



# Simvastatin Significantly Reduced Alcohol-Induced Cardiac Damage in Adolescent Mice

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## Abstract

Alcohol abuse by adolescents is becoming a serious health concern as they often progress to becoming alcoholics later in life which may lead to heart problems. Chronic alcohol use alters the cardiac function and structure, such as haemodynamic changes, weakening and loss of cardiomyocytes, myocardial fibrosis, and inflammation. Simvastatin is a commonly used drug for the treatment and management of various cardiovascular problems but information on its protective effects against alcohol-induced cardiomyocyte hypertrophy, fibrosis, and inflammation is lacking in the literature. Four-week-old male ( $n = 5$ ) and female ( $n = 5$ ) C57BL/6 J mice were assigned to each experimental group: (I) NT—no administration of alcohol or Simvastatin; (II) ALC—2.5 g/Kg/day of 20% alcohol via intraperitoneal injection (i.p.); (III) SIM—5 mg/Kg/day of Simvastatin via oral gavage; (iv) ALC + SIM5—5 mg/Kg/day of Simvastatin via oral gavage followed by 2.5 g/Kg/day of 20% alcohol via i.p.; and (v) ALC + SIM15—15 mg/Kg/day Simvastatin via oral gavage followed by 2.5 g/Kg/day of 20% alcohol via i.p. After the 28-day treatment period, the heart was removed and processed for H&E, Masson's trichrome, or TNF- $\alpha$  immunolabelling. The area and diameter of cardiomyocytes were measured on the H&E-stained sections. The distribution of collagen or TNF- $\alpha$  expression was quantified using the deconvolution tool of ImageJ software. The results confirmed alcohol-induced toxicity on the cardiomyocytes and Simvastatin reduced alcohol-induced cardiomyocyte hypertrophy, fibrosis, and inflammation in both sexes. This study demonstrated that Simvastatin, an FDA approved and easily accessible drug, may be beneficial in lowering the prevalence of alcohol-induced cardiovascular diseases (especially in adolescents) which will have a huge financial implication on health systems worldwide.

**Keywords** Alcohol · Adolescents · Simvastatin · Cardiomyocyte morphometry · Fibrosis · Inflammation · Cardiovascular disease

## Introduction

Adolescence is a period of growth and development characterized by physical, psychosocial, and psychological changes [1, 2]. At this age, adolescents tend to experiment with alcohol resulting in heavy episodic drinking, especially where there are lapses in policies to regulate access to alcohol [1, 2]. Alcohol misuse is higher in male than female adolescents and individuals that begin drinking during adolescence are

more likely to become alcoholics later in life [3]. Apart from the social economic impact of alcohol abuse, alcohol misuse causes several metabolic diseases which include cardiac, renal, and hepatic diseases [2–6].

Chronic alcohol use increases the incidence of cardiovascular diseases, which is a huge financial burden on health systems worldwide [4, 7]. Alcohol abuse changes the cardiac function and structure which may result in the weakening and loss of cardiomyocytes [8]. In addition, the mitochondria, plasma membrane, ribosomes, and receptors of cardiomyocytes are also damaged due to the high reactivity, high diffusion rate, and the ability of alcohol to easily cross membranes [9]. Alcohol furthermore disrupts signalling processes, activates apoptosis, and decreases heart contraction [7, 9]. Likewise, the repair mechanism of cardiomyocytes is hindered by alcohol (i.e. by reducing regeneration and proliferation of cardiomyocytes) whilst promoting cardiac

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lesions (i.e. by increasing apoptosis and necrosis of cardiomyocytes), the outcome of which leads to myocardial fibrosis [9] and ventricular dysfunction [10]. Alcohol is also known to induce inflammation and promote the secretion of cytokines such as tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) [7], a pro-inflammatory cytokine that regulates myocardial homeostasis by signalling inflammation and cell abnormality (e.g. apoptosis) [7]. It must be noted that an increased level of TNF- $\alpha$  in chronic alcoholism is an indication of cardiac pathologies, fibrosis, and necrosis [7, 11, 12].

