of simvastatin on coenzyme Q and antioxidant/oxidant balance in diabetic-hypercholesterolaemic rats. Gen Physiol Biophys 2008;27:291-8.
12. Adameová A, Kuželová1 M, Fáberová V, Svec P. Protective effect of simvastatin and VULM 1457 in ischaemic-reperfused myocardium of the diabetic-hypercholesterolemic rats. Pharmazie 2006;61:807-8.
13. Adameová A, Harčárová1 A, Matejíková J, Pancza D, Kuželová1 M, Carnická S, et al. Simvastatin Alleviates Myocardial Contractile Dysfunction and Lethal Ischemic Injury in Rat Heart Independent of Cholesterol-Lowering Effects. Physiol Res 2009;58:449-54.
14. Zheng X, Hu SJ. Effects of simvastatin on cardiohemodynamic responses to ischemia–reperfusion in isolated rat hearts. Heart Vessels 2006;21:116-23.
15. Szárszoi O, Malý J, Ošťádal P, Netuka I, Bešík J, Kolář F, et al. Effect of Acute and Chronic Simvastatin Treatment on Post-Ischemic Contractile Dysfunction in Isolated Rat Heart. Physiol Res 2008;57:793-6.
16. Langendorff O. Untersuchungen am uberlebenden Saugethierherzen. Archiv fur die gesammte Physiologie des Menschen und der Thiere Bonn 1895,61:291-332.
17. Parikh V, Singh M. Possible role of adrenergic component and cardiac mast cell degranulation in preconditioning-induced cardioprotection. Pharmacol Res 1999;40:129-37.
18. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
19. Wang HD, Pagano PJ, Du Y, Cayatte AJ, Quinn MT, Brecher P, et al. Superoxide anion from the adventitia of the rat thoracic aorta inactivates nitric oxide. Circ Res 1998;82:810-8.
20. Beutler E, Duron O, Kefly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963;61:882-8.
21. Pepine CJ, Nichols WW. The pathophysiology of chronic ischemic heart disease. Clin Cardiol 2007;30:I-4-9.
22. Kaur H, Parikh V, Sharma A, Singh M. Effect of amiloride a Na+/H+ exchange inhibitor on cardioprotective effect of ischaemic preconditioning: Possible involvement of resident cardiac mast cells. Pharmacol Res 1997;36:95-102.
23. Sharma A, Singh M. Possible mechanism of cardioprotective effect of ischaemic preconditioning in isolated rat heart. Pharmacol Res 2000;41:635-40.
24. Balakumar P, Rohilla A, Singh G, Singh K, Singh M. Modulation of Cardioprotective Effect of Ischemic Pre- and Postconditioning in the Hyperhomocysteinemic Rat Heart. Meth Find Exp Clin Pharmacol 2009;31:71-9.
25. Singh G, Rohilla A, Singh M, Balakumar P. Possible Role of JAK-2 in Attenuated Cardioprotective Effect of Ischemic Preconditioning in Hyperhomocysteinemic Rat Hearts. Yakugaku Zasshi 2009;129:523-35.
26. Ungvari Z, Csiszar A, Edwards JG. Increased superoxide production in coronary arteries in hyperhomocysteinemia: Role of tumor necrosis factor-α, NAD(P)H oxidase, and inducible nitric oxide synthase. Arterioscler Thromb Vasc Biol 2003;23:418-24.
27. Devi S, Kennedy RH, Joseph L, Shekhawat NS, Melchert RB, Joseph J. Effect of long-term hyperhomocysteinemia on myocardial structure and function in hypertensive rats. Cardiovasc Pathol 2006;15:75-82.
28. Kettawan A, Takahashi T, Kongkachuichai R, Charoenkiatkul S, Kishi T, Okamoto T. Protective effects of coenzyme q(10) on decreased oxidative stress resistance induced by simvastatin. J Clin Biochem Nutr 2007;40:194-202.
29. Tong XK, Nicolakakis N, Fernandes P, Ongali B, Brouillette J, Quirion R, et al. Simvastatin improves cerebrovascular function and counters soluble amyloid-beta, inflammation and oxidative stress in aged APP mice. Neurobiol Dis 2009;35:406-14.

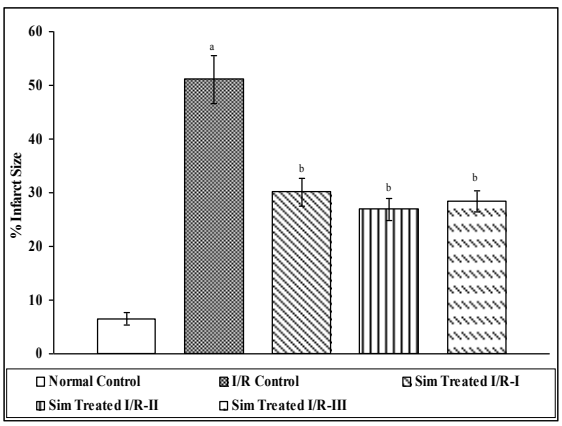

Figure 1. Effect of Simvastatin on increases in infarct size induced by ischemia–reperfusion (I/R). Values are expressed as mean ± SD.
a= P < 0.05 vs. normal control; b= P < 0.05 vs. I/R control. Sim Treated I/R-I= 3 µMol; Sim Treated I/R-II= 10 µMol; Sim Treated I/R-III= 30 µMol.

