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TIME SUBMITTED	16-FEB-2021 02:37PM (UTC+0700)	WORD COUNT	5595
SUBMISSION ID	1510611003	CHARACTER COUNT	26665

ISSN 0975-2366
DOI: https://doi.org/10.31838/ijpr/2021.13.02.071

Research Article

Combination Use of Simvastatin and Metformin in Male Wistar Rats Following the Atherogenic Diet

ABDURRAHMAN HASYMI*[1,2], BATARI TODJA UMAR[3,4], RAHMAWATI MINHAJAT[3,5], AND ABDI DZUL IKRAM HASANUDDIN[1,6], AMELIA RUMI[7]

[1]Postgraduate Program Hasanuddin Universitiy, Makassar, Indonesia
[2]Histology Department, Medical Faculty Alkhairaat University, Palu, Indonesia
[3]Histology Department, [4] Ophtalmology Department, [5] Internal Medicine Department Hasanuddin University, Makassar, Indonesia
[6]Medical Study Program Gorontalo State University, Gorontalo, Indonesia
[7]Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Tadulako University, Palu, Indonesia
*Corresponding Author
Email ID: hasymia19p@student.unhas.ac.id
Received: 16.11.20, Revised: 11.12.20, Accepted: 13.01.21

ABSTRACT

Atherosclerosis is a vascular disease that is the main cause of hypercholesterolemia. Statins are the most effective cholesterol-lowering drugs to date and metformin is also thought to have anti-atherosclerotic effects in addition to hypoglycemic effects. Statin-associated muscle symptoms (SAMS) are the effect of statin use up to 72%. To determine the effectiveness of the combination of simvastatin and metformin following the atherogenic diet in male Wistar rats. All rats were divided into five experimental groups. The negative control group (n = 5), the positive control group were given the atherogenic diet alone (n = 5), the P1 group were treated with simvastatin 20 mg / BW / day (n = 5), the P2 group were treated with simvastatin 40 mg / BW / day (n = 5), the P3 group were treated with simvastatin 40 mg / BW / day and added with metformin 2 g /BW. All drugs were given after a two-week atherogenic diet. All groups of rats were measured for body weight and length before the diet, in the second week, and after four weeks. Before the rats were sacrificed, blood is drawn to be measured for cholesterol, creatinine, ALT, and AST. After four weeks, the animals were sacrificed and collected for the aorta, liver, and muscles of the right leg organ. All the organs were then measured for their weight. In the measurement of the aortic organ weight (P = 0.041), there was a significant difference in the results in each treatment group, while the examination of the weight of the liver (P = 0.017) and leg muscles (P = 0.831) was not a significant difference in each group. On cholesterol measurement, there was a significant difference (P = 0.000) in each treatment group, while there was no significant difference in creatinine (P = 0.052), ALT (P = 0.059), and AST (P = 0.824) levels. On body weight (P = 0.003), Body Mass Index (BMI) (P = 0.002) and Lee index (P = 0.002) measurement, there were significant differences after the diet in each group, while body length (P = 0.249) was not significantly different after diet in each group. ***Conclusion*** We conclude, there is an association between simvastatin and metformin combination administration in male Wistar rats after being given an atherogenic diet.

Keywords: Atherogenic diet, atherosclerosis, hepatotoxic, metformin, simvastatin, statin-associated muscle symptoms

INTRODUCTION

Atherosclerosis is a vascular disease that is the main cause of hypercholesterolemia (Libby & Libby, 2019). Cardiovascular disease risk factors, including dyslipidemia, smoking, hypertension, diabetes mellitus, and lack of activity play an important role in the occurrence of atherosclerosis (Boucher et al., 2020). During atherosclerosis, abnormal serum lipid levels accumulate to become cholesterol cells through absorption of LDL particles that form foam cells (Tedjokusumo, 2016). This accumulation forms the basis of progression to various diseases (Lily *et al.*, 2019). The most common cause of death from atherosclerosis is reduced oxygen supply to the coronary arteries, causing myocardial ischemia (Indonesian Heart Association, 2018).

The statin class is the drug of choice and the most effective way to lower LDL cholesterol (Rumi & Safaruddin, 2016)(Mann *et al.*, 2015). The agents of this group most frequently used are simvastatin and atorvastatin (Rumi & Safaruddin, 2016). The effect of statins reduces serum LDL levels by 20 to 55%, depending on the type of drug used and the dose (Ward et al., 2019).

