

APPENDICES

Appendix 1. Ethical clearance



**KOMISI ETIK PENELITIAN
FAKULTAS KEDOKTERAN HEWAN UNIVERSITAS AIRLANGGA
*Animal Care and Use Committee (ACUC)***

**KETERANGAN KELAIKAN ETIK
“ ETHICAL CLEARANCE ”**

No : 1.KE.179.10.2019

**KOMISI ETIK PENELITIAN (ANIMAL CARE AND USE COMMITTEE)
FAKULTAS KEDOKTERAN HEWAN UNIVERSITAS AIRLANGGA SURABAYA,
TELAH MEMPELAJARI SECARA SEKSAMA RANCANGAN PENELITIAN YANG
DIUSULKAN, MAKA DENGAN INI MENYATAKAN BAHWA :**

PENELITIAN BERJUDUL : The Protective Effect of *Ocimum sanctum* Leaf Extract
Against Lead Acetate Induced Nephrotoxicity and
Hepatotoxicity in Mice (*Mus musculus*)

PENELITI UTAMA : Nina Krismaharani

**UNIT/LEMBAGA/TEMPAT
PENELITIAN** : Program Studi Kedokteran Hewan
Fakultas Kedokteran Hewan Universitas Airlangga

DINYATAKAN : LAIK ETIK

Surabaya, 10 Oktober 2019


Ketua 

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Mengetahui,
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Appendix 2. *Ocimum sanctum* identification



UNIT LAYANAN BIOLOGI
FAKULTAS SAINS DAN TEKNOLOGI
UNIVERSITAS AIRLANGGA

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 Kristania Celia (061611133128)
 Kharisma Dwi Veteriananta (061611133129)
 Nina Krismaharani (06161133053)

Jenis uji : Identifikasi tanaman

Berdasarkan sampel yang diterima, kemudian dideterminasi dengan hasil sebagai berikut :

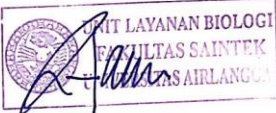
Kingdom	: Plantae
Divisio	: Magnoliophyta
Classis	: Magnoliopsida
Ordo	: Lamiales
Familia	: Lamiaceae
Genus	: <i>Ocimum</i>
Species	: <i>Ocimum sanctum</i>

Deskripsi:

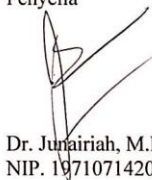
Terna kecil, daun tidak lengkap terdiri atas tangkai daun (petiolus) dan helaian daun (lamina), panjang daun 2,8 cm-6,4 cm, lebar daun 1,6-3,6 cm, bentuk daun ovalis atau ellipticus, ujung daun runcing, pangkal daun tumpul, tepi daun rata, tulang daun menyirip, helaian daun dan tangkai daun berwarna hijau muda, tangkai daun panjang 0,5-2,5 cm, daun mempunyai aroma khas, daging daun tipis lunak.

Surabaya, 13 November 2019

Mengetahui,



Dr. Sucipto Hariyanto, DEA
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Appendix 3. Dosage conversion from rat to mice

Based on Manikandan *et al.*, (2007) research, the treatment dose used was 100 mg / kg BW, 200 mg / kg BW and 400 mg / kg BW for rat. The dosage was converted to mice dosage using calculations with dosage conversion tables by Laurence and Bachrach (1964).

	Mice 20 g	Rat 200 g	Guinea pig 400 g	Rabbit 1.5 kg	Cat 2 kg	Monkey 4 kg	Dog 12 kg	Human 70 kg
Mice 20 g	1.0	7.0	12.25	27.8	29.7	64.1	124.2	387.9
Rat 200 g	0.14	1.0	1.74	3.9	4.2	9.2	17.8	56.0
Guinea pig 400 g	0.08	0.57	1.0	2.25	2.4	5.2	10.2	31.5
Rabbit 1.5 kg	0.04	0.25	0.44	1.0	1.08	2.4	4.5	14.2
Cat 2 kg	0.03	0.23	0.41	0.92	1.0	2.2	4.1	13.0
Monkey 4 kg	0.016	0.11	0.19	0.42	0.45	1.0	1.9	6.1
Dog 12 kg	0.008	0.06	0.1	0.22	0.24	0.52	1.0	3.1
Human 70 kg	0.0026	0.018	0.031	0.07	0.076	0.16	0.32	1.0

T1 : 100 mg/kg BW = 100 mg/1000g BW = 20 mg/200g BW (rat)

mice = 20 mg/200g BW × 0.14 = 2.8 mg/20g BW

= 140 mg/kg BW (mice)

