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LAMPIRAN

1. Perhitungan Pemberian Larutan Simvastatin I, Simvastatin II, dan Metformin

Simvastatin I

$$\begin{aligned} \text{Stok} &= \text{Dosis} \times \text{BB max} / 1/2 \text{Volume Pemberian} \\ &= \frac{0,72 \text{mg}}{200 \text{gr}} \times 300 \text{gr} \\ &= \frac{0,72 \text{mg}}{1/2 \times 5 \text{ml}} = 0,432 \text{ mg/ml} = 10,8 \text{ mg/25 ml} \\ \text{Penimbangan} &= \frac{x \text{ mg}}{20 \text{ mg}} \times 10,8 \text{ mg} = 0,54x \text{ mg} \\ &= 0,54x \text{ mg serbuk tablet simvastatin} \approx 10,8 \text{ mg zat murni simvastatin.} \end{aligned}$$

$$\begin{aligned} \text{Volume Pemberian} &= \frac{\text{Dosis} \times \text{BB}}{\text{Stok}} \\ &= \frac{0,72 \text{mg}}{200 \text{gr}} \times 300 \text{gr} \\ &= \frac{0,72 \text{mg}}{0,432 \text{mg/ml}} = 2,5 \text{ ml} \end{aligned}$$

$$\begin{aligned} \text{Contoh VP} &= \frac{\text{BB Tikus}}{\text{BB Max}} \times 1/2 \text{Vp} \\ &= \frac{200 \text{ gr}}{300 \text{ gr}} \times 2,5 \text{ ml} = 1,67 \text{ ml} \end{aligned}$$

Jadi, tikus dengan BB 200 gr diberikan larutan simvastatin 1,67 ml per hari melalui sonde.

Simvastatin II

$$\begin{aligned} \text{Stok} &= \text{Dosis} \times \text{BB max} / 1/2 \text{Volume Pemberian} \\ &= \frac{0,36 \text{mg}}{200 \text{gr}} \times 300 \text{gr} \\ &= \frac{0,36 \text{mg}}{1/2 \times 5 \text{ml}} = 0,216 \text{ mg/ml} = 5,4 \text{ mg/25 ml} \\ \text{Penimbangan} &= \frac{x \text{ mg}}{20 \text{ mg}} \times 5,4 \text{ mg} = 0,27x \text{ mg} \end{aligned}$$

= 0,27x mg serbuk tablet simvastatin \approx 5,4mg
zat murni simvastatin.

$$\text{Volume Pemberian} = \frac{\text{Dosis} \times \text{BB}}{\text{Stok}}$$

$$= \frac{\frac{0,72\text{mg}}{200\text{gr}} \times 300\text{gr}}{0,432\text{mg/ml}} = 2,5 \text{ ml}$$

$$\text{Contoh VP} = \frac{\text{BB Tikus}}{\text{BB Max}} \times \frac{1}{2} \text{Vp}$$

$$= \frac{200\text{ gr}}{300\text{ gr}} \times 2,5 \text{ ml} = 1,67 \text{ ml}$$

Jadi, tikus dengan BB 200 gr diberikan larutan simvastatin 1,67 ml per hari melalui sonde.

Metformin

$$\text{Stok} = \text{Dosis} \times \text{BB max}/1/2\text{Volume Pemberian}$$

$$= \frac{\frac{36\text{mg}}{200\text{gr}} \times 300\text{gr}}{1/2 \times 5\text{ml}} = 21,6 \text{ mg/ml} = 540 \text{ mg}/25 \text{ ml}$$

$$\text{Penimbangan} = \frac{x \text{ mg}}{500 \text{ mg}} \times 540 \text{ mg} = 1,08x \text{ mg}$$

$$= 1,08x \text{ mg serbuk tablet metformin} \approx 21,6 \text{ mg}$$

zat murni metformin.

$$\text{Volume Pemberian} = \frac{\text{Dosis} \times \text{BB}}{\text{Stok}}$$

$$= \frac{\frac{36}{200\text{gr}} \times 300\text{gr}}{21,6/\text{ml}} = 2,5 \text{ ml}$$

$$\text{Contoh VP} = \frac{\text{BB Tikus}}{\text{BB Max}} \times \frac{1}{2} \text{Vp}$$

$$= \frac{200\text{ gr}}{300\text{ gr}} \times 2,5 \text{ ml} = 1,67 \text{ ml}$$

Jadi, tikus dengan BB 200 gr diberikan larutan metformin 1,67 ml perhari melalui sonde.

2. Surat Rekomendasi Persetujuan Etik Penelitian



KEMENTERIAN PENDIDIKAN DAN KEBUDAYAAN
 UNIVERSITAS HASANUDDIN FAKULTAS KEDOKTERAN
 KOMITTEE ETIK PENELITIAN KESEHATAN
 RSPTN UNIVERSITAS HASANUDDIN
 RSUP Dr. WAHIDIN SUDIROHUSODO MAKASSAR
 Sekretariat : Lantai 2 Gedung Laboratorium Terpadu
 Jl. PERINTIS KEMERDEKAAN KAMPUS TAMALANREA KM 10 MAKASSAR 90245.
 Contact Person: dr. Agussalim Bukhari, M.Med.Phd, Sp.GK. Telp. 081241850950, 0411 5780103, Fax: 0411-581431



REKOMENDASI PERSETUJUAN ETIK

Nomor : 685/UN4.6.4.5.31/ PP36/ 2020

Tanggal: 26 Oktober 2020

Dengan ini Menyatakan bahwa Protokol dan Dokumen yang Berhubungan Dengan Protokol berikut ini telah mendapatkan Persetujuan Etik :