Myalgia is the most common cause of patients taking statins, making up 72% of all statin side effects (Kabo, 2014)(Taylor and Thompson,

2018). Among serious but rare muscle side effects are rhabdomyolysis and statin-induced necrotizing autoimmune myopathy (SINAM) (Golomb and Evans, 2010). Rhabdomyolysis is usually diagnosed with a creatine kinase (CK) level> 10 times the upper limit of normal, with evidence of renal impairment, and no other cause of muscle injury (Thankumar, Raghunath and Raghunath, 2016). Simvastatin is more likely to cause a tenfold increase in the level of kidney function (Luo et al., 2017). Statin toxicity can also give rise to an increase in aminotransferase with symptoms that may be asymptomatic but may increase in liver enzyme activity, with an increase in aminotransferase activity> 3X (Taylor and Thompson, 2018).

Metformin is the only class of oral hypoglycemic biguanides available and used to date (Van Stee, de Graaf and Groen, 2018). Metformin also has anti-atherosclerotic properties beyond its glucose-lowering effects (Brunton, Hilal-Dandan and Knollmann, 2018). This drug was also found to reduce LDL and VLDL (Rang *et al.*, 2012).

The combination of metformin with statins can enhance the anti-atherosclerotic effect and counteract some of the unwanted side effects of statins (Van Stee, de Graaf and Groen, 2018). In this study, we aimed to investigate the effectiveness of the simvastatin and metformin combination following the atherogenic diet in male Wistar rats.

METHODS

This research has been approved by the Health Research Ethics Committee of the Medical Faculty, Hasanuddin University with letter number 685 / UN4.6.4.5.31 / PP36 / 2020. Twenty-five male Wistar rats weighing 200 ± 10 g at two months of age were adapted for one week with standard feed. Then divided into five experimental groups. Negative control (KN, n = 5), positive control (KP, n = 5), the intervention group: P1 was given simvastatin 20 mg / day (n = 5), P2 was given simvastatin 40 mg / day (n = 5), and P3 was given simvastatin 40 mg / day in combination with metformin 2 g / day (n = 5). All intervention groups will be given drugs after the second week of the atherogenic diet. All groups except KN were given an atherogenic diet (Modified Western Type Diet (MWTD) ± 15.8% fat and ± 1.25% cholesterol) using sonde for four weeks (Getz and Reardon, 2006). All rats were kept in open drums, in good ventilation conditions, light / dark cycle of 12 hrs / 12 hrs. Each cage contains three mice with access to AD2 food and free drinking ad libitum.

To treat or prevent atherosclerotic plaque formation by lowering cholesterol levels. We prepared simvastatin at a dose of 20 - 40 mg/day (Alberton et al., 2012)(Newman et al., 2018), and to achieve an anti-atherosclerotic effect metformin 2 g / day was added (with two administrations) (Petrie, Chaturvedi, Ford, and Martijn, 2018)(Luo et al., 2019). Administration of simvastatin and metformin was previously dissolved in 0.5% CMC after that given orally using a sonde.

After four weeks, all the rats were sacrificed. Along the aortic arch to the border of the iliac artery, the liver, and muscles of the right leg (up to the ankle) were collected. All tissues that had been collected were then rinsed and cleaned using 0.9% NaCl. After the cleaning process, all the tissues were weighed using analytical scales.

Measurement of body weight and body length was carried out before the diet, the second week, and after the diet was completed. The results of measurements and anthropometric calculations are body weight, body length (naso-anal), *BMI* (gr/cm^2), *Lee index* ($\frac{\sqrt{BBx10}}{PB\,(mm)}$) (Ridwan et al., 2019)(Rabiu et al., 2017).

Before all the rats were sacrificed, 3-4 cc of venous blood was taken from the tails. The blood sample was then centrifuged to produce serum. The serum is then measured for cholesterol, creatinine, ALT, and AST. Serum was examined at the Clinical Pathology Laboratory of Hasanuddin University Hospital.