T2 : 200 mg/kg BW = 200 mg/1000g BW = 40 mg/200g BW (rat)

mice = 40 mg/200g BW \times 0.14 = 5.6 mg/20g BW

= 280 mg/kg BW (mice)

T3 : 400 mg/kg BW = 400 mg/1000g BW = 80 mg/200g BW (rat)

mice = 80 mg/200g BW \times 0.14 = 11.2 mg/20g BW

= 560 mg/kg BW (mice)

Appendix 4. Dosage calculation for *Ocimum sanctum* leaf extract

$$\begin{aligned} \text{T1} &= 140 \text{ mg/kg BW} \\ &= 0.14 \text{ mg/g BW} \times 25 \text{ g} \times 6 \text{ mice} \times 24 \text{ days} \\ &= 504 \text{ mg/6 mice/24 days} \\ \text{T2} &= 280 \text{ mg/kg BW} \\ &= 0.28 \text{ mg/g BW} \times 25 \text{ g} \times 6 \text{ mice} \times 24 \text{ days} \\ &= 1008 \text{ mg/6 mice/24 days} \\ \text{T3} &= 560 \text{ mg/kg BW} \\ &= 0.56 \text{ mg/g BW} \times 25 \text{ g} \times 6 \text{ mice} \times 24 \text{ days} \\ &= 2016 \text{ mg/6 mice/24 days} \\ \text{Total} &= 504 + 1008 + 2016 \\ &= 3528 \text{ mg/18 mice/24 days} \end{aligned}$$

Appendix 5. Dosage calculation for lead acetate

The dosage of lead acetate administration for mice by per oral is 20 mg/kg BW. Lead acetate needed for this experiment is calculated below:

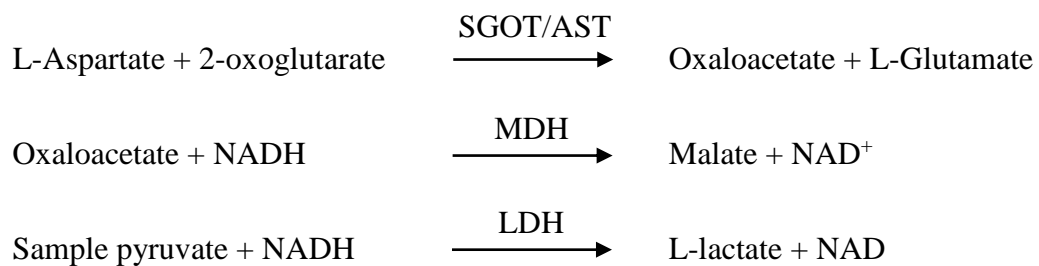
$$\begin{aligned} 20 \text{ mg/kg BW} &= 20 \text{ mg}/1000\text{g BW} \\ &= 0.02 \text{ mg/g BW/mice/day} \\ &= 0.02 \text{ mg} \times 25 \text{ g} \times 24 \text{ mice} \times 21 \text{ days} \\ &= 252 \text{ mg}/24 \text{ mice}/21 \text{ days} \end{aligned}$$

$$\begin{aligned} \text{Volume: } 0.01 \text{ ml/g BW} \times 25 \text{ g} \times 24 \text{ mice} \times 21 \text{ days} \\ = 126 \text{ ml}/24 \text{ mice}/21 \text{ days} \end{aligned}$$

Appendix 6. Procedure of SGOT measurement

Blood collection was done through the heart (intracardiac) with 1 ml syringe. The blood taken was stored in a plain vacuum tube without anticoagulants. Afterwards, the blood was centrifuged at a speed of 3000 rpm for 10 minutes to obtain the serum. The clear liquid above the clotted blood cells was taken with a micro pipette and putted into an Eppendorf tube. Then the SGOT level was measured using a reagent kit according to the photometric system method.

Principle:



SGOT/AST which present in the sample catalyses the transfer of the amino group from L-aspartate to 2-oxoglutarate forming oxaloacetate and L-glutamate. Oxaloacetate in the presence of NADH and Malate dehydrogenase (MDH) is reduced to L-malate. In this reaction NADH is oxidized to NAD. The reaction is monitored by measuring the rate of decrease in absorbance at 340 nm due to the oxidation of NADH to NAD. Addition of Lactate dehydrogenase (LDH) to the reagent is necessary to achieve rapid and complete reduction of endogenous pyruvate so that it does not interfere with the assay.

