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Simvastatin Improves The Renal Function and Glomerulosclerosis (Cahyawati PN, *et al.*)
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RESEARCH ARTICLE

Simvastatin Improves Renal Function and Glomerulosclerosis in Ischemic-reperfusion Injury

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Abstract

BACKGROUND: Statin is an anti-cholesterol drug that is widely prescribed throughout the world. Statins are mainly used to treat and prevent cardiovascular disease. Several studies have found the pleiotropic effect of statin. However, related effect of statin in kidney failure is still unclear. Ischemic-reperfusion (I/R) injury is a major cause of acute kidney failure. This study aims to determine the effect of simvastatin on kidney function and glomerular conditions by periodic acid-schiff staining in I/R injury.

METHODS: Eighteen male Swiss mice were grouped into sham operation group (GSO), I/R injury group (GIRI), and simvastatin group (GSIM). The GSO group was performed by sham operation and pretreatment of 1% carboxymethylcellulose (CMC) for 3 days. The GIRI group was performed by I/R procedure and pretreatment of 1% CMC for 3 days and the GSIM group was performed by I/R procedure and pretreatment of 10 mg/kg BW simvastatin for 3 days. Blood urea nitrogen (BUN) and creatinine serum were assessed to determine kidney function. Histopathological analysis of glomerulosclerosis was assessed by the extent of glomerular damage (sclerosis), capillary loops, and synechia. The data were analyzed by one-way ANOVA followed by post hoc Tukey's test ($p < 0.05$).

RESULTS: The creatinine and BUN levels in the GIRI group were the highest (0.97±0.48) compared with the other groups. The glomerulosclerosis index in the GSO group was 0.75±0.56, the GIRI group was 3.55±0.61, and the GSIM group was 2.08±1.37. There was a significant difference in the glomerulosclerosis index between the GSO and GIRI groups, but there was no significant difference between the GIRI and GSIM groups. These differences include the formation of sclerosis in the glomerulus, capillary loop, and synechiae.

CONCLUSION: Simvastatin improves kidney function and glomerulosclerosis in I/R injury.

KEYWORDS: ischemic-reperfusion injury, simvastatin, glomerulosclerosis

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Introduction

Statins, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors is an anti-cholesterol drug that is widely prescribed throughout the world. Statins used to treat and prevent cardiovascular diseases, such as coronary heart diseases. This drug can decrease cholesterol synthesis and increase the number of low-density lipoproteins (LDL) receptors, thereby speeding up the cleansing of cholesterol from circulation.(1,2) Statins work by inhibiting the enzyme HMG-CoA reductase, so blocking the mevalonate pathway includes inhibition of cholesterol biosynthesis and isoprenoid metabolites such as farnesyl-diphosphate (FPP) and geranylgeranyl pyrophosphate (GGPP).(1,3,4)

Many studies have found good effects of statin in various conditions. Not only limited to cardiovascular disease but also in infectious diseases, neurological disorders, and tumors. Statins are also have been reported to have antioxidant and anti-inflammatory effects. This condition makes the statin therapy does not only limited to cardiovascular diseases, but also to the condition or disease that have a direct impact on dyslipidemia, as it does in the condition of kidney failure.(1,2)

Kidney failure is a condition where there is a failure on kidney function. This condition can occur both in acute conditions (acute kidney injury/AKI) or chronic (chronic kidney disease/CKD). The research about the effects of statin on kidney failure is quite a lot with varying results. Previous research found that simvastatin had a remedial effect on creatinine, histopathological conditions and expression of several proteins such as α-smooth muscle actin (α-SMA), platelet-derived growth factor receptor beta (PDGF-Rβ), monocyte chemoattractant protein-1 (MCP-1), intracellular adhesion molecule-1 (ICAM-1), nephrin, and podocin on mice with the 5/6 subtotal nephrectomy procedure.(5) Similar results were also found in research using the sepsis-induced AKI model.(6) In patients who will undergo surgery (abdomen, cardiac, thoracic and vascular), statins therapy can actually reduce the risk of postoperative AKI.(7)

However, other studies have found that statins are not proven to affects kidney function, although it can prevent heart attack and stroke.(8) The use of high-dose statins (≥ 40 mg simvastatin, ≥ 20 mg atorvastatin, and ≥ 10 mg rosuvastatin) are also associated with increased risk of AKI. The administration 150 mg/kg/d for 7 days atorvastatin also reported has a nephrotoxic effect in the I/R injury.(9,10)