The results of statistical data were analyzed using SPSS version 20 with a confidence interval of 95% (α = 0.05). All data were compared for each test group using One Way ANOVA or the Kruskal Wallis method. Followed by Tukey's Post hoc test to see the significant comparison of each group. The p-value ≤ of 0.05 is considered significant.

RESULTS

The effect of the combination of simvastatin and metformin after being given MWTD on the weight of aortic, liver, and muscles organs can be seen in Table 1. It revealed the mean aortic organ weight at KN 1.43 gr and KP 2.01 gr. Meanwhile, the mean aortic organ weight at P1 1.74 gr; P2 3.35 gr, and P3 1.62 gr., where there was a significant difference (P = 0.041) in the weight of the aortic organ in each group.

For the liver organ, the mean weight in KN 7.28 gr and KP 9.34 gr. While the mean weight in the 8.51 gr; P2 9.58 gr and P3 9.27 gr, where there was no significant difference (P = 0.077) in liver weight in each group. Also, for the muscle organ, the mean weight in KN 9.07 gr and KP 8.98 gr. While the mean weight in the 8.56 gr; P2 8.44 gr and P3 7.65 gr, where there was

no significant difference (P = 0.831) in muscle organs weight in each group.

The effect of the combination of simvastatin and metformin after being given MWTD on anthropometric measurements (weight, length, BMI, and Lee index) can be seen in Table 2. Before the MWTD diet, mean body weight for KN 193.8 gr and KP 209.2 gr, while for P1 190 gr; P2 203 gr and P3 186.4 gr, while there was no significant difference (P = 0.146) for bodyweight before diet for each group. For the second week's of MWTD diet, mean body weight for KN 200 gr and KP 237.6 gr diet, while for P1 208.6 gr; P2 238,2 gr and P3 223 g, while there was no significant difference (P = 0.059) for each group. Finally, the mean body weight after diet for KN was 209 gr and KP was 284 gr, while P1 was 223.8 gr, P2 250.6 gr, and P3 222.8 gr, while there was a significant difference (P = 0.003) for each group. In table 3, to see the comparison of the significance of the average body weight of each test group. Where each group was significantly different from KN to KP (P = 0.003), KP to P1 (P = 0.021) and KP to P3 (P = 0.018). Meanwhile, the rest of the group did not have a significant difference.

The mean body length of each group before, the second week, and after the MWTD diet. Before the diet, the mean body length of KN was 18.7 cm and KP was 19.5 cm, while P1 was 19.9 cm, P2 was 19.9 cm, and P3 was 20 cm, while there was no significant difference (P = 0.117) in each group. At the second week of the MWTD diet, the mean body length of KN was 19.3 cm and KP was 19.7 cm, while P1 was 20.1 cm, P2 was 20.4 cm, and P3 was 20.5 cm, while there was no significant difference (P = 0.26) in each group. At the time after the MWTD diet, the mean body length of KN was 20 cm and KP was 19.7 cm, while P1 was 20.6 cm, P2 was 16.7 cm, and P3 was 20.5 cm, while there was no significant difference (P = 0.249) in each group.

The mean BMI of each group before, the second week, and after the MWTD diet. Before the diet, the mean BMI of KN was 0.56 and KP was 0.55, while P1 was 0.4 P2 was 0.51, and P3 was 0.47, while there was a significant difference (P = 0.036) in each group. At the second week of the diet, the mean BMI of KN was 0.54 and KP was 0.61, while P1 was 0.52, P2 was 0.57, and P3 was 0.53, while there was a significant difference (P = 0.032) in each group. At the time after the diet, the mean BMI of KN was 0.52 and KP was 0.73, while P1 was 0.53, P2 was 0.6, and P3 was 0.53, while there was a significant difference (P = 0.002) in each group.

The Lee index of each group before, the second week, and after the MWTD diet. Before the diet, the mean Lee index of KN was 0.235 and KP was 0.23, while P1 was 0.4, P2 was 0.23, and P3 was 0.21, while there was a significant difference (P = 0.041) in each group. At the second week of the diet, the mean Lee index of KN was 0.23 and KP was 0.24, while P1 was 0.23; P2 was 0.24 and P3 was 0.23, while there was no significant difference (P = 0.104) in each group. After the diet, the mean Lee index of KN was 0.23 and KP was 0.27, while P1 was 0.23, P2 was 0.24, and P3 was 0.23, where there was a significant difference (P = 0.002) in each group.