With the prevalence of chronic alcoholism (especially in adolescents) and its associated cardiovascular diseases, it is important to investigate interventions that may mask or reduce the damaging effects of alcohol on the heart tissues. One such intervention that is gaining popularity, due to its anti-inflammatory and immune-regulatory properties, is Simvastatin [13–15], an FDA-approved drug, primarily used for lowering blood cholesterol levels (hypercholesterolemia) [16–19]. Simvastatin has also been shown to prevent coronary heart disease [19, 20], cardiomyocyte death [21], myocardial inflammation, and fibrosis [14, 22–24]. Furthermore, Simvastatin also prevents myocarditis [25], improves endothelial functions [14, 15], and inhibits the TGF- $\beta$ 1 pathway which triggers the development of interstitial fibrosis and cardiomyocyte hypertrophy [14].

Simvastatin may therefore be beneficial against alcohol-induced myocardial damage by preventing or potentially reversing the structural alterations caused by chronic alcohol use. Thus, this study investigated the protective capabilities of Simvastatin against alcohol-induced damage on the heart of adolescent mice that were administered alcohol. Preventing and/or reversing alcohol-induced damage could alleviate the financial burden caused by alcohol-related diseases [4].

## Materials and Methods

### Experimental Animal Study

This study received ethical approval (Ethics Clearance No: 2019/11/63/C) from the Animal Research Ethics Committee (AREC) of the University of the Witwatersrand Johannesburg, South Africa. Mice of the same sex and belonging to the same experimental group were housed together in a group of five mice per cage (cage dimensions: 200 × 200 × 300 mm) and kept under a reversed 12-h-day/12-h dark cycle (with the light switched off from 06:00 to 18:00). For this study, the period of adolescence in mice was taken as the period between 4 and 8 weeks old [26].

Four-week-old male ( $n = 25$ ; 5 per group) and female ( $n = 25$ ; 5 per group) C57BL/6 J mice housed in the Witwatersrand Research Animal Facility (WRAF), University of the Witwatersrand were assigned to each experimental group: (I) Non-treatment group (NT); (II) Alcohol-only group (ALC)—2.5 g/Kg/day of 20% alcohol via intraperitoneal injection (i.p.); (III) Simvastatin-only group (SIM)—5 mg/Kg/day Simvastatin via oral gavage; (IV) Alcohol + Simvastatin 5 (ALC + SIM5)—5 mg/Kg/day of Simvastatin via oral gavage followed by 2.5 g/Kg/day of 20% alcohol via i.p.; and (V) Alcohol + Simvastatin 15 (ALC + SIM15)—15-mg/Kg/day Simvastatin via oral gavage followed by 2.5 g/Kg/day of 20% alcohol via i.p. Simvastatin (Cat no: 1612700, Merck, South Africa) was prepared according to McKay et al. [27] to ensure complete dissolution. In addition, a pharmacological-grade absolute alcohol (99.9%) (Sigma-Aldrich, South Africa; Cat No: 24105) was serially diluted in saline (0.9% NaCl) into a 20% alcohol solution. Both Simvastatin and alcohol were prepared daily and then filter sterilized before administering. Any unused solution per day was discarded. All treatments were performed for 28 consecutive days. Oral gavage and i.p. were performed with the utmost care by the trained staff of WRAF to reduce introducing stress into the animal.

On the last day of treatment, 50- $\mu$ L saphenous blood was collected in heparinized capillary tubes within 30 min after the administration of alcohol before being centrifuged with Vivaspin500© 100- $\mu$ m membrane tubes (Biotech, South Africa) at 5000 rpm for 15 min to separate the serum. The serum was extracted, and the Blood Alcohol Concentration (BAC) was determined using an EnzyChrom™ Ethanol Assay Kit (BioVision, South Africa). The average BAC level was in the range of 182.5–253.4 mg/dL for the groups that were administered alcohol.

Following blood collection, the mice were euthanized using Euthanaze (sodium pentobarbital, 80 mg/Kg, i.p.). Thereafter, the mice were transcardially perfused with 4% paraformaldehyde (in 0.1-M phosphate buffer, PB) (PFA), the heart was removed, and then further fixed in 4% PFA at 4 °C before further processing.