I/R injury is the main cause of AKI. This injury can make a temporary decrease in blood flow in the kidneys and changes in renal cellular and vascular reactivity, so it may cause inflammation, cell death, and AKI.(11) Based on a meta-analysis the incidence of AKI is 21.6% in adults and 33.7% in children, with a mortality rate of 23.9% (adults) and 13.8% (children).(12) Other studies mention the degree of mortality is between 25-80%, depending on the cause and clinical condition of the patient.(13) This condition is also associated with high health costs due to prolonged periods of hospitalization, increased examination costs and long-term complications that can occur. The result can be even worse in developing countries compared with developed countries.(14)

Based on the data above, it is known that there is still controversy on the effects of statin towards kidney conditions. For this reason, research needs to be done to determine the effect of statins on kidney conditions in animal models with AKI conditions.

Methods

Animals
Eighteen male Swiss mice (3-4 months, weight 30-40 g) were used in this study. Animals were kept in a standard room for animal studies (temperature $23\pm2°C$ and 12 h dark-light cycle). All animals got standard feed and ad libitum access to tap water. All the animal experiments were performed under protocol from The Ethics Committe for Research Faculty of Medicine Universitas Udayana/Sanglah Central General Hospital No: 2458/UN14.2.2.VII.14/LP/2019.

I/R Injury Model of AKI and Experimental Designs
Eighteen male Swiss mice were randomly and equally divided into 3 treatment groups: sham operation (GSO, n= 6) as negative control, I/R injury (GIRI, n = 6) as positive control, and simvastatin (GSIM, n = 6). For GSIM group, mice was given pretreatment of 10 mg/kg BW simvastatin (Sigma-Aldrich, Science Park Road, Singapore) for 3 days before I/R procedure based on the previous protocol.(12) Simvastatin was dissolved in 1% carboxymethylcellulose (CMC) solution (Sigma-Aldrich, St Louis, MA, USA), then was given to the mice 1x/day by oral gavage. The dose of simvastatin in this study based on the median dose of the previous study.(5) The GSO group received 1% CMC solution and sham operation. The GIRI group received 1% CMC solution and I/R procedure. I/R procedure was performed as follows, incisions were made at both the lumbar (flank) regions. After renal pedicles were exposed, both renal pedicles were clamped using Dieffenbach bulldog clamps (RZ Medizintechnik, Tuttlingen, Germany) for 30 min to induce ischemia. Then the clamps were removed to induce reperfusion. The incision sites were sutured and the mice were left conscious. The I/R procedure was performed after anesthetized with a combination of 90 mg/kg BW ketamine and 10 mg/kb BW xylazine through intraperitoneal injection.(15)

Biochemistry Analysis and Body Weight Determinations
Blood samples were collected from the retroorbital vein for the determination of blood urea nitrogen (BUN) and creatinine serum (DiaSys, Holzheim, Germany). About 0.5-1 mL blood was centrifuged at a speed of 10,000 rpm for 10 min. Serum was obtained and stored at -20°C for further

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analyses. For BUN examination, blood was reacted with Tris, 2-oxoglutarate, adenosine diphosphate (ADP), urease, glutamate dehydrogenase (GLDH), nicotinamide adenine dinucleotide hydride (NADH) for 1 min. The reaction was measured using a spectrophotometer at 340 nm wavelength. Then urea concentration and BUN value were calculated. For creatinine examination, blood was reacted with sodium hydroxide and picric acid 1 min. The reaction was measured using a spectrophotometer at 492 nm wavelength. Then creatinine concentration was calculated. Changes in the body weight of each group were determined during the acclimatization period until the end of the study.

Histological Preparation and Assessments of Glomerulo-sclerosis in Periodic Acid-Schiff (PAS) Staining

One day after I/R procedure mice were euthanized by 300 mg/kg BW intraperitoneal injection of ketamine. Then the kidney was excised, cleaned carefully and fixed in 10% buffered paraformaldehyde and embedded in paraffin for histological processing sections. For histopathology analysis, block paraffin was cut (4 μm), then stained with PAS. Images were taken at 400× magnifications under a light microscope (Olympus, Tokyo, Japan).

Histopathological analysis of glomerulosclerosis scores was assessed by the extent of glomerular damage (sclerosis), capillary loops, and synechia. Ten fields of view glomerulus will be examined and glomerulosclerosis scores classified from 0 to 4 (0 = normal glomeruli, 1 = area of sclerosis <25%, 2 = area of sclerosis 25-50%, 3 = area sclerosis >50-75%, 4 = area of sclerosis >75%).(5) The morphological measurement was performed blindly by 2 observers. To avoid bias between observers, we performed a discussion to equalize the perception before starting the assessment.