Table 4 shows the differences in the mean body weight, body length, BMI, and Lee index of each group after the diet. Mean body weight for KN was 209 gr, and KP was 284 gr, while P1 was 223.8 gr, P1 was 250.6 gr, and P3 was 222.8 gr, while there was a significant difference (P = 0.003) in each group. Mean body length for KN was 20 cm, and KP was 19.7 cm, while P1 was 20.6 cm, P2 was 16.7 cm, and P3 was 20.5 cm, while there was no significant difference (P = 0.472) in each group. Mean BMI for KN was 0.52, and KP was 0.73, while P1 was 0.53, P2 was 0.6, and P3 was 0.53, while there was a significant difference (P = 0.000) in each group. Mean Lee index for KN was 0.23, and KP was 0.27, while P1 was 0.23, P2 was 0.24, and P3 was 0.23, while there was a significant difference (P = 0.000) in each group.

The effect of the combination of simvastatin and metformin on cholesterol and other biochemical markers after the MWTD diet can be seen in Table 5. The mean cholesterol for the KN was 43.84 and KP was 63.12, while P1 was 40.84, P2 was 25.64, and P3 was 30.64, while there was a significant difference (P = 0.000) in each group. The mean creatinine levels for the KN was 0.39 and KP was 0.46, while P1 was 0.22, P2 was 052, and P3 was 0.28, while there was no significant difference (P = 0.052) in each group. The mean ALT levels for KN was 85.76 and KP was 76.88, while P1 was 0.98, P2 was 70.56, and P3 was 45.52, while there was no significant difference (P = 0.059) in each group. The mean AST levels for KN was 123.2 and KP was 199.6, while P1 was 20.2, P2 was 151.8, and P3 was 114.4, while there was no significant difference (P = 0.824) in each group. Table 6 shows the comparison of the significant values of the mean cholesterol levels in each t group. Where KP was significantly different to P1 (P = 0.028), P2 (P = 0.000) and also P3 (P = 0.001). The rest of the group did not differ significantly in mean cholesterol levels.

Table 1: Mean weight of organ (gram) in each group

	AORTA	LIVER	MUSCLE
KN	1.4266	7.2800	9.0660
KP	2.0080	9.3400	8.9820
P1	1.7420	8.5120	8.5600
P2	3.3520	9.6840	8.4440
P3	1.6160	9.2700	7.6460
P-VALUE	0.041**#	0.077*	0.831**

KN (negative control group), KP (positive control group), P1 (simvastatin 20 mg / kg BW/ day group), P2 (simvastatin 40 mg / kg BW/ day group), P3 (simvastatin 40 mg / kg BW/ day and metformin 2 gr / kg BW/ day group). *One-way ANOVA. **Kruskal wallis test. Data are mean ± SD, n = 5 for each group. #P < 0.05, compared with each group.

Tabel 2: Mean body weight (gram), body length (cm), BMI (gram/cm^2) and Lee index in each group before, the second week, and after the MWTD diet

Antropometri	ID Group	Before diet	2nd Week	After diet
Weight (g)	KN	193.80	200.00	209.00
	KP	209.20	237.60	284.00
	P1	190.00	208.60	223.80
	P2	203.00	238.20	250.60
	P3	186.40	223.00	222.80
	P-Value	0.146*	0.059*	0.003*#
Length (cm)	KN	18.700	19.300	20.000
	KP	19.500	19.700	19.700
	P1	19.900	20.100	20.600
	P2	19.900	20.400	16.710
	P3	20.000	20.500	20.500
	P-Value	0.117**	0.260**	0.249**
BMI (g/cm^2)	KN	0.5563	0.5372	0.5210
	KP	0.5491	0.6129	0.7340
	P1	0.4814	0.5183	0.5275
	P2	0.5121	0.5728	0.6024
	P3	0.4673	0.5280	0.5260
	P-Value	0.036**#	0.032**#	0.002**#
Lee Index	KN	0.2354	0.2312	0.2280
	KP	0.2338	0.2450	0.2698
	P1	0.2182	0.2274	0.2296
	P2	0.2262	0.2390	0.2454
	P3	0.2158	0.2284	0.2276
	P-Value	0.041**#	0.104**	0.002**#

KN (negative control group), KP (positive control group), P1 (simvastatin 20 mg / kg BW/ day group), P2 (simvastatin 40 mg / kg BW/ day group), P3 (simvastatin 40 mg / kg BW/ day and metformin 2 gr / kg BW/ day group). *One-way ANOVA. **Kruskal wallis test. Data are mean ± SD, n = 5 for each group. #P < 0.05, compared with each group.