### Tissue Processing and Morphometries

The PFA-fixed heart was cut midway across the ventricles. The lower portion of the ventricles was processed for histology and sectioned horizontally across the ventricles at 2  $\mu$ m thickness. Serial sections were prepared for haematoxylin and eosin (H&E) staining (for general morphology and morphometry of cardiomyocytes), Masson's trichrome (MT) staining (for evaluating myocardial fibrosis), or TNF- $\alpha$  immunolabelling (for quantifying the expression of TNF- $\alpha$ ;

38. Fernandez Sola, J., & Estruch Riba, R. (2017). Alcoholic cardiomyopathy: Old and new insights. *Journal of Alcoholism & Drug Dependence*, 5(06), 1–6. <https://doi.org/10.4172/2329-6488.1000293>
39. Kawano, Y. (2010). Physio-pathological effects of alcohol on the cardiovascular system: Its role in hypertension and cardiovascular disease. *Hypertension Research*, 33(3), 181–191. <https://doi.org/10.1038/hr.2009.226>
40. Alleyne, J., & Dopic, A. M. (2021). Alcohol use disorders and their harmful effects on the contractility of skeletal, cardiac and smooth muscles. *Advances in Drug and Alcohol Research*, 1, 1–18. <https://doi.org/10.3389/adar.2021.10011>
41. Calabrese, F., Carturan, E., Chimenti, C., Pieroni, M., Agostini, C., Angelini, A., Crosato, M., Valente, M., Boffa, G. M., Frustaci, A., & Thiene, G. (2004). Overexpression of tumor necrosis factor (TNF)- $\alpha$  and TNF- $\alpha$  receptor I in human viral myocarditis: Clinicopathologic correlations. *Modern Pathology*, 17(9), 1108–1118. <https://doi.org/10.1038/modpathol.3800158>
42. Irwin, M. W., Mak, S., Mann, D. L., Qu, R., Penninger, J. M., Yan, A., Dawood, F., Wen, W.-H., Shou, Z., & Liu, P. (1999). Tissue expression and immunolocalization of tumor necrosis factor- $\alpha$  in postinfarction dysfunctional myocardium. *Circulation*, 99(11), 1492–1498.
43. Lee, J. H., Lee, T.-K., Kim, I. H., Lee, J. C., Won, M.-H., Park, J. H., Ahn, J. H., Shin, M. C., Ohk, T. G., Moon, J. B., Cho, J. H., Park, C. W., & Tae, H.-J. (2017). Changes in histopathology and tumor necrosis factor- $\alpha$  levels in the hearts of rats following asphyxial cardiac arrest. *Clinical and Experimental Emergency Medicine*, 4(3), 160–167. <https://doi.org/10.15441/ceem.17.220>
44. Li, X., Nie, Y., Lian, H., & Hu, S. (2018). Histopathologic features of alcoholic cardiomyopathy compared with idiopathic dilated cardiomyopathy. *Medicine*, 97(39), 1–5. <https://doi.org/10.1097/MD.00000000000012259>
45. Qin, Y. W., Ye, P., He, J. Q., Sheng, L., Wang, L. Y., & Du, J. (2010). Simvastatin inhibited cardiac hypertrophy and fibrosis in apolipoprotein E-deficient mice fed a western-style diet by increasing PPAR  $\alpha$  and  $\gamma$  expression and reducing TC, MMP-9, and Cat S levels. *Acta Pharmacologica Sinica*, 31(10), 1350–1358. <https://doi.org/10.1038/aps.2010.109>
46. Su, F., Shi, M., Zhang, J., Zheng, Q., Zhang, D., Zhang, W., Wang, H., & Li, X. (2018). Simvastatin protects heart from pressure overload injury by inhibiting excessive autophagy. *International Journal of Medical Sciences*, 15(13), 1508–1516. <https://doi.org/10.7150/ijms.28106>
47. Chen, Y. Q., Zhao, L. Y., Zhang, W. Z., & Li, T. (2015). Simvastatin reverses cardiomyocyte hypertrophy via the upregulation of phosphatase and tensin homolog expression. *Experimental and Therapeutic Medicine*, 10(2), 797–803. <https://doi.org/10.3892/ETM.2015.2550>
48. Al-Rasheed, N. M., Al-Oteibi, M. M., Al-Manee, R. Z., Al-Shareef, S. A., Al-Rasheed, N. M., Hasan, I. H., Mohamad, R. A., & Mahmoud, A. M. (2015). Simvastatin prevents isoproterenol-induced cardiac hypertrophy through modulation of the JAK/STAT pathway. *Drug Design, Development and Therapy*, 9, 3217–3229. <https://doi.org/10.2147/DDDT.S86431>
49. Ahmed, L. A., Darwish, H. A., Abdelsalam, R. M., & Amin, H. A. A. (2016). Role of rho kinase inhibition in the protective effect of fasudil and simvastatin against 3-nitropropionic acid-induced striatal neurodegeneration and mitochondrial dysfunction in rats. *Molecular Neurobiology*, 53(6), 3927–3938. <https://doi.org/10.1007/s12035-015-9303-2>
