

1. Ekstrak mengkudu kelompok X2 dapat menurunkan kadar albumin urin secara bermakna dibandingkan kelompok lain dan kelompok kontrol (+)..
2. Ekstrak mengkudu dapat menurunkan skor ekspresi VEGF jaringan ginjal hewan coba sesuai dosis kelompok X1, X2, X3 dan yang paling rendah adalah kelompok X4, dibandingkan dengan kelompok kontrol (+).
3. Penurunan ekspresi VEGF jaringan ginjal tidak berpengaruh terhadap kadar albumin dalam urin..

## 7.2.Saran

Perlu penelitian lanjutan untuk melengkapi konsep dalam pemikiran penelitian ini antara lain :

Pengaruh *Morinda citrifolia L* terhadap variasi lain seperti Nitrit oksida, TGF  $-\beta$ , dan ekspansi matriks mesangial laminin, kolagen, fibronectin pada ginjal diabetes nefropati, dan senyawa kimia apa yang terkandung dalam mengkudu yang paling berkhasiat terhadap penyakit lainnya.

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### **Lampiran 1:**

## **Prosedur Pengukuran Kadar Gula Darah Pada Hewan Coba**

1. Pemeriksaan kadar gula darah; masing-masing tikus dipuasakan selama 10-12 jam. Pemeriksaan kadar gula darah dilakukan melalui pengambilan cuplikan darah dari vena di ekor tikus dengan cara memotong sedikit ujung ekor. Darah yang pertama kali dibuang, tetesan darah berikutnya diperiksa dengan menggunakan alat POCT (*point of care test*) Super Glucocard II. Reagen carik celup yang telah ditetesi darah vena dimasukkan ke bagian alat pemeriksa, kemudian hasilnya dibaca pada layar dalam waktu kurang dari 30 detik. Nilai yang tertera pada layar adalah nilai konsentrasi gula darah dalam mg/dl.
2. Prinsip pemeriksaan kadar gula darah adalah berdasarkan reaksi oksidasi enzimatik yaitu sampel darah vena dalam reagen strip yang mengandung glucose oksidase (GOD) dan potassium ferrisianida. Gula darah di dalam reagen strip bereaksi dengan glukosa oksidase dan potassium ferisianida sehingga terbentuk potassium ferosianida. Banyaknya potassium ferosianida akan menghasilkan arus listrik yang dapat dideteksi oleh alat dan kemudian diubah menjadi angka yang ditampilkan pada layar.
3. Pemeriksaan kadar gula darah dilakukan sebelum penelitian dilakukan, setelah diinduksi Streptozotocin, untuk menentukan keberhasilan induksi STZ terhadap peningkatan kadar gula darah dalam proses hiperglikemi.

**Lampiran 2:**

**Prosedur Pemeriksaan MAU (Mikroalbuminuria);  
Diperiksa dengan Metode ELISA:**

1. MAU diperiksa dari urin 24 jam dari tiap tikus.
2. Albumin konsentrat diperiksa dengan metode ELISA (*enzyme linkeimmunosorbent assay*). Plate dengan 96-well (Nunc, Naperville, IL, USA) diprecoat dengan *sheep antirat albumin* (250mg/ml), diinkubasi 2 jam dengan larutan standar albumin tikus atau sampel urin tikus yang diencerkan
3. Standar atau sampel diencerkan 200µl dengan buffer reagen dan equilibrating 60 menit, kemudian ditambah *horseradish peroxidase-labeled antirat albumin* dan reaksi diproses 30 menit pada temperatur ruang.
4. Kemudian plate dicuci tiga kali dengan PBST (PBS berkisik 0.05% Tween-20), dan substrat larutan (dipreparasi dengan melarutkan O-phenilenediamin dalam etanol pada konsentrasi 10mg/ml, dilarutkan dengan 1:100 deionized water dan ditambah 0.01 ml dari 30% H<sub>2</sub>O<sub>2</sub> per 100ml solusion)
5. Inkubasi selama 3 jam. Kemudian setelah diberi stop reagen 4M, absorben dibaca pada 495 nm dengan ELISA reader.
6. *Sheep antirat albumin antibodies* dan standar diperoleh dari Cappel Laboratories (West Chester, PA, USA).