No Protokol	UH20090530	No Sponsor Protokol	
Peneliti Utama	dr. Abdurrahman Hasymi, S.Ked	Sponsor	
Judul Peneliti	Efek Kombinasi Simvastatin dan Metformin Terhadap Pertumbuhan Aterosklerosis Pada Aorta Rattus norvegicus Jantan Galur Wistar Setelah Pemberian Diet Aterogenik.		
No Versi Protokol	1	Tanggal Versi	29-Sep-20
No Versi PSP		Tanggal Versi	
Tempat Penelitian	Fakultas Farmasi Universitas Hasanuddin Makassar		
Jenis Review	<input type="checkbox"/> Exempted <input checked="" type="checkbox"/> Expedited <input type="checkbox"/> Fullboard Tanggal	Masa Berlaku 26 Oktober 2020 sampai 26 Oktober 2021	Frekuensi review lanjutan
Ketua Komisi Etik Penelitian Kesehatan FKUH	Nama Prof.Dr.dr. Suryani As'ad, M.Sc.,Sp.GK (K)	Tanda tangan	
Sekretaris Komisi Etik Penelitian Kesehatan FKUH	Nama dr. Agussalim Bukhari, M.Med.,Ph.D.,Sp.GK (K)	Tanda tangan	

Kewajiban Peneliti Utama:

- Menyerahkan Amandemen Protokol untuk persetujuan sebelum di implementasikan
- Menyerahkan Laporan SAE ke Komisi Etik dalam 24 Jam dan dilengkapi dalam 7 hari dan Lapor SUSAR dalam 72 Jam setelah Peneliti Utama menerima laporan
- Menyerahkan Laporan Kemajuan (progress report) setiap 6 bulan untuk penelitian resiko tinggi dan setiap setahun untuk penelitian resiko rendah
- Menyerahkan laporan akhir setelah Penelitian berakhir
- Melaporkan penyimpangan dari protokol yang disetujui (protocol deviation / violation)
- Mematuhi semua peraturan yang ditentukan

3. Kondisi Kandang Hewan Coba

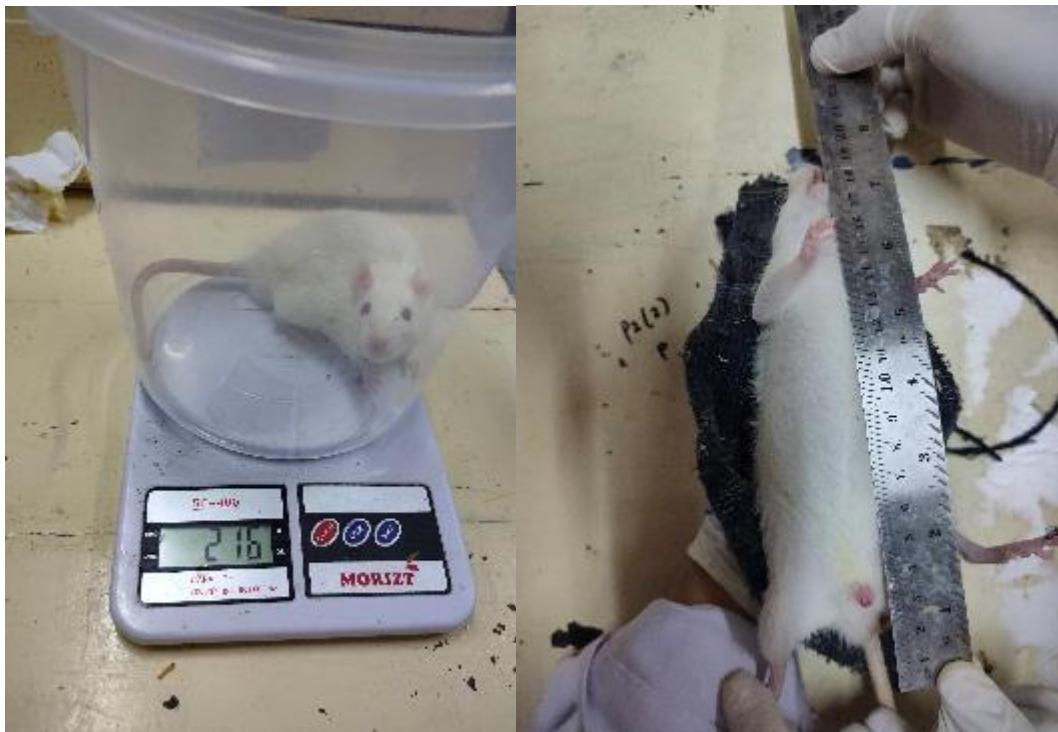


Jenis dan ukuran kandang hewan coba yang menunjukkan ventilasi yang terbuka.



Kondisi kandang yang sangat renggang dengan berisi 2-3 ekor tikus di tiap kandang, serta mendapat akses bebas ke air minum *ad libitum*.

4. Dokumentasi Prosedur Penelitian



Pengukuran berat badan dan panjang badan hewan coba. Pada pengukuran berat dengan menggunakan timbangan digital, terlihat seluruh badan tikus menyentuh pada dasar badan untuk memastikan pengukuran yang tepat. Pada pengukuran panjang badan menggunakan penggaris, terlihat pengukuran dilakukan dengan posisi tikus dorsal rekumben dan mengukur panjang badan tikus dari hidung – anus.



Pengukuran berat organ dengan menggunakan timbangan analitik. Terlihat pada organ di atas aorta, hati, dan otot paha yang di masukkan ke dalam timbangan analitik yang kedap udara untuk mendapat pengukuran yang tepat.



Proses pengambilan darah vena pada pembuluh darah vena ekor tikus dengan menggunakan spoit 3 cc.



Proses terminasi tikus coba dengan Teknik inhalasi eter. Terlihat tikus coba ditempatkan pada tertutup berisikan eter.