50. Refaie, M. M. M., El-Hussieny, M., Bayoumi, A. M. A., Shehata, S., Welson, N. N., & Abdelzaher, W. Y. (2022). Simvastatin cardioprotection in cyclophosphamide-induced toxicity via the modulation of inflammasome/caspase1/interleukin1 $\beta$  pathway. *Human and Experimental Toxicology*, 41, 1–11. <https://doi.org/10.1177/09603271221111440>
51. Nežić, L., Škrbić, R., Amidžić, L., Gajanin, R., Kuća, K., & Jačević, V. (2018). Simvastatin protects cardiomyocytes against endotoxin-induced apoptosis and up-regulates survivin/NF- $\kappa$ B/p65 expression. *Scientific Reports*, 8(1), 1–18. <https://doi.org/10.1038/S41598-018-32376-4>
52. Rohilla, A., Ahmad, A., Khan, M. U., & Khanam, R. (2015). A comparative study on the cardioprotective potential of atorvastatin and simvastatin in hyperhomocysteinemic rat hearts. *European Journal of Pharmacology*, 764, 48–54. <https://doi.org/10.1016/J.EJPHAR.2015.06.045>
53. Goldstein, J. L., & Brown, M. S. (1990). Regulation of the mevalonate pathway. *Nature*, 343(6257), 425–430. <https://doi.org/10.1038/343425a0>
54. Segatto, M., Manduca, A., Lecis, C., Rosso, P., Jozwiak, A., Swiezewska, E., Moreno, S., Trezza, V., & Pallottini, V. (2014). Simvastatin treatment highlights a new role for the isoprenoid/cholesterol biosynthetic pathway in the modulation of emotional reactivity and cognitive performance in rats. *Neuropsychopharmacology*, 39(4), 841–854. <https://doi.org/10.1038/npp.2013.284>
55. Fracassi, A., Marangoni, M., Rosso, P., Pallottini, V., Fioramonti, M., Siteni, S., & Segatto, M. (2019). Statins and the brain: More than lipid lowering agents? *Current Neuropharmacology*, 17(1), 59–83. <https://doi.org/10.2174/1570159X15666170703101816>
56. Luo, J. D., Zhang, W. W., Zhang, G. P., Guan, J. X., & Chen, X. (1999). Simvastatin inhibits cardiac hypertrophy and angiotensin-converting enzyme activity in rats with aortic stenosis. *Clinical and Experimental Pharmacology and Physiology*, 26(11), 903–908. <https://doi.org/10.1046/j.1440-1681.1999.03165.x>
57. Scalia, R., Gooszen, M. E., Jones, S. P., Hoffmeyer, M., Rimmer, D. M., III, Trocha, S. D., Huang, P. L., Smith, M. B., Lefer, A. M., & Lefer, D. J. (2001). Simvastatin exerts both anti-inflammatory and cardioprotective effects in apolipoprotein E-deficient mice. *Circulation*, 103(21), 2598–2603. <https://doi.org/10.1161/01.CIR.103.21.2598>
58. Takemoto, M., Node, K., Nakagami, H., Liao, Y., Grimm, M., Takemoto, Y., Kitakaze, M., & Liao, J. K. (2001). Statins as antioxidant therapy for preventing cardiac myocyte hypertrophy. *The Journal of Clinical Investigation*, 108(10), 1429–1437. <https://doi.org/10.1172/JCI13350>
59. Nakagami, H., Jensen, K., & Liao, J. (2003). A novel pleiotropic effect of statins: Prevention of cardiac hypertrophy by cholesterol-independent mechanisms. *Annals of Medicine*, 35(6), 398–403. <https://doi.org/10.1080/07853890310001294>
60. Liu, J., Shen, Q., & Wu, Y. (2008). Simvastatin prevents cardiac hypertrophy in vitro and in vivo via JAK/STAT pathway. *Life Sciences*, 82(19–20), 991–996. <https://doi.org/10.1016/j.lfs.2008.02.012>
61. Zhai, Y., Gao, X., Wu, Q., Peng, L., Lin, J., & Zuo, Z. (2008). Fluvastatin decreases cardiac fibrosis possibly through regulation of TGF- $\beta$ 1/Smad 7 expression in the spontaneously hypertensive rats. *European Journal of Pharmacology*, 587(1–3), 196–203. <https://doi.org/10.1016/j.ejphar.2008.03.006>
62. Blum, A., & Shamburek, R. (2009). The pleiotropic effects of statins on endothelial function, vascular inflammation, immunomodulation and thrombogenesis. *Atherosclerosis*, 203(2), 325–330. <https://doi.org/10.1016/j.atherosclerosis.2008.08.022>
63. Yamamoto, C., Fukuda, N., Jumabay, M., Saito, K., Matsumoto, T., Ueno, T., Soma, M., Matsumoto, K., & Shimosawa, T. (2011). Protective effects of statin on cardiac fibrosis and apoptosis in adrenomedullin-knockout mice treated with angiotensin II and high salt loading. *Hypertension Research*, 34(3), 348–353. <https://doi.org/10.1038/hr.2010.243>