Table 3: Significant comparison of body weight after the MWTD diet in each group

MULTIPLE COMPARISONS		P-VALUE
KN	KP	0.003*
	P1	0.915
	P2	0.169
	P3	0.933
KP	KN	0.003*
	P1	0.021*
	P2	0.353
	P3	0.018*
P1	P2	0.563
	P3	1.000
P2	P3	0.529

KN (negative control group), KP (positive control group), P1 (simvastatin 20 mg / kg BW/ day group), P2 (simvastatin 40 mg / kg BW/ day group), P3 (simvastatin 40 mg / kg BW/ day and metformin 2 gr / kg BW/ day group). P < 0.05, compared with KP group; KN group; P1 group; P3 group.

Table 4: Comparison of mean body weight, body length, BMI, and Lee index in each group after the MWTD diet

	WEIGHT (GRAM)	LENGTH (CM)	BMI (GRAM/CM^2)	LEE INDEX
KN	209.00	20.000	0.5210	0.2280
KP	284.00	19.700	0.7340	0.2698
P1	223.80	20.600	0.5275	0.2296
P2	250.60	16.710	0.6024	0.2454
P3	222.80	20.500	0.5260	0.2276
P-VALUE	0.003*#	0.472**	0.000**#	0.000**#

KN (negative control group), KP (positive control group), P1 (simvastatin 20 mg / kg BW/ day group), P2 (simvastatin 40 mg / kg BW/ day group), P3 (simvastatin 40 mg / kg BW/ day and metformin 2 gr / kg BW/ day group). *One-way ANOVA. **Kruskal wallis test. Data are mean ± SD, n = 5 for each group. #P < 0.05, compared with each group.

Table 5: Mean cholesterol, creatinine, ALT, and AST levels in each group

	KOLESTEROL	KREATININ	ALT	AST
KN	43.8400	0.3880	85.7600	123.2000
KP	63.1200	0.4620	76.8800	199.6000
P1	40.8400	0.2180	50.9800	120.2000
P2	25.6400	0.5220	70.5600	151.8000
P3	30.6400	0.2760	45.5200	114.4000
P-VALUE	0.000*#	0.052**	0.059*	0.824**

KN (negative control group), KP (positive control group), P1 (simvastatin 20 mg / kg BW/ day group), P2 (simvastatin 40 mg / kg BW/ day group), P3 (simvastatin 40 mg / kg BW/ day and metformin 2 gr / kg BW/ day group). *One-way ANOVA. **Kruskal wallis test. Data are mean ± SD, n = 5 for each group. #P < 0.05, compared with each group.

Table 6: Significant comparison of mean cholesterol levels in each group

MULTIPLE COMPARISONS		P-VALUE
KN	KP	0.069
	P1	0.992
	P2	0.094
	P3	0.330
KP	KN	0.069
	P1	0.028*
	P2	0.000*
	P3	0.001*
P1	P2	0.208
	P3	0.575
P2	P3	0.946

KN (negative control group), KP (positive control group), P1 (simvastatin 20 mg / kg BW/ day group), P2 (simvastatin 40 mg / kg BW/ day group), P3 (simvastatin 40 mg / kg BW/ day and metformin 2 gr / kg BW/ day group). $P < 0.05$, compared with KP group; P1 group; P2 group; P3 group.

DISCUSSION

In this study, we used the MWTD diet to induce plaque atherosclerosis, then evaluated the effect during the administration of simvastatin and metformin combination. In general, the layers of large blood vessels contain three basic components: a flattened layer of endothelium, smooth muscle, and connective tissue with elastic fibers and collagen fibers (Mescher, 2013). In this study, there was an increase in the weight of the aortic organ which may be due to the accumulation of cholesterol cells under the endothelium which also triggers a local inflammatory response (Wolf & Ley, 2019)(Bergheanu et al., 2017). In the next stage, this process will attract monocytes to form cholesterol together with foam cells which are the basis for atherosclerosis formation (Stary et al., 1994)(Marchio et al., 2019).