5. Gambar Representatif Pemeriksaan Biomarker Darah.



Gambar representatif specimen darah vena tikus. Darah vena tikus selanjutnya akan disentrifuge untuk mendapatkan hasil pemeriksaan yang akurat.

6. Tabulasi Data

	BeratOrgan Aorta	BeratOrgan Hati	BeratOrgan Otot	KimiaDarah Kol	KimiaDarah Cr	KimiaDarah ALT	KimiaDarah AST	KlpUji
1	2,00	5,64	5,79	36,50	,28	129,00	130,00	1
2	1,13	8,71	9,47	33,40	,26	79,40	127,00	1
3	,97	6,31	7,77	46,80	,19	70,80	112,00	1
4	1,92	8,75	11,35	38,50	,86	59,50	103,00	1
5	1,11	6,99	10,95	64,00	,35	90,10	144,00	1
6	1,96	9,94	10,02	51,80	,45	82,40	96,00	2
7	1,69	8,12	8,52	73,60	,51	53,70	139,00	2
8	2,57	10,45	10,64	68,40	,41	52,70	94,00	2
9	1,64	8,33	7,12	53,40	,40	80,30	180,00	2
10	2,18	9,86	8,61	68,40	,54	115,30	489,00	2
11	1,23	9,58	10,84	23,70	,15	58,80	83,00	3
12	2,56	8,14	9,41	41,30	,30	56,30	80,00	3
13	2,20	8,09	7,71	59,80	,29	59,20	111,00	3
14	1,66	7,60	7,56	45,60	,14	39,20	189,00	3
15	1,06	9,15	7,28	33,80	,21	41,40	138,00	3
16	1,98	8,90	5,54	18,60	1,81	120,80	372,00	4
17	5,19	9,86	8,88	28,90	,28	83,10	87,00	4
18	4,87	11,43	9,57	26,70	,18	59,80	121,00	4
19	2,60	7,99	9,50	26,30	,14	52,80	65,00	4
20	2,12	10,24	8,73	27,70	,20	36,30	114,00	4
21	1,54	11,20	10,51	33,90	,30	65,40	116,00	5
22	1,38	5,99	5,97	43,40	,23	43,60	179,00	5
23	2,29	9,79	10,14	37,10	,30	48,00	105,00	5
24	1,34	10,04	4,84	25,00	,30	39,70	112,00	5
25	1,53	9,33	6,77	13,80	,25	30,90	60,00	5

	KlpUji	BBpre Diet	BBmgg2	BBmgg4	PB_pre Diet	PB_mgg2	PB_mgg 4	BMI_preDiet	BMI_mgg2	BMI_mgg... Lee_pre Diet	Lee_mgg2	Lee_mgg 4
1	1	189	162	170	18,0	18,0	18,5	,58	,50	,50	,24	,22
2	1	192	180	206	20,0	20,5	21,0	,48	,43	,47	,22	,21
3	1	201	195	185	18,5	19,0	20,0	,59	,54	,46	,24	,23
4	1	186	223	224	18,0	19,0	20,0	,57	,62	,56	,24	,25
5	1	201	240	260	19,0	20,0	20,5	,56	,60	,62	,24	,25
6	2	211	262	320	19,5	19,5	19,5	,55	,69	,84	,24	,26
7	2	184	225	280	19,0	19,0	19,0	,51	,62	,78	,23	,25
8	2	185	220	260	19,5	19,5	19,5	,49	,58	,68	,22	,23
9	2	226	230	270	19,5	20,0	20,0	,59	,58	,68	,24	,26
10	2	240	251	290	20,0	20,5	20,5	,60	,60	,69	,25	,24
11	3	191	187	238	21,0	21,0	21,5	,43	,42	,51	,20	,21
12	3	203	218	230	19,5	20,0	20,5	,53	,55	,55	,23	,23
13	3	201	225	232	19,5	20,0	20,5	,53	,56	,55	,23	,24
14	3	171	200	203	20,0	20,0	20,5	,43	,50	,48	,21	,22
15	3	184	213	216	19,5	19,5	20,0	,48	,56	,54	,22	,23
16	4	223	244	248	20,5	20,5	20,5	,53	,58	,59	,23	,24
17	4	197	243	264	20,0	20,5	21,1	,49	,58	,63	,22	,24
18	4	200	241	253	19,5	20,0	20,0	,53	,60	,63	,23	,25
19	4	210	233	240	20,0	20,5	20,5	,53	,55	,57	,23	,24
20	4	185	230	248	19,5	20,5	20,5	,49	,55	,59	,22	,23
21	5	197	262	260	21,5	22,0	22,0	,43	,54	,54	,21	,23
22	5	190	213	247	19,5	20,5	20,5	,50	,51	,59	,22	,23
23	5	189	250	253	20,5	21,0	21,0	,45	,57	,57	,21	,24
24	5	168	180	164	18,5	19,0	19,0	,49	,50	,45	,22	,21
25	5	188	210	190	20,0	20,0	20,0	,47	,53	,48	,22	,23

	kelompok	area	mean	ketebalan	derajatkerusakan	LeucocyteCount
1	1	73,800	131,107	72,695	1	81
2	1	91,000	60,569	89,953	1	45
3	1	95,200	82,297	94,190	1	54
4	1	32,200	98,374	31,339	1	74
5	1	58,000	82,021	57,007	1	77
6	2	88,200	81,386	87,284	4	75
7	2	91,800	79,874	90,685	4	78
8	2	90,400	71,952	89,482	4	70
9	2	152,200	121,955	151,200	4	141
10	2	123,600	96,273	122,422	3	153
11	3	126,800	120,841	126,125	3	47
12	3	248,600	101,775	247,405	2	77
13	3	86,800	102,736	86,110	3	49
14	3	84,800	101,099	83,668	2	70
15	3	165,800	94,977	164,693	2	45
16	4	121,800	112,393	120,880	4	63
17	4	109,600	83,609	108,544	3	82
18	4	150,600	126,376	149,479	3	69
19	4	74,000	80,540	72,984	2	70
20	4	126,000	71,079	124,857	3	135