64. Antonopoulos, S., Margaritis, M. A., Lee, R., Channon, K., & Antoniades, C. (2012). Statins as anti-inflammatory agents in atherogenesis: Molecular mechanisms and lessons from the recent clinical trials. *Current Pharmaceutical Design*, 18(11), 1519–1530. <https://doi.org/10.2174/138161212799504803>
65. Akahori, H., Tsujino, T., Naito, Y., Matsumoto, M., Sasaki, N., Iwasaku, T., Eguchi, A., Sawada, H., Hirotsu, S., & Masuyama, T. (2014). Atorvastatin ameliorates cardiac fibrosis and improves left ventricular diastolic function in hypertensive diastolic heart failure model rats. *Journal of Hypertension*, 32(7), 1534–1541. <https://doi.org/10.1097/HJH.0000000000000184>
66. Kudo, S., Satoh, K., Nogi, M., Suzuki, K., Sunamura, S., Omura, J., Kikuchi, N., Kurosawa, R., Satoh, T., Minami, T., & Ikeda, S. (2016). SmgGDS as a crucial mediator of the inhibitory effects of statins on cardiac hypertrophy and fibrosis: Novel mechanism of the pleiotropic effects of statins. *Hypertension*, 67(5), 878–889. <https://doi.org/10.1161/HYPERTENSIONAHA.115.07089>
67. Oesterle, A., Laufs, U., & Liao, J. K. (2017). Pleiotropic effects of statins on the cardiovascular system. *Circulation Research*, 120(1), 229–243. <https://doi.org/10.1161/CIRCRESAHA.116.308537>
68. Kuo, H. F., Hsieh, C. C., Wang, S. C., Chang, C. Y., Hung, C. H., Kuo, P. L., Liu, Y. R., Li, C. Y., & Liu, P. L. (2019). Simvastatin attenuates cardiac fibrosis via regulation of cardiomyocyte-derived exosome secretion. *Journal of Clinical Medicine*, 8(6), 794. <https://doi.org/10.3390/jcm8060794>
69. Koushki, K., Shahbaz, S. K., Mashayekhi, K., Sadeghi, M., Zayeri, Z. D., Taba, M. Y., Banach, M., Al-Rasadi, K., Johnston, T. P., & Sahebkar, A. (2021). Anti-inflammatory action of statins in cardiovascular disease: The role of inflammasome and toll-like receptor pathways. *Clinical Reviews in Allergy and Immunology*, 60(2), 175–199. <https://doi.org/10.1007/s12016-020-08791-9>

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