**Lampiran 3:**

**Prosedur Pemeriksaan VEGF diukur dari sampel darah  
imunohistokimia (Quantikine, USA). (Cha, 2004):**

1. Jaringan ginjal difiksasi segera pada 10% netral buffer formalin, *cast* dalam paraffin dan dibuat irisan 4- $\mu$ m dan diletakkan pada slide mikroskop. Setelah dihilangkan dan dehidrasi dalam xylene dan *graded* alkohol, slide dialiri air destilasi.
2. Irisan ginjal ditransfer ke 10mmol/l sitrat buffer untuk antigen *retrieve* pada pH 6.0 dan *microwave* 10 menit. Setelah dicuci air, 0.05% peroksid/etanol dituangkan selama 15 menit untuk memblok *endogenous peroksidase*.
3. Primer *antibody*, poliklonal *rabbit antirat* VEGF (Biogenex, san Ramon, Ca, USA) antibodi ditambahkan dengan perbandingan 1:20 untuk 2 jam pada suhu kamar.
4. Negatif kontrol diwarnai dengan kondisi yang sama dengan primer antibodi. LSB kit/HRP (DAKO, Carpiknteria, CA, USA), irisan ginjal sekuensial ditreat dengan *goat* serum, primer antibodi, *link* antibodi. *Streptvidin-biotin horseradish peroksidase* dan amino-etilkarbamilisol (*Chromogen*).
5. Irisan dikonterstain dengan *Mayer's hematoksilin*.
6. Untuk evaluasi pewarnaan VEGF, glomerulus di *grading* semikuantitatif, setiap skor menunjukkan perubahan sesuai dengan intensitas pewarnaan.
7. Tahap pertama . Menentukan persentase sklerosis pada tiap glomerulus, yaitu :

Skor 0 : sangat lemah atau tak ada pewarnaan dan tak ada peningkatan lokasi pewarnaan.

Skor 1 : diffus, pewarna lemah dengan 1-25% glomerulus fokal warna meningkat

Skor 2 : 25-50% glomerulus menunjukkan fokal dengan pewarnaan kuat

Skor 3 : 50-75% glomerulus terwarna kuat pada menurut fokal

Skor 4 : >75% glomerulus tercat kuat.

Sampel diperiksa dari evaluasi 60 glomerulus. Dan rata-rata skor dikalkulasi.

Setiap slide diskor oleh 2 pengamat /pembaca.

8. Tahap kedua. Menentukan indeks sklerosis (IS) dengan rumus :

$$IS = \left[ \frac{0 \times n_0 + 1 \times n_1 + 2 \times n_2 + 3 \times n_3 + 4 \times n_4}{60} \right] \times 100$$

Keterangan :

$n_0$  = Jumlah glomerulus dengan skor 0

$n_1$  = Jumlah glomerulus dengan skor 1

$n_2$  = Jumlah glomerulus dengan skor 2

$n_3$  = Jumlah glomerulus dengan skor 3

$n_4$  = Jumlah glomerulus dengan skor 4

#### **Lampiran 4:**

### **Preparasi *Morinda citrifolia L***



1. *Morinda citrifolia L* segar (tanpa biji dan *core*) diperoleh dari *Agriculture Park*, Jawa Tengah. Sampel dibersihkan dan dicuci dengan air mengalir sebelum dicacah menjadi bagian kecil.
2. Sampel *dioven-dried* menggunakan oven konvensional, Memmert UFB (Memmert GmbH Co.KG, Schabach, Germany pada 45°C; 48 jam.
3. Kemudian sampel dipulverisasi menjadi bubuk.
4. *Morinda citrifolia L* kemudian diekstrak menggunakan modifikasi Chang dkk. (1977)
5. 10g sampel diekstrak dengan etanol pada 24jam; 45°C.
6. Ekstrak difiltrasi dengan selulose membran Whatman filter paper dan ekstrak solven dibuang dengan alat *rotary evaporator* dibawah kedap udara.
7. Sampel dibuat baru setiap hari.