21	5	72,800	74,748	71,912	3	66
22	5	112,600	79,281	111,566	4	60
23	5	69,200	61,105	68,143	3	124
24	5	88,600	63,382	87,452	2	156
25	5	108,333	104,751	107,125	2	60

7. Output Hasil Analisis Statistik

Uji Normalitas dan Nilai Mean Status Antropometri

Tests of Normality

	Kelompok Uji	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
BB Pre Diet	KN	,251	5	,200*	,868	5	,257
	KP	,236	5	,200*	,904	5	,434
	P1	,199	5	,200*	,936	5	,635
	P2	,183	5	,200*	,986	5	,962
	P3	,358	5	,035	,825	5	,127
	KN	,167	5	,200*	,968	5	,864
BB Minggu 2	KP	,263	5	,200*	,898	5	,401
	P1	,214	5	,200*	,955	5	,775
	P2	,272	5	,200*	,865	5	,246
	P3	,219	5	,200*	,942	5	,682
	KN	,153	5	,200*	,972	5	,887
	KP	,197	5	,200*	,943	5	,685
BB Post Diet	P1	,269	5	,200*	,919	5	,522
	P2	,216	5	,200*	,946	5	,709
	P3	,313	5	,123	,842	5	,170

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	
					Lower Bound	Upper Bound			
BB Pre Diet	KN	5	193,80	6,907	3,089	185,22	202,38	186	201
	KP	5	209,20	24,773	11,079	178,44	239,96	184	240
	P1	5	190,00	13,115	5,865	173,72	206,28	171	203
	P2	5	203,00	14,300	6,395	185,24	220,76	185	223
	P3	5	186,40	10,877	4,864	172,89	199,91	168	197
	Total	25	196,48	16,330	3,266	189,74	203,22	168	240
BB Minggu 2	KN	5	200,00	31,615	14,139	160,74	239,26	162	240
	KP	5	237,60	18,036	8,066	215,21	259,99	220	262
	P1	5	208,60	15,143	6,772	189,80	227,40	187	225
	P2	5	238,20	6,301	2,818	230,38	246,02	230	244
	P3	5	223,00	33,045	14,778	181,97	264,03	180	262
	Total	25	221,48	26,290	5,258	210,63	232,33	162	262
BB Post Diet	KN	5	209,00	35,114	15,704	165,40	252,60	170	260
	KP	5	284,00	23,022	10,296	255,41	312,59	260	320
	P1	5	223,80	14,149	6,328	206,23	241,37	203	238
	P2	5	250,60	8,820	3,945	239,65	261,55	240	264
	P3	5	222,80	43,055	19,255	169,34	276,26	164	260
	Total	25	238,04	37,261	7,452	222,66	253,42	164	320

Tests of Normality

	Kelompok Uji	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
PB Pre Diet	KN	,201	5	,200*	,881	5	,314
	KP	,300	5	,161	,883	5	,325
	P1	,330	5	,079	,735	5	,021
	P2	,231	5	,200*	,881	5	,314
	P3	,127	5	,200*	,999	5	1,000
	KN	,468	5	,001	,560	5	,000
PB Minggu 2	KP	,237	5	,200*	,961	5	,814
	P1	,372	5	,022	,828	5	,135
	P2	,473	5	,001	,552	5	,000
	P3	,127	5	,200*	,999	5	1,000
	KN	,470	5	,001	,557	5	,000
	KP	,237	5	,200*	,961	5	,814
PB Post Diet	P1	,372	5	,022	,828	5	,135
	P2	,456	5	,001	,570	5	,000
	P3	,127	5	,200*	,999	5	1,000

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Report

Kelompok Uji		PB Pre Diet	PB Minggu 2	PB Post Diet
KN	Mean	18,700	19,300	20,000
	N	5	5	5
KP	Std. Deviation	,8367	,9747	,9354
	Mean	19,500	19,700	19,700
P1	N	5	5	5
	Std. Deviation	,3536	,5701	,5701
P2	Mean	19,900	20,100	20,600
	N	5	5	5
P3	Std. Deviation	,6519	,5477	,5477
	Mean	19,900	20,400	16,710
Total	N	5	5	5
	Std. Deviation	,4183	,2236	8,1980
	Mean	20,000	20,500	20,500
	N	25	25	25
	Std. Deviation	,8292	,8292	3,7151

Tests of Normality

	Kelompok Uji	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
BMI Pre Diet	KN	,304	5	,146	,771	5	,046
	KP	,216	5	,200*	,906	5	,444
	P1	,230	5	,200*	,859	5	,226
	P2	,331	5	,076	,803	5	,086
	P3	,184	5	,200*	,957	5	,786
	KN	,193	5	,200*	,951	5	,742
BMI Minggu 2	KP	,232	5	,200*	,858	5	,221
	P1	,276	5	,200*	,832	5	,145
	P2	,226	5	,200*	,931	5	,602
	P3	,183	5	,200*	,951	5	,741
	KN	,241	5	,200*	,886	5	,338
	KP	,328	5	,085	,806	5	,091
BMI Post Diet	P1	,269	5	,200*	,877	5	,296
	P2	,277	5	,200*	,876	5	,293
	P3	,193	5	,200*	,933	5	,616