In this study, we also found there was no difference in the weight of liver and muscle tissue in the group of simvastatin and metformin or simvastatin alone. This is possible, statin only affects myalgia in muscle tissue (Taylor and Thompson, 2018), while in the liver, it takes approximately three months of statin therapy for macroscopic changes to occur (Wang et al., 2017).

In this study, we found an increase in the weight of the aortic organ due to atherosclerosis formation. Also, the combination of simvastatin and metformin made the weight of the aortic organ almost the same as the simvastatin group alone. These results are in line with the study of Wang Q et al, who found that metformin can reduce Drp1 expression and mitochondrial fission which is mediated by the AMPK pathway, resulting in inhibition of endothelial oxidative stress, increased endothelial function, and reduction of atherosclerotic lesions (Marchio et al., 2019)(Forouzandeh et al., 2014).

For a long time, statins have been known as the main cholesterol-lowering drugs and are the choice for anti-atherosclerotic (Rumi & Safaruddin, 2016)(Mann, Zipes, Libby, and Bonow, 2015). In this study, we showed that the combination of simvastatin and metformin lowered cholesterol almost as well as high-dose simvastatin. In patients with dyslipidemia, statins function to inhibit cholesterol synthesis in the liver and increase plaque stability (Rumi, Atthobari, Pinzon and Nugroho, 2014). he research of Luo et al found that metformin can reduce fat levels (Luo et al., 2017). The CAMERA study also found that high doses of metformin (> 1700 g day) can lower blood cholesterol levels (Petrie et al., 2018)(Luo et al., 2019).

This study also found that the combination of simvastatin and metformin had a weight loss effect comparable to that of simvastatin administration alone. This is also in line with the preclinical studies which found that metformin improved obesity-related hypertriglyceridemia in some rate via the apolipoprotein A5 pathway (Petrie et al., 2018)(Luo et al., 2019).

In this study, it was found that there was no significant increase in renal function in all experimental groups. This shows that myalgia can occur without any abnormalities in kidney function. We also found elevated liver enzymes which may be due to a side effect of simvastatin or from MWTD (Stroes et al., 2015). Mild elevations of the liver transaminase enzyme occur in 0.5–2.0% of patients on any statin, and this

effect is in a dose-dependent manner (Kapar et al., 2013). According to data from the Drug-Induced Liver Injury Network (DILIN), histopathological disorders of Drug-Induced Liver Injury (DILI) are predominantly such as hepatocellular disorders (Menon et al., 2020). A further diagnostic approach is needed to determine whether the abnormality is caused by the use of statins (Taylor and Thompson, 2018).

CONCLUSION

The combination of simvastatin and metformin had been associated with antiatherosclerotic and lipid-lowering effects. This combination was also associated with bodyweight loss. Additional tests are needed to assess the side effects of this drug combination.

ACKNOWLEDGMENT

A higher appreciation for full funded in this study from Faculty of Medicine, University of Alkhairaat, Palu. We also thank for helping until completion study to staff the Laboratory of Biopharmacy from Faculty of Pharmacy, Hasanuddin University, Makassar.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