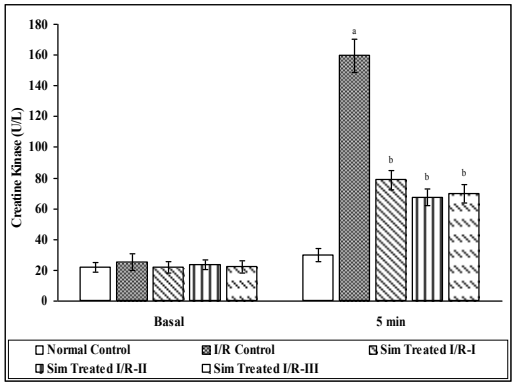

Figure 2. Effect of Simvastatin on increases in creatine kinase (CK) levels induced by ischemia–reperfusion (I/R). Values are expressed as mean ± SD. a= P < 0.05 vs. normal control; b= P < 0.05 vs. I/R control. Sim Treated I/R-I= 3 µMol; Sim Treated I/R-II= 10 µMol; Sim Treated I/R-III= 30 µMol.

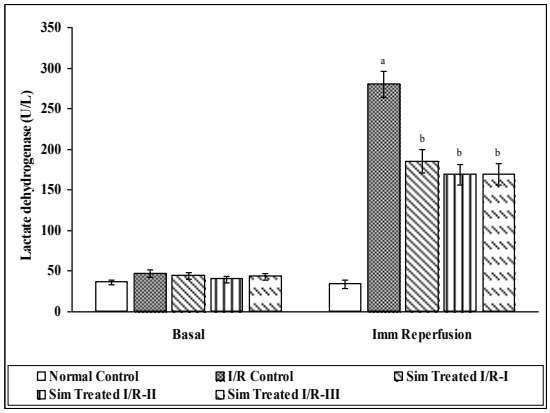

Figure 3. Effect of Simvastatin on increases in lactate dehydrogenase (LDH) levels induced by ischemia–reperfusion (I/R). Values are expressed as mean ± SD. a= P < 0.05 vs. normal control; b= P < 0.05 vs. I/R control. Sim Treated I/R-I= 3 µMol; Sim Treated I/R-II= 10 µMol; Sim Treated I/R-III= 30 µMol.

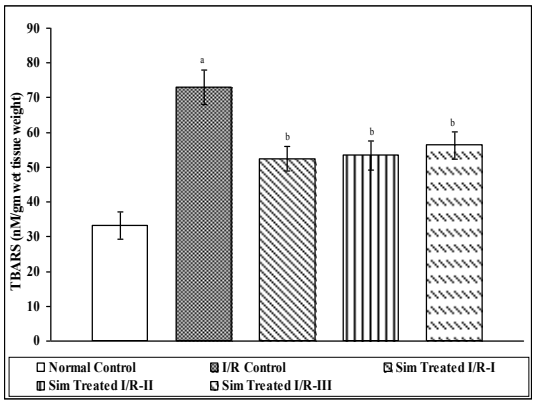

Figure 4. Effect of Simvastatin on increases in thiobarbituric acid reactive substance (TBARS) levels induced by ischemia–reperfusion (I/R). Values are expressed as mean ± SD. a= P < 0.05 vs. normal control; b= P < 0.05 vs. I/R control. Sim Treated I/R-I= 3 µMol; Sim Treated I/R-II= 10 µMol; Sim Treated I/R-III= 30 µMol.

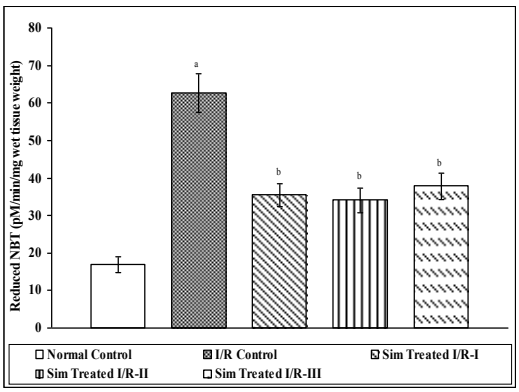

Figure 5. Effect of Simvastatin on increases in superoxide anion levels induced by ischemia–reperfusion (I/R). Values are expressed as mean ± SD. a= P < 0.05 vs. normal control; b= P < 0.05 vs. I/R control. Sim Treated I/R-I= 3 µMol; Sim Treated I/R-II= 10 µMol; Sim Treated I/R-III= 30 µMol.

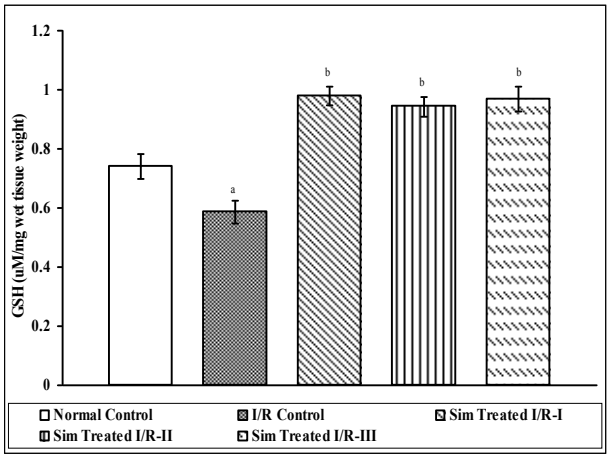

Figure 6. Effect of Simvastatin on decreases in reduced glutathione (GSH) levels induced by ischemia–reperfusion (I/R). Values are expressed as mean ± SD. a= P < 0.05 vs. normal control; b= P < 0.05 vs. I/R control. Sim Treated I/R-I= 3 µMol; Sim Treated I/R-II= 10 µMol; Sim Treated I/R-III= 30 µMol.

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