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Report

Kelompok Uji		BMI Pre Diet	BMI Minggu 2	BMI Post Diet
KN	Mean	,5563	,5372	,5210
	N	5	5	5
KP	Std. Deviation	,04424	,07694	,06704
	Mean	,5491	,6129	,7340
P1	N	5	5	5
	Std. Deviation	,05035	,04654	,07266
P2	Mean	,4814	,5183	,5275
	N	5	5	5
P3	Std. Deviation	,05056	,05839	,02866
	Mean	,5121	,5728	,6024
Total	N	5	5	5
	Std. Deviation	,02086	,02221	,02671
	Mean	,4673	,5280	,5260
	N	5	5	5
	Std. Deviation	,03004	,02707	,05941
	Mean	,5132	,5539	,5822
Total	N	25	25	25
	Std. Deviation	,05199	,05815	,09707

Tests of Normality

	Kelompok Uji	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Lee Pre Diet	KN	,325	5	,090	,762	5	,038
	KP	,204	5	,200*	,928	5	,580
	P1	,220	5	,200*	,881	5	,313
	P2	,319	5	,105	,793	5	,071
	P3	,174	5	,200*	,951	5	,745
	KN	,196	5	,200*	,940	5	,668
Lee Minggu 2	KP	,141	5	,200*	,995	5	,993
	P1	,266	5	,200*	,823	5	,122
	P2	,212	5	,200*	,936	5	,635
	P3	,135	5	,200*	,989	5	,977
	KN	,233	5	,200*	,890	5	,357
	KP	,341	5	,059	,792	5	,069
Lee Post Diet	P1	,251	5	,200*	,887	5	,344
	P2	,273	5	,200*	,852	5	,201
	P3	,222	5	,200*	,953	5	,758

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Report

Kelompok Uji		Lee Pre Diet	Lee Minggu 2	Lee Post Diet
KN	Mean	,2354	,2312	,2280
	N	5	5	5
KP	Std. Deviation	,00945	,01666	,01466
	Mean	,2338	,2450	,2698
P1	N	5	5	5
	Std. Deviation	,01076	,01221	,01365
P2	Mean	,2182	,2274	,2296
	N	5	5	5
P3	Std. Deviation	,01287	,01309	,00619
	Mean	,2262	,2390	,2454
Total	N	5	5	5
	Std. Deviation	,00482	,00453	,00537
	Mean	,2158	,2284	,2276
	N	5	5	5
	Std. Deviation	,00691	,00799	,01092
	Mean	,2259	,2342	,2401
	N	25	25	25
	Std. Deviation	,01179	,01270	,01933

Uji One-Way ANOVA dan Pos Hoc Tukey Berat Badan

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
BB Pre Diet	Between Groups	1775,440	4	443,860	1,919	,146
	Within Groups	4624,800	20	231,240		
	Total	6400,240	24			
BB Minggu 2	Between Groups	5845,040	4	1461,260	2,720	,059
	Within Groups	10743,200	20	537,160		
	Total	16588,240	24			
BB Post Diet	Between Groups	17742,160	4	4435,540	5,694	,003
	Within Groups	15578,800	20	778,940		
	Total	33320,960	24			

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Kelompok Uji	(J) Kelompok Uji	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
BB Pre Diet	KN	KP	-15,400	9,617	,514	-44,18	13,38
		P1	3,800	9,617	,994	-24,98	32,58
		P2	-9,200	9,617	,871	-37,98	19,58
		P3	7,400	9,617	,936	-21,38	36,18
	KP	KN	15,400	9,617	,514	-13,38	44,18
		P1	19,200	9,617	,303	-9,58	47,98
		P2	6,200	9,617	,966	-22,58	34,98
		P3	22,800	9,617	,164	-5,98	51,58
	P1	KN	-3,800	9,617	,994	-32,58	24,98
		KP	-19,200	9,617	,303	-47,98	9,58
		P2	-13,000	9,617	,664	-41,78	15,78
		P3	3,600	9,617	,995	-25,18	32,38
BB Minggu 2	P2	KN	9,200	9,617	,871	-19,58	37,98
		KP	-6,200	9,617	,966	-34,98	22,58
		P1	13,000	9,617	,664	-15,78	41,78
		P3	16,600	9,617	,441	-12,18	45,38
	P3	KN	-7,400	9,617	,936	-36,18	21,38
		KP	-22,800	9,617	,164	-51,58	5,98
		P1	-3,600	9,617	,995	-32,38	25,18
		P2	-16,600	9,617	,441	-45,38	12,18
	KN	KP	-37,600	14,658	,116	-81,46	6,26
		P1	-8,600	14,658	,976	-52,46	35,26
		P2	-38,200	14,658	,107	-82,06	5,66
		P3	-23,000	14,658	,533	-66,86	20,86
BB Minggu 2	KP	KN	37,600	14,658	,116	-6,26	81,46
		P1	29,000	14,658	,312	-14,86	72,86
		P2	-,600	14,658	1,000	-44,46	43,26
		P3	14,600	14,658	,854	-29,26	58,46
	P1	KN	8,600	14,658	,976	-35,26	52,46
		KP	-29,000	14,658	,312	-72,86	14,86
		P2	-29,600	14,658	,293	-73,46	14,26
		P3	-14,400	14,658	,860	-58,26	29,46