SGOT reagents consist of reagent 1: Tris Buffer (pH 7.8), L-Aspartate, Lactate Dehydrogenase (LDH), Malate Dehydrogenase (MDH) and reagent 2: CAPSO, 2-Oxoglutarate, Nicotinamide Adenine Dinucleotide (NADH). Reagent 1

(buffer) 1 ml was mixed with 0.1 ml sample and incubated it at 37°C for one minute. Afterwards, the mixture of reagent 1 and sample was mixed with reagent 2 (substrate) 0.25 ml and incubated again at 37°C for one minute. Then, the initial absorbance of the calibrator and sample was measured against reagent blank. Absorbance change was measured exactly after one, two, and three minutes. Measurement was done by using clinical chemical analyzer (ERBA Mannheim GmbH XL 600) with 340 nm wavelength. Absorbance change was calculated per one minute ($\Delta A/\text{min}$).

Calculation:

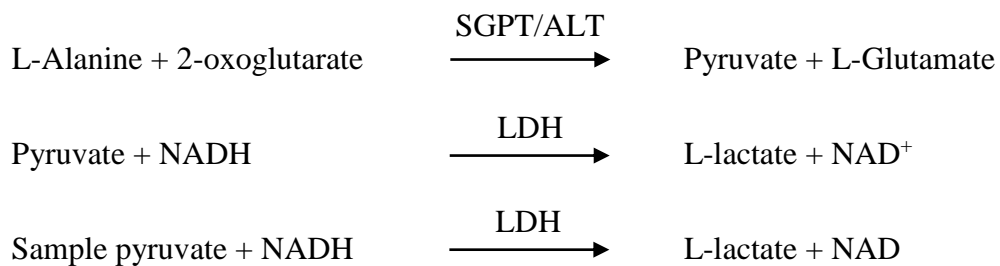
$$\text{SGOT/AST (U/L)} = \frac{\Delta A_{\text{sam}}/\text{min}}{\Delta A_{\text{cal}}/\text{min}} \times C_{\text{cal}}$$

C_{cal} = Calibrator concentration

Appendix 7. Procedure of SGPT measurement

Blood collection was done through the heart (intracardiac) with 1 ml syringe. The blood taken was stored in a plain vacuum tube without anticoagulants. Afterwards, the blood was centrifuged at a speed of 3000 rpm for 10 minutes to obtain the serum. The clear liquid above the clotted blood cells was taken with a micro pipette and putted into an Eppendorf tube. Then the SGPT level was measured using a reagent kit according to the photometric system method.

Principle:



The amino group is enzymatically transferred by SGPT/ALT which present in the sample from alanine to the carbon atom of 2-oxoglutarate yielding pyruvate and L-glutamate. Pyruvate is reduced to lactate by LDH present in the reagent with the simultaneous oxidation of NADH to NAD⁺. The reaction is monitored by measuring the rate of decrease in absorbance at 340 nm due the oxidation of NADH. Endogenous sample pyruvate is rapidly and completely reduced by LDH during initial incubation period to avoid interference during the assay

SGPT reagents consist of reagent 1: Tris Buffer (pH 7.5), L-Alanine, Lactate Dehydrogenase (LDH) microbial and reagent 2: CAPSO, 2-Oxoglutarate, Nicotinamide Adenine Dinucleotide (NADH). Reagent 1 (buffer) 1 ml was mixed with 0.1 ml sample and incubated it at 37°C for five minutes. Afterwards, mixed

with reagent 2 (substrate) 0.25 ml and incubated again at 37°C for one minute. Then, the initial absorbance of the calibrator and sample was measured against reagent blank. Absorbance change was measured exactly after one, two, and three minutes. Measurement was done by using clinical chemical analyzer (ERBA Mannheim GmbH XL 600) with 340 nm wavelength. Absorbance change was calculated per one minute ($\Delta A / \text{min}$).

Calculation:

$$\text{SGPT/ALT (U/L)} = \frac{\Delta A_{\text{sam/min}}}{\Delta A_{\text{cal/min}}} \times C_{\text{cal}}$$

C_{cal} = Calibrator concentration

Appendix 8. SGOT and SGPT test result**TEST RESULT**

No.	Groups	SGOT (U/L)	SGPT (U/L)
1	C(-)	106	71
2	C(-)	155	89
3	C(-)	154	100
4	C(-)	163	101
5	C(+)	157	123
6	C(+)	288	109
7	C(+)	214	137
8	C(+)	257	115
9	T1	211	144
10	T1	144	82
11	T1	204	139
12	T1	227	112
13	T2	154	100
14	T2	139	85
15	T2	180	94
16	T2	192	100
17	T3	180	128
18	T3	192	127
19	T3	190	105
20	T3	176	116

Appendix 9. Statistical analysis of SGOT level

Summarize

Case Summaries^a

			SGOT
Groups	C(-)	1	106
		2	155
		3	154
		4	163
	Total	N	4
		Sum	578
		Mean	144.50
		Std. Deviation	25.981
	C(+)	1	157
		2	288
		3	214
		4	257
	Total	N	4
		Sum	916
		Mean	229.00
		Std. Deviation	56.786
T1	1	211	
	2	144	
	3	204	
	4	227	
Total	N	4	
	Sum	786	
	Mean	196.50	
	Std. Deviation	36.300	
T2	1	154	
	2	139	