Statistical Analyses

Data were analyzed using Statistical Package for the Social Sciences (SPSS) software version 20.0 (IBM Corporation, New York, USA). Data were expressed as mean±SEM and analyzed by one-way ANOVA followed by post hoc Tukey's test. Statistical significance was set at $p<0.05$.

Results

Characteristics of Animals

All animals were survived until the end of the study. During the acclimatization period, the body weight of the mice on treatment groups appeared stable (A1-A7). There was a slight increase in body weight at the beginning of the treatment (D2-D4), but there was a decrease in body weight at the end of the treatment (D5), as shown in Figure 1. Based on the statistical analysis there was no significant difference in the mean bodyweight of the experimental group at the beginning of the treatment (D1) and the end of the treatment (D5). The mean body weight D1 in the GSO group = 32.67±1.36, GIRI = 32.3±1.51, GSIM = 33.33±1.21; $p=0.55$, while the mean body weight of D5 in the GSO group = 32±1.26, GIRI = 1.17±1.6, GSIM = 31.83±1.16; $p=0.54$.

Biochemical Analysis

Based on the results we found that creatinine levels in the GIRI group were the highest (0.97±0.48) compared with
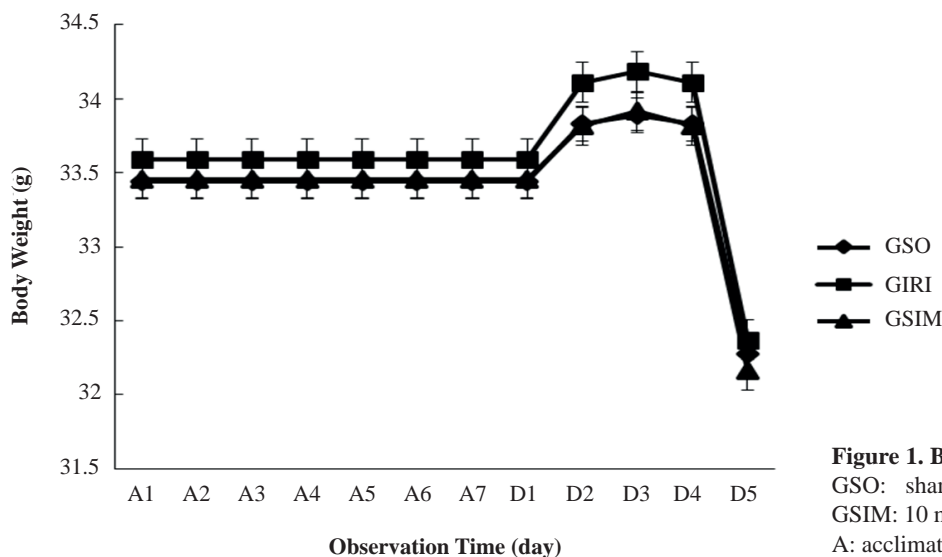

Figure 1. Body weight on treatment groups. GSO: sham operation; GIRI: I/R injury; GSIM: 10 mg/kg BW simvastatin + I/R injury; A: acclimatization day, D: treatment day.
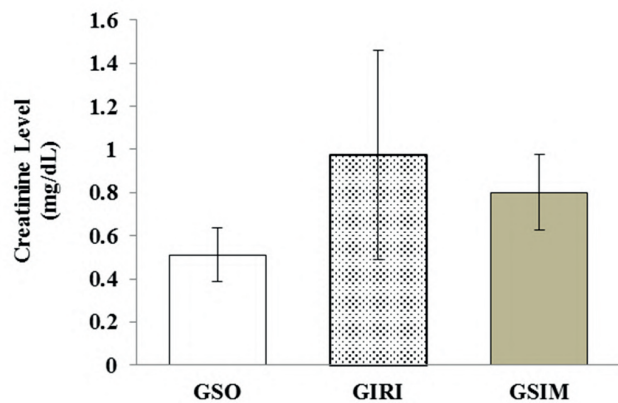
Figure 2. Creatinine serum levels in the treatment groups. GSO: sham operation; GIRI: I/R injury; GSIM: 10 mg/kg BW simvastatin + I/R injury. Tested with one way ANOVA, p=0.058.

the GSO group (0.51±0.12) and GSIM group (0.8±0.17) (Figure 2). The BUN levels in the GIRI group (36±22.17) were also the highest compared with GSO (23.55±5.53) and GSIM (18.59±5.0) (Figure 3). However, statistical analysis showed that there were no significant differences between treatment groups p>0.05.