		KN	38,200	14,658	,107	-5,66	82,06
	P2	KP	,600	14,658	1,000	-43,26	44,46
		P1	29,600	14,658	,293	-14,26	73,46
		P3	15,200	14,658	,835	-28,66	59,06
		KN	23,000	14,658	,533	-20,86	66,86
	P3	KP	-14,600	14,658	,854	-58,46	29,26
		P1	14,400	14,658	,860	-29,46	58,26
		P2	-15,200	14,658	,835	-59,06	28,66
		KP	-75,000*	17,652	,003	-127,82	-22,18
	KN	P1	-14,800	17,652	,915	-67,62	38,02
		P2	-41,600	17,652	,169	-94,42	11,22
		P3	-13,800	17,652	,933	-66,62	39,02
		KN	75,000*	17,652	,003	22,18	127,82
	KP	P1	60,200*	17,652	,021	7,38	113,02
		P2	33,400	17,652	,353	-19,42	86,22
		P3	61,200*	17,652	,018	8,38	114,02
		KN	14,800	17,652	,915	-38,02	67,62
BB Post Diet	P1	KP	-60,200*	17,652	,021	-113,02	-7,38
		P2	-26,800	17,652	,563	-79,62	26,02
		P3	1,000	17,652	1,000	-51,82	53,82
		KN	41,600	17,652	,169	-11,22	94,42
	P2	KP	-33,400	17,652	,353	-86,22	19,42
		P1	26,800	17,652	,563	-26,02	79,62
		P3	27,800	17,652	,529	-25,02	80,62
		KN	13,800	17,652	,933	-39,02	66,62
	P3	KP	-61,200*	17,652	,018	-114,02	-8,38
		P1	-1,000	17,652	1,000	-53,82	51,82
		P2	-27,800	17,652	,529	-80,62	25,02

*. The mean difference is significant at the 0.05 level.

Uji Kruskall-Wallis Panjang Badan

Test Statistics^{a,b}

	PB Pre Diet	PB Minggu 2	PB Post Diet
Chi-Square	7,382	5,275	5,401
df	4	4	4
Asymp. Sig.	,117	,260	,249

a. Kruskal Wallis Test

b. Grouping Variable: Kelompok Uji

Uji Kruskall-Wallis Body Mass Index

Test Statistics^{a,b}

	BMI Pre Diet	BMI Minggu 2	BMI Post Diet
Chi-Square	10,267	10,570	17,084
df	4	4	4
Asymp. Sig.	,036	,032	,002

a. Kruskal Wallis Test

b. Grouping Variable: Kelompok Uji

Uji Kruskall-Wallis Lee Index

Test Statistics^{a,b}

	Lee Pre Diet	Lee Minggu 2	Lee Post Diet
Chi-Square	9,963	7,676	17,128
df	4	4	4
Asymp. Sig.	,041	,104	,002

a. Kruskal Wallis Test

b. Grouping Variable: Kelompok Uji

Uji Normalitas dan Nilai Mean Biomarker Darah

Tests of Normality

	Kelompok Uji	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Kimia Darah Kolesterol	KN	,268	5	,200*	,859	5	,224
	KP	,304	5	,147	,845	5	,179
	P1	,162	5	,200*	,993	5	,989
	P2	,365	5	,029	,782	5	,057
	P3	,211	5	,200*	,962	5	,823

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Descriptives

Kimia Darah Kolesterol

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
KN	5	43,8400	12,31434	5,50714	28,5497	59,1303	33,40	64,00
KP	5	63,1200	9,85150	4,40572	50,8878	75,3522	51,80	73,60
P1	5	40,8400	13,46934	6,02367	24,1156	57,5644	23,70	59,80
P2	5	25,6400	4,06177	1,81648	20,5966	30,6834	18,60	28,90
P3	5	30,6400	11,51707	5,15059	16,3397	44,9403	13,80	43,40
Total	25	40,8160	16,48157	3,29631	34,0127	47,6193	13,80	73,60

Tests of Normality

	Kelompok Uji	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Kimia Darah Creatinin	KN	,356	5	,037	,748	5	,029
	KP	,201	5	,200*	,911	5	,471
	P1	,231	5	,200*	,866	5	,250
	P2	,431	5	,003	,617	5	,001
	P3	,362	5	,031	,760	5	,036

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Report

Kimia Darah Creatinin

Kelompok Uji	Mean	N	Std. Deviation
KN	,3880	5	,26994
KP	,4620	5	,06140
P1	,2180	5	,07530
P2	,5220	5	,72182
P3	,2760	5	,03362
Total	,3732	25	,33768

Tests of Normality

	Kelompok Uji	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Kimia Darah ALT	KN	,235	5	,200*	,910	5	,466
	KP	,217	5	,200*	,892	5	,368
	P1	,306	5	,143	,790	5	,066
	P2	,229	5	,200*	,940	5	,666
	P3	,223	5	,200*	,953	5	,755

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Descriptives

Kimia Darah ALT

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
KN	5	85,7600	26,65695	11,92135	52,6610	118,8590	59,50	129,00
KP	5	76,8800	25,69167	11,48966	44,9796	108,7804	52,70	115,30
P1	5	50,9800	9,84337	4,40209	38,7578	63,2022	39,20	59,20
P2	5	70,5600	32,73474	14,63942	29,9145	111,2055	36,30	120,80
P3	5	45,5200	12,77368	5,71257	29,6594	61,3806	30,90	65,40
Total	25	65,9400	26,36467	5,27293	55,0572	76,8228	30,90	129,00

Tests of Normality

	Kelompok Uji	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Kimia Darah AST	KN	,194	5	,200*	,972	5	,889
	KP	,347	5	,049	,729	5	,019
	P1	,195	5	,200*	,903	5	,428
	P2	,397	5	,010	,720	5	,015
	P3	,285	5	,200*	,924	5	,559

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Report

Kimia Darah AST

Kelompok Uji	Mean	N	Std. Deviation
KN	123,2000	5	16,02186
KP	199,6000	5	165,59982
P1	120,2000	5	45,09656
P2	151,8000	5	125,09876
P3	114,4000	5	42,51235
Total	141,8400	25	94,36263

Uji One-Way ANOVA dan Pos Hoc Tukey Biomarker Darah ANOVA

Kimia Darah Kolesterol

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4202,378	4	1050,594	9,068	,000
Within Groups	2317,036	20	115,852		
Total	6519,414	24			