	3		180
	4		192
	Total	N	4
		Sum	665
		Mean	166.25
		Std. Deviation	24.116
T3	1		180
	2		192
	3		190
	4		176
	Total	N	4
		Sum	738
		Mean	184.50
		Std. Deviation	7.724
Total	N		20
		Sum	3683
		Mean	184.15
		Std. Deviation	42.179

a. Limited to first 100 cases

Tests of Normality

Groups	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
SGOT C(-)	.393	4	.	.764	4	.052
C(+)	.189	4	.	.976	4	.880
T1	.332	4	.	.857	4	.250
T2	.216	4	.	.952	4	.727
T3	.262	4	.	.895	4	.408

a. Lilliefors Significance Correction

Oneway**Descriptives**

SGOT

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
C(-)	4	144.50	25.981	12.990	103.16	185.84	106	163
C(+)	4	229.00	56.786	28.393	138.64	319.36	157	288
T1	4	196.50	36.300	18.150	138.74	254.26	144	227
T2	4	166.25	24.116	12.058	127.88	204.62	139	192
T3	4	184.50	7.724	3.862	172.21	196.79	176	192
Total	20	184.15	42.179	9.432	164.41	203.89	106	288

Test of Homogeneity of Variances

SGOT

Levene Statistic	df1	df2	Sig.
2.702	4	15	.071

ANOVA

SGOT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	16226.800	4	4056.700	3.462	.034
Within Groups	17575.750	15	1171.717		
Total	33802.550	19			

Post Hoc Tests**Homogeneous Subsets****SGOT**Duncan^a

Kelompok	N	Subset for alpha = 0.05	
		1	2
C(-)	4	144.50	
T2	4	166.25	
T3	4	184.50	184.50
T1	4	196.50	196.50
C(+)	4		229.00
Sig.		.065	.101

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Appendix 10. Statistical analysis of SGPT level

Summarize

Case Summaries^a

			SGPT	
Groups	C(-)	1	71	
		2	89	
		3	100	
		4	101	
		Total	N	4
			Sum	361
			Mean	90.25
			Std. Deviation	13.937
		C(+)	1	123
			2	109
			3	137
			4	115
			Total	N
			Sum	484
			Mean	121.00
			Std. Deviation	12.111
		T1	1	144
			2	82
			3	139
			4	112
	Total		N	4
		Sum	477	
		Mean	119.25	
		Std. Deviation	28.535	
	T2	1	100	
		2	85	

	3		94
	4		100
	Total	N	4
		Sum	379
		Mean	94.75
		Std. Deviation	7.089
T3	1		128
	2		127
	3		105
	4		116
	Total	N	4
		Sum	476
		Mean	119.00
		Std. Deviation	10.801
Total	N		20
		Sum	2177
		Mean	108.85
		Std. Deviation	19.974

a. Limited to first 100 cases.

Tests of Normality

Groups	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
SGPT C(-)	.258	4	.	.865	4	.277
C(+)	.190	4	.	.962	4	.792
T1	.256	4	.	.908	4	.473
T2	.271	4	.	.848	4	.220
T3	.271	4	.	.889	4	.377

a. Lilliefors Significance Correction

Oneway

Descriptives

SGPT

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
C(-)	4	90.25	13.937	6.969	68.07	112.43	71	101
C(+)	4	121.00	12.111	6.055	101.73	140.27	109	137
T1	4	119.25	28.535	14.268	73.84	164.66	82	144
T2	4	94.75	7.089	3.544	83.47	106.03	85	100
T3	4	119.00	10.801	5.401	101.81	136.19	105	128
Total	20	108.85	19.974	4.466	99.50	118.20	71	144

Test of Homogeneity of Variances

SGPT

Levene Statistic	df1	df2	Sig.
3.034	4	15	.051

ANOVA

SGPT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3614.300	4	903.575	3.417	.036
Within Groups	3966.250	15	264.417		
Total	7580.550	19			

Post Hoc Tests**Homogeneous Subsets****SGPT**Duncan^a

Kelompok	N	Subset for alpha = 0.05	
		1	2
C(-)	4	90.25	
T2	4	94.75	94.75
T3	4		119.00
T1	4		119.25
C(+)	4		121.00
Sig.		.701	.051

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Appendix 11. Research documentations



Ocimum sanctum leaves



Mice



Blood sample



Oral administration of *Ocimum sanctum* leaf extract



Ocimum sanctum simplicia preparation



Suspension of *Ocimum sanctum* leaf extract preparation



Necropsy and blood sampling of mice