REFERENCES

1. Alberton, M., Wu, P., Druyts, E., Briel, M., & Mills, E. J. (2012). *Adverse events associated with individual statin treatments for cardiovascular disease : an indirect comparison*. *September 2011*, 145–157. https://doi.org/10.1093/qjmed/hcr158
2. Association, I. H. (2018). *Guidelines for The Management of Acute Coronary Syndrome* (4th ed.).
3. Bergheanu, S. C., Bodde, M. C., & Jukema, J. W. (2017). Pathophysiology and treatment of atherosclerosis: Current view and future perspective on lipoprotein modification treatment. *Netherlands Heart Journal*, *25*(4), 231–242. https://doi.org/10.1007/s12471-017-0959-2
4. Boucher, P., Matz, R. L., & Terrand, J. (2020). Atherosclerosis: Gone with the Wnt? *Atherosclerosis*, *301*(February), 15–22. https://doi.org/10.1016/j.atherosclerosis.2020.03.024
5. Forouzandeh, F., Salazar, G., Patrushev, N., Xiong, S., Hilenski, L., Fei, B., & Wayne Alexander, R. (2014). Metformin beyond diabetes: Pleiotropic benefits of metformin in attenuation of atherosclerosis. *Journal of the American Heart Association*, *3*(6), 1–13. https://doi.org/10.1161/JAHA.114.001202
6. G, B. A., & E, M. A. (2010). Statin Adverse Effects: A Review of the Literature and Evidence for a Mitochondrial Mechanisme. *Am J Cardiovasc Drugs*, *8*(6), 373–418.
7. Getz, G. S., & Reardon, C. A. (2006). *Diet and Murine Atherosclerosis*. 242–249. https://doi.org/10.1161/01.ATV.0000201071.49029.17
8. L, B. L. (2018). *Goodman & Gilman's: The Pharmacological Basis Of Therapeutic* (13th ed.). McGraw-Hill Education.
9. L, M. D., P, Z. D., P, L., & O, B. R. (2015). *Braunwald's Heart Diseases: A Textbook of Cardiovascular Medicine* (10th ed.). elsevier saunders.
10. Luo, F., Das, A., Chen, J., Wu, P., Li, X., & Fang, Z. (2019). Metformin in patients with and without diabetes: A paradigm shift in cardiovascular disease management. *Cardiovascular Diabetology*, *18*(1), 1–9. https://doi.org/10.1186/s12933-019-0860-y
11. Luo, F., Guo, Y., Ruan, G., Long, J., Zheng, X., Xia, Q., & Zhao, S. (2017). Combined use of metformin and atorvastatin attenuates atherosclerosis in rabbits fed a high-cholesterol diet. *Scientific Reports*, *April*, 1–10. https://doi.org/10.1038/s41598-017-02080-w
12. Marchio, P., Guerra-Ojeda, S., Vila, J. M., Aldasoro, M., Victor, V. M., & Mauricio, M. D. (2019). Targeting early atherosclerosis: A focus on oxidative stress and inflammation. *Oxidative Medicine and Cellular Longevity*, *2019*(Ldl). https://doi.org/10.1155/2019/8563845
13. Menon, P. D., Singh, T., Hubbard, H., Hackman, S., & Sharkey, F. E. (2020). Cholangiolytic Changes in Statin-Induced Liver Injury. *Case Reports in Pathology*, *2020*, 1–4. https://doi.org/10.1155/2020/9650619
14. Mescher, A. L. (2013). *Junqueira's Basic Histology TEXT AND ATLAS* (thirteenth). McGraw-Hill Education.
15. Newman, C. B., Preiss, D., Tobert, J. A., Jacobson, T. A., Ii, R. L. P., Goldstein, L. B., Chin, C., Tannock, L. R., Miller, M., Raghuveer, G., Duell, P. B., Brinton, E. A., Pollak, A., & Braun, L. T. (2018). *A Scientific Statement From the American Heart Association*. https://doi.org/10.1161/ATV.0000000000000073
16. P, K. (2014). *How To Use Cardiovascular Medicines Rationally* (1st ed.). Medical Faculty UI.
17. P, R. H. (2012). *Rang and Dale's Pharmacology* (7th ed.). Elsevier Churchill Livingstone.
18. P, T. (2016). Pathogenesis of Atherosclerosis. In R. A. U (Ed.), *Cardiovascular Diseases (CVD) 5 Secrets* (4th ed., p. 128). Medical Faculty UI.
19. Petrie, P. J. R., Chaturvedi, P. N., Ford, P. I., & Martijn, C. G. J. (2018). *Europe PMC Funders Group Cardiovascular and metabolic effects of metformin in patients with type 1 diabetes (REMOVAL): a double-blind , randomised ,*. *5*(8), 597–609. https://doi.org/10.1016/S2213-8587(17)30194-8.Cardiovascular
20. R, S., R, A., & R, S. (2016). Statin Therapy :

Review of Safety and Potential Side Effects. *Acta Cardiol Sin*, *January 2013*, 631–639. https://doi.org/10.6515/ACS20160611A