Multiple Comparisons

Dependent Variable: Kimia Darah Kolesterol

Tukey HSD

(I) Kelompok Uji	(J) Kelompok Uji	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
KN	KP	-19,28000	6,80740	,069	-39,6503	1,0903
	P1	3,00000	6,80740	,992	-17,3703	23,3703
	P2	18,20000	6,80740	,094	-2,1703	38,5703
KP	P3	13,20000	6,80740	,330	-7,1703	33,5703
	KN	19,28000	6,80740	,069	-1,0903	39,6503
	P1	22,28000*	6,80740	,028	1,9097	42,6503
P1	P2	37,48000*	6,80740	,000	17,1097	57,8503
	P3	32,48000*	6,80740	,001	12,1097	52,8503
	KN	-3,00000	6,80740	,992	-23,3703	17,3703
P2	KP	-22,28000*	6,80740	,028	-42,6503	-1,9097
	P2	15,20000	6,80740	,208	-5,1703	35,5703
	P3	10,20000	6,80740	,575	-10,1703	30,5703
P2	KN	-18,20000	6,80740	,094	-38,5703	2,1703
	KP	-37,48000*	6,80740	,000	-57,8503	-17,1097
	P1	-15,20000	6,80740	,208	-35,5703	5,1703
P3	P3	-5,00000	6,80740	,946	-25,3703	15,3703
	KN	-13,20000	6,80740	,330	-33,5703	7,1703
	KP	-32,48000*	6,80740	,001	-52,8503	-12,1097
P3	P1	-10,20000	6,80740	,575	-30,5703	10,1703
	P2	5,00000	6,80740	,946	-15,3703	25,3703

*. The mean difference is significant at the 0.05 level.

Test Statisticsa,b

Uji Kruskal-Wallis Kreatinin

Kimia Darah Creatinin	
Chi-Square	9,379
df	4
Asymp. Sig.	,052

a. Kruskal Wallis Test

b. Grouping Variable: Kelompok Uji

Uji One-Way ANOVA

ANOVA

Kimia Darah ALT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5873,192	4	1468,298	2,717	,059
Within Groups	10809,108	20	540,455		
Total	16682,300	24			

Uji Kruskal-Wallis AST

Test Statistics^{a,b}

	Kimia Darah AST
Chi-Square	1,513
df	4
Asymp. Sig.	,824

a. Kruskal Wallis Test

b. Grouping Variable: Kelompok Uji

Uji Normalitas dan Nilai Mean Berat Organ

Tests of Normality

	Kelompok Uji	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Berat Organ Aorta	KN	,326	5	,088	,800	5	,081
	KP	,197	5	,200*	,928	5	,583
	P1	,190	5	,200*	,940	5	,664
	P2	,286	5	,200*	,811	5	,099
	P3	,378	5	,019	,749	5	,029

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Report

Berat Organ Aorta

Kelompok Uji	Mean	N	Std. Deviation
KN	1,4266	5	,49304
KP	2,0080	5	,38219
P1	1,7420	5	,63500
P2	3,3520	5	1,55308
P3	1,6160	5	,38708
Total	2,0289	25	1,02571

Tests of Normality

	Kelompok Uji	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Berat Organ Hati	KN	,245	5	,200*	,884	5	,329
	KP	,291	5	,194	,859	5	,223
	P1	,275	5	,200*	,916	5	,507
	P2	,153	5	,200*	,991	5	,983
	P3	,312	5	,125	,860	5	,230

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Descriptives

Berat Organ Hati

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
KN	5	7,2800	1,40716	,62930	5,5328	9,0272	5,64	8,75
KP	5	9,3400	1,04535	,46749	8,0420	10,6380	8,12	10,45
P1	5	8,5120	,82096	,36714	7,4926	9,5314	7,60	9,58
P2	5	9,6840	1,31081	,58621	8,0564	11,3116	7,99	11,43
P3	5	9,2700	1,95897	,87608	6,8376	11,7024	5,99	11,20
Total	25	8,8172	1,52260	,30452	8,1887	9,4457	5,64	11,43

Tests of Normality

	Kelompok Uji	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Berat Organ Otot	KN	,193	5	,200*	,933	5	,619
	KP	,206	5	,200*	,955	5	,770
	P1	,312	5	,127	,852	5	,202
	P2	,368	5	,025	,740	5	,024
	P3	,237	5	,200*	,881	5	,315

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Report

Berat Organ Otot

Kelompok Uji	Mean	N	Std. Deviation
KN	9,0660	5	2,30982
KP	8,9820	5	1,38250
P1	8,5600	5	1,52330
P2	8,4440	5	1,66494
P3	7,6460	5	2,54325
Total	8,5396	25	1,84429

Uji Kruskal-Wallis pada Berat Organ Aorta

Test Statistics^{a,b}

	Berat Organ Aorta
Chi-Square	9,969
df	4
Asymp. Sig.	,041

a. Kruskal Wallis Test

b. Grouping Variable: Kelompok Uji

Uji One-Way ANOVA pada Berat Organ Hati

ANOVA

Berat Organ Hati

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	18,429	4	4,607	2,476	,077
Within Groups	37,210	20	1,861		
Total	55,640	24			

Uji Kruskal-Wallis pada Berat Organ Aorta

Test Statistics^{a,b}

	Berat Organ Otot
Chi-Square	1,477
df	4
Asymp. Sig.	,831

a. Kruskal Wallis Test

b. Grouping Variable: Kelompok Uji

Uji Normalitas dan Homogenitas Ketebalan Dinding Aorta

Descriptives

Ketebalan

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Kelompok 1	5	46,8701	17,47265	7,81401	25,1750	68,5653	21,28	63,95
Kelompok 2	5	123,2911	29,82254	13,33704	86,2616	160,3207	85,63	167,97
Kelompok 3	5	73,4685	19,03955	8,51475	49,8278	97,1093	59,26	102,65
Kelompok 4	5	78,3121	18,98218	8,48909	54,7426	101,8815	49,55	101,48
Kelompok 5	5	60,5861	13,43696	6,00919	43,9019	77,2703	46,26	75,74
Total	25	76,5056	32,31199	6,46240	63,1679	89,8433	21,28	167,97