Glomerulosclerosis Index Analysis

The glomerular histopathology of the GSO group showed normal glomerulus without synechia, capillary loops still open and no sclerosis. This was different from the GIRI group. The glomerulus was very sclerotic, there was an attachment to the Bowman's capsule and all capillaries loop were closed. The absorption of red color was also very strong compared to the GSO group. In the GSIM group, the contour of glomerular was still clearly observed and there was no attachment to the Bowman's capsule. Some capillary loops were still open, as shown in Figure 4.

The mean of glomerulosclerosis index in each group was: GSO (0.75±0.56), GIRI (3.55±0.61), and GSIM (2.08±1.37) (Figure 5). There was significant difference between the GSO and GIRI groups (p=0.007), but there was no significant difference between the GIRI and GSIM groups. Glomerulosclerosis index in the GSIM group showed a decrease, but not statistically significant. These results indicated that damage occurred in the glomerulus was very minimal in the group without AKI (GSO group), compared with the group with AKI (GIRI group), shown by >4 times increase of the glomerulosclerosis index in the GIRI group. In the GSIM group, glomerular damage was also occurred due to the I/R procedure but to a lesser degree than the GIRI group.

Discussion

In this study, BUN and creatinine serum levels were increased one day after the I/R procedure. These results are consistent with the previous studies showing the BUN and serum creatinine levels are increased at 2, 4, and 6 h post-ischemia and peak elevations at 24 h post-ischemia.[16]

Increased BUN and creatinine indicated damage to the filtration process on the kidneys. Filtration is an important process associated with increased blood levels of BUN and creatinine. The structure involved in the process is glomerulus. The glomerulus is a structure with multiple interactions, namely endothelial cells, mesangial cells and epithelial cells which together form a filtration webbing (capillary loops).[17] Fibrosis that occurs in the glomerulus is known as glomerulosclerosis. Glomerulosclerosis is caused by the accumulation of collagen in the extracellular matrix. Glomerular injury can be the basis for this condition. Glomerular injury causes endothelial dysfunction and hemodynamic changes. The initial recruitment of neutrophils and immune response can cause by the activation of the renin-angiotensin-aldosterone system (RAAS). The presence of transforming growth factor-β (TGF-β), activation of connective tissue growth factor (CTGF), plasminogen activator inhibitor-1 (PAI-1) and nuclear factor κB (NFκB) are factors that also exacerbate the immune response.[18] In this study, glomerulosclerosis has occurred with varying degrees. Pretreatment of simvastatin can reduce the degree of sclerosis on the GSIM group because of its renoprotection action. The renoprotective effect of statins has been confirmed by other studies. This effect may be obtained through their anti-inflammatory and antioxidant
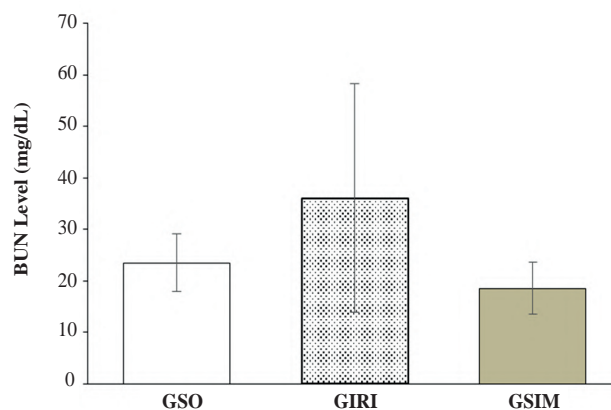

Figure 3. BUN levels in the treatment groups. GSO: sham operation; GIRI: I/R injury; GSIM: 10 mg/kg BW simvastatin + I/R injury. Tested with one way ANOVA, p=0.058