21. Rabiu, A., Wale, H., Garba, K., Sabo, A., Hassan, Z., Shugaba, A., Egesie, U., & Odeh, S. (2017). Body mass index of male and female Wistar rats following administration of leptin hormone after a dietary regime. *Annals of Bioanthropology*, *5*(1), 22. https://doi.org/10.4103/aoba.aoba_17_16
22. Ridwan, R., Natzir, R., Rasyid, H., Patellongi, I., Hatta, M., Linggi, E. B., Bukhari, A., & Bahrun, U. (2019). Decreased renal function induced by high-fat diet in Wistar rat: The role of plasma angiotensin converting enzyme 2 (ACE2). *Biomedical and Pharmacology Journal*, *12*(3), 1279–1287. https://doi.org/10.13005/bpj/1756
23. Rumi, A., Atthobari, J., Pinzon, R., & Nugroho, A. E. (2014). Predictors of unachieved LDL levels in ischemic stroke patients treated with statins in Yogyakarta Indonesia. *Journal of Applied Pharmaceutical Science*, *4*(4), 97–102. https://doi.org/10.7324/JAPS.2014.40417
24. Rumi, A., & Safaruddin. (2016). *Easy Learning Clinical Pharmacy*. PT Kanisius.
25. S, L. L. (2019). Cardiovascular Drugs. In L. S. L (Ed.), *Pathophysiology of The Heart Diseases* (6th ed., p. 466).
26. Shahawy, S., & Libby, P. (2019). Atherosclerosis. In Lily SL (Ed.), *Pathophysiology of The Heart Diseases* (6th ed., p. 116). Medik.
27. Stary, H. C., Chandler, A. B., Glagov, S., Guyton, J. R., Insull, W., Rosenfeld, M. E., Schaffer, S. A., Schwartz, C. J., Wagner, W. D., & Wissler, R. W. (1994). A definition of initial, fatty streak, and intermediate lesions of atherosclerosis: A report from the committee on vascular lesions of the council on arteriosclerosis, American Heart Association. *Arteriosclerosis and Thrombosis*, *14*(5), 840–856. https://doi.org/10.1161/01.atv.14.5.840
28. Stee, M. F. Van, Graaf, A. A. De, & Groen, A. K. (2018). Actions of metformin and statins on lipid and glucose metabolism and possible benefit of combination therapy. *Cardiovascular Diabetology*, 1–22. https://doi.org/10.1186/s12933-018-0738-4
29. Stroes, E. S., Thompson, P. D., Corsini, A., Vladutiu, G. D., Raal, F. J., Ray, K. K., Roden, M., Stein, E., Tokgözoğlu, L., Nordestgaard, B. G., Bruckert, E., De Backer, G., Krauss, R. M., Laufs, U., Santos, R. D., Hegele, R. A., Hovingh, G. K., Leiter, L. A., Mach, F., … Ginsberg, H. N. (2015). Statin-associated muscle symptoms: impact on statin therapy - European Atherosclerosis Society Consensus Panel Statement on Assessment, Aetiology and Management. *European Heart Journal*, *36*(17), 1012–1022. https://doi.org/10.1093/eurheartj/ehv043
30. Taylor, B. A., & Thompson, P. (2018). *Statin-Associated Muscle Disease : Advances in Diagnosis and Management of the treatment of cholesterol expanded the number of US*.
31. Thapar, M., Russo, M. W., & Bonkovsky, H. L. (2013). Statins and liver injury. *Gastroenterology and Hepatology*, *9*(9), 605–606.
32. Wang, Q., Zhang, M., Torres, G., Wu, S., Ouyang, C., Xie, Z., & Zou, M. H. (2017). Metformin suppresses diabetes-accelerated atherosclerosis via the inhibition of Drp1-mediated mitochondrial fission. *Diabetes*, *66*(1), 193–205. https://doi.org/10.2337/db16-0915
33. Ward, N. C., Watts, G. F., & Eckel, R. H. (2019). *Mechanistic Insights and Clinical Implications*. 328–350. https://doi.org/10.1161/CIRCRESAHA.118.312782
34. Wolf, D., & Ley, K. (2019). Immunity and Inflammation in Atherosclerosis. *Circulation Research*, *124*(2), 315–327. https://doi.org/10.1161/CIRCRESAHA.118.313591

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