Uji One-Way ANOVA dan Pos Hoc Tukey Ketebalan Dinding Aorta

ANOVA

Ketebalan

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	16665,321	4	4166,330	9,929	,000
Within Groups	8392,228	20	419,611		
Total	25057,549	24			

Multiple Comparisons

Dependent Variable: Ketebalan

Tukey HSD

(I) Kelompok Aorta	(J) Kelompok Aorta	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Kelompok 1	Kelompok 2	-76,42100*	12,95548	,000	-115,1887	-37,6533
	Kelompok 3	-26,59840	12,95548	,278	-65,3661	12,1693
	Kelompok 4	-31,44192	12,95548	,149	-70,2096	7,3257
	Kelompok 5	-13,71599	12,95548	,825	-52,4836	25,0517
Kelompok 2	Kelompok 1	76,42100*	12,95548	,000	37,6533	115,1887
	Kelompok 3	49,82260*	12,95548	,008	11,0549	88,5903
	Kelompok 4	44,97908*	12,95548	,018	6,2114	83,7467
	Kelompok 5	62,70502*	12,95548	,001	23,9374	101,4727
Kelompok 3	Kelompok 1	26,59840	12,95548	,278	-12,1693	65,3661
	Kelompok 2	-49,82260*	12,95548	,008	-88,5903	-11,0549
	Kelompok 4	-4,84352	12,95548	,995	-43,6112	33,9241
	Kelompok 5	12,88242	12,95548	,855	-25,8852	51,6501
Kelompok 4	Kelompok 1	31,44192	12,95548	,149	-7,3257	70,2096
	Kelompok 2	-44,97908*	12,95548	,018	-83,7467	-6,2114
	Kelompok 3	4,84352	12,95548	,995	-33,9241	43,6112
	Kelompok 5	17,72593	12,95548	,654	-21,0417	56,4936
Kelompok 5	Kelompok 1	13,71599	12,95548	,825	-25,0517	52,4836
	Kelompok 2	-62,70502*	12,95548	,001	-101,4727	-23,9374
	Kelompok 3	-12,88242	12,95548	,855	-51,6501	25,8852
	Kelompok 4	-17,72593	12,95548	,654	-56,4936	21,0417

*. The mean difference is significant at the 0.05 level.

Nilai Mean dan Uji Kruskal-Wallis Derajat Kerusakan Hati

Report

Derajat Kerusakan Hati

Kelompok Uji	Mean	N	Std. Deviation
1	1,00	5	,000
2	3,80	5	,447
3	2,40	5	,548
4	3,00	5	,707
5	2,80	5	,837
Total	2,60	25	1,080

Test Statistics^{a,b}

	Derajat Kerusakan Hati
Chi-Square	17,407
df	4
Asymp. Sig.	,002

a. Kruskal Wallis Test

b. Grouping Variable: Kelompok

Uji

Uji Normalitas, Homogenitas dan Nilai Mean Jumlah Leukosit Otot Lurik

Tests of Normality

	Kelompok	Kolmogorov-Smirnova ^a			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Leucocyte Count	Kelompok 1	,290	5	,198	,874	5	,281
	Kelompok 2	,337	5	,066	,785	5	,060
	Kelompok 3	,320	5	,105	,822	5	,121
	Kelompok 4	,324	5	,093	,750	5	,030
	Kelompok 5	,331	5	,078	,801	5	,083

a. Lilliefors Significance Correction

Test of Homogeneity of Variances

Leucocyte Count

Levene Statistic	df1	df2	Sig.
4,666	4	20	,008

Descriptives

Leucocyte Count

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Kelompok 1	5	66,20	15,770	7,053	46,62	85,78	45	81
Kelompok 2	5	97,60	47,569	21,273	38,54	156,66	41	153
Kelompok 3	5	57,60	14,792	6,615	39,23	75,97	45	77
Kelompok 4	5	82,80	30,103	13,463	45,42	120,18	63	135
Kelompok 5	5	90,20	47,468	21,228	31,26	149,14	45	156
Total	25	78,88	34,819	6,964	64,51	93,25	41	156

Uji Kruskal-Wallis Jumlah Leukosit Otot Lurik

Test Statistics^{a,b}

	Leucocyte Count
Chi-Square	6,116
df	4
Asymp. Sig.	,191

a. Kruskal Wallis Test

b. Grouping Variable: Kelompok

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

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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 ($P = 0.077$) 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 (Shahawy & 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 (Ramkumar, 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) $\pm 15.8\%$ fat and $\pm 1.25\%$ 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* ($\frac{\sqrt{BB \times 10}}{PB (\text{mm})}$) (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% ($\alpha = 0.05$). 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 \leq 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 P1 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 P1 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 0.48, 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 0.22, 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 0.52, 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 50.98, 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 120.2, 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 test 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 \pm SD, n = 5 for each group. #P < 0.05, compared with each group.

Tabel 2. Mean body weight (gram), body length (cm), BMI (gram/cm²) and Lee index in each group before, the second week, and after the MWTD diet.

Antropometri	ID Group	Before diet	2 nd 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 \pm SD, n = 5 for each group. #P < 0.05, compared with each group.

Table 3. Significant comparison of body weight after the MWT 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 MWT 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 \pm 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 \pm 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 MWT 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). The 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 mice 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 (Thapar 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.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest

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