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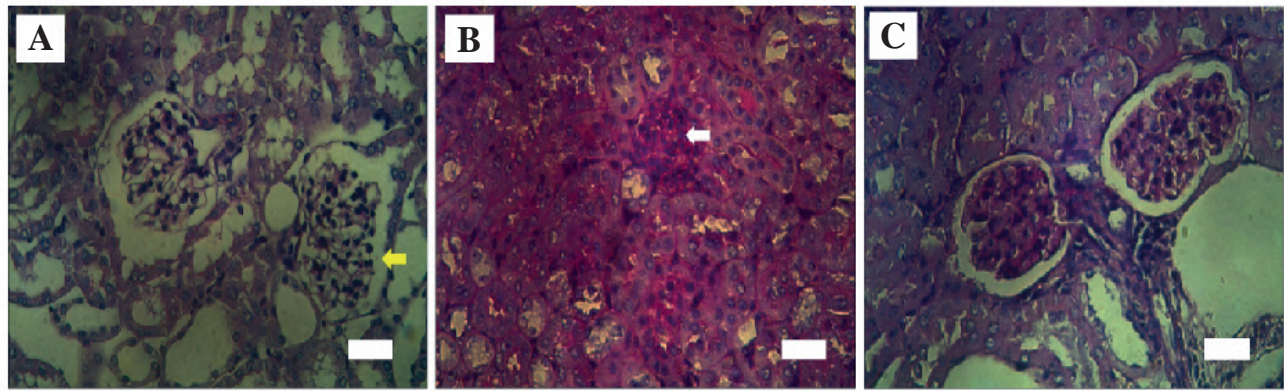Indones Biomed J. 2020; 12(2): 143-8

Figure 4. Kideny glomerulus on PAS staining. Yellow arrow: normal glomeruli; White arrow: sclerotic glomeruli. White bar: 50 μm.

actions. On the kidney, statin can increase the filtration rate of the glomerulus, tubular function and reducing markers of oxidative injury. Through these actions, the condition of the kidney will gradually improve.(19) Other studies have found that the protective effect of statin caused by inhibition in the mevalonate pathway, independent of its lipid-lowering action.(20)

An increasing dose of simvastatin was reported to have a renoprotective effect. At a dose of 5.2 mg/kg BW, 10.4 mg/kg BW and 20.8 mg/kg BW, simvastatin was reported to improve glomerulosclerosis, tubular injury and interstitial fibrosis in the 5/6 subtotal nephrectomy model significantly.5 Simvastatin has also been reported to improve the severity of proteinuria, BUN and histological changes at doses of 1 mg/kg, 10 mg/kg and 25 mg/kg in a dose-dependent manner.(21) On a clinical trial, atorvastatin also demonstrated improve kidney function over time in a dose-dependent manner.(22)

Weakness in this study is the limited number of the treatment group. The researchers only used one dose of the drug, so the effect that occurs in increasing doses of simvastatin not yet known. This research is also still limited to histopathological studies and not yet reached studies at the level of molecular biology. This causes the data is still limited. Therefore, further research is needed to determine the effective dose of simvastatin in this model, as well as research related to the effects of drugs from the molecular aspect.

Conclusion

The result of this study shows that simvastatin improves kidney function and glomerulosclerosis in I/R injury.

Acknowledgements

References

Figure 5. Glomerulosclerosis index in the treatment groups.
GSO: sham operation; GIRI: I/R injury; GSIM: 10 mg/kg BW simvastatin + I/R injury. Tested with one way ANOVA, $p=0.01$ GSO *vs.* GIRI)

1. Gazzerro P, Proto MC, Gangemi G, Malfitano AM, Ciaglia E, Pisanti S, *et al.* Pharmacological actions of statins: a critical appraisal in the management of cancer. Pharmacol Rev. 2012; 64: 102-46.
2. Tristano AG, Fuller K. Immunomodulatory effects of statins and autoimmune rheumatic diseases: novel intracellular mechanism involved. Int J Immunopharmaco. 2006; 6: 1833-46.
3. Pleşea CF, Tânţu M, Grigorescu A, Cîrlig V. Statins–between the cholesterol lowering and pleiotropic effect. Current Health Sciences Journal. 2013; 39: 210-3.
4. Yano M, Matsumura T, Senokuchi T, Ishii N, Murata Y, Taketa K. *et al.* Statins activate peroxisome proliferator-activated receptor through extracellular signal-regulated kinase 1/2 and p38 mitogen-activated protein kinase–dependent cyclooxygenase-2 expression in macrophages. Circ Res. 2007; 100: 1442-51.
5. Cahyawati PN, Ngatidjan, Sari DCR, Romi MM, Arfian N. Simvastatin attenuates renal failure in mice with a 5/6 subtotal nephrectomy. Int J Pharm Pharm Sci. 2017; 9: 12-7.
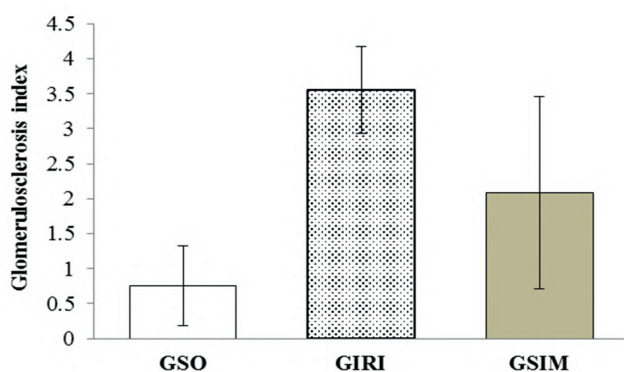
6. Santos FN, Watanabe M, Vasco CF, Fonseca CD, Vattimo MFF. Antioxidant protection of statins in acute kidney injury induced by sepsis. Rev Esc Enferm USP. 2014; 48: 820-6.

7. Brunelli SM, Waikar SS, Bateman BT, Chang TI, Lii J, Garg AX, *et al.* Preoperative statin use and postoperative acute kidney injury. Am J Med. 2012; 125: 1195-204.

8. Haynes R, Lewis D, Emberson J, Reith C, Agodoa L, Cass A, *et al.* Effects of lowering LDL cholesterol on progression of kidney disease. J Am Soc Nephrol 25. 2014; 25: 1825-33.

9. Dormuth CR, Hemmelgarn BR, Paterson JM, James MT, Teare GF, Raymond CB, Lafrance JP, *et al.* Use of high potency statins and rates of admission for cute kidney injury: multicenter, retrospective observational analysis of administrative databases. BMJ. 2013; 346: f880. doi: 10.1136/bmj.f880.

10. Layton JB, Brookhart A, Funk MJ, Simpson RJ, Pate V, Stürmer T, *et al.* Acute kidney injury in statin initiators. Pharmacoepidemiol Drug Saf. 2013; 22: 1-21.

11. Malek M, Nematbakhsh M. Renal ischemia/reperfusion injury; from pathophysiology to treatment. J Renal Inj Prev. 2015; 4: 20-7.

12. Gueler F, Park JK, Rong S, Kirsch T, Lindschau C, Zheng W, *et al.* Statins attenuate ischemia-reperfusion injury by inducing heme oxygenase-1 in infiltrating macrophages. Am J Pathol. 2007; 170: 1192-9.

13. Rahman M, Shad F, Smith MC. Acute kidney injury: a guide to diagnosis and management. Am Fam Physician. 2012; 86: 631-9.

14. Ostermann M, Cerdá J. The burden of acute kidney injury and related financial issues. Contrib Nephrol. 2018; 193: 100-12.

15. Cahyawati PN. Effect of simvastatin on histopathology of the heart after 5/6 subtotal nephrectomy. Int J App Pharm. 2019; 1: 131-3.

16. Williams P, Lopez H, Britt D, Chan C, Ezrin A, Hottendorf R. Characterization of renal ischemia-reperfusion injury in rats. J Pharmacol Toxicol Methods. 1997; 37: 1-7.

17. Fogo AB. Progression and potential regression of glomerulosclerosis. Kidney Int. 2001; 59: 804-19.

18. Nogueira A, Pires MJ, Oliveira PA. Pathophysiological mechanisms of renal fibrosis: a review of animal models and therapeutic strategies. In Vivo. 2017; 31: 1-22.

19. Teshima CAS, Watanabe M, Fonseca CD, Vattimo MFF. Simvastatin and acute ischemic renal injury in rats. Acta Paulista de Enfermagem. 2012; 25: 86-9.

20. Sharyo S, Yokota-Ikeda N, Mori M, Kumagai K, Uchida K, Ito K, *et al.* Pravastatin improves renal ischemia–reperfusion injury by inhibiting the mevalonate pathway. Kidney International. 2008; 7: 577-84.

21. Christensen M, Su AW, Snyder RW, Greco A, Lipschutz JH, Madaio MP. Simvastatin protection against acute immune-mediated glomerulonephritis in mice. Kidney International. 2006; 69: 457-63.

22. Vogt L, Bangalore S, Fayyad R, Melamed S, Hovingh GK, DeMicco DA, *et al.* Atorvastatin has a dose-dependent beneficial effect on kidney function and associated cardiovascular outcomes: post hoc analysis of 6 double-blind randomized controlled trials. J Am Heart Assoc. 2019; 8: e010827. doi: 10.1161/JAHA.118.010827.