#### **Lampiran 5:**

**Konversi Perhitungan Dosis untuk Berbagai Jenis Hewan dan Manusia  
(Laurence & Bacharach, 1964)**

	Mencit 20g	Tikus 200g	Marmot 200g	Kelinci 1,5 kg	Kucing 2 kg	Kera 4 kg	Anjing 12 kg	Manusia 70 kg
Mencit 20 g	1,0	7,0	12,25	27,8	29,7	64,1	124,2	387,9
Tikus 200 g	0,14	1,0	1,74	3,9	4,2	9,2	17,8	56,0
Marmot 200 g	0,08	0,57	1,0	2,25	2,4	5,2	10,2	31,5
Kelinci 1,5 kg	0,04	0,25	0,4	1,0	1,08	2,4	4,5	14,2
Kucing 2 kg	0,03	0,23	0,41	0,92	1,0	2,2	4,1	13,0
Kera 4 kg	0,016	0,11	0,19	0,42	0,45	1,0	1,9	6,1
Anjing 12 kg	0,008	0,06	0,1	0,22	0,24	0,52	1,0	3,1
Manusia 70kg	0,0026	0,018	0,031	0,07	0,076	0,16	0,32	1,0

**Lampiran 6 : Uji statistik Kappa terhadap kalibrasi 2 pembaca**

Sebelum kedua pembaca melakukan pembacaan ekspresi VEGF pada preparat imunohistokimia, terlebih dahulu dilakukan kalibrasi untuk melihat kesesuaian hasil pembacaan dari kedua pembaca tersebut. Kalibrasi dilakukan dengan melakukan diagnosis pada jaringan ginjal tikus yang telah diinduksi

dengan STZ untuk memicu terjadinya diabetes nefropati jaringan ginjal dibuat preparat HE. Hasil diagnosis dari kedua pembaca dinyatakan sebagai positif apabila terdapat kerusakan pada jaringan ginjal.

**Hasil diagnosis :**

No	Diagnosis	
	Pembaca 1	Pembaca 2
1	+++	+++
2	+++	+++
3	+++	+++
4	+++	+++
5	+++	+++
6	+++	+++
7	++	++
8	+++	+++
9	++	++
10	++	++
11	+++	+++
12	+++	+++
13	++	++
14	++	++

15	++	++
16	+++	+++
17	+++	+++
18	++	++
19	++	++
20	+	+
21	++	++
22	++	++
23	+	+
24	++	++
25	+++	+++
26	+++	+++
27	+++	+++
28	+++	+++
29	+++	+++
30	+++	+++

**Kedua pembaca memperlihatkan tidak ada perbedaan hasil diagnosis, maka disimpulkan bahwa kedua pembaca memiliki kemampuan yang sama.**

**Lampiran 7 : Uji statistik Hasil analisis deskriptif**

**Means**

**Report**

Kelompok	BB awal	GD awal	vol.urin awal
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K (+)	Mean	193,350	85,17	19,5667
	Std. Deviation	5,9052	3,920	6,05629
	Median	195,850	85,50	18,4000
	Minimum	182,0	79	13,20
	Maximum	197,8	89	30,00
X1	Mean	245,733	92,33	22,4000
	Std. Deviation	3,8521	10,463	4,12602
	Median	245,950	88,00	24,0000
	Minimum	240,4	82	15,00
	Maximum	250,0	106	25,60
X2	Mean	232,433	84,00	22,2667
	Std. Deviation	1,5526	5,621	5,85104
	Median	233,150	83,00	21,7000
	Minimum	230,0	77	16,00
	Maximum	233,8	92	32,00
X3	Mean	213,217	83,83	17,8333
	Std. Deviation	10,1115	5,154	3,32846
	Median	212,850	84,50	16,4000
	Minimum	202,8	77	14,20
	Maximum	223,6	91	22,00
X4	Mean	217,317	86,17	20,6167
	Std. Deviation	5,3177	7,627	3,10897
	Median	218,000	85,50	20,7000
	Minimum	210,8	78	17,20
	Maximum	223,0	100	25,60
Total	Mean	220,410	86,30	20,5367
	Std. Deviation	18,9277	7,193	4,66731
	Median	222,600	85,00	20,4000
	Minimum	182,0	77	13,20
	Maximum	250,0	106	32,00

## Explore

### Descriptives

			Statistic	Std. Error
BB_awal	Mean		220,410	3,4557
	95% Confidence	Lower Bound	213,342	

	Interval for Mean	Upper Bound	227,478	
	5% Trimmed Mean		220,726	
	Median		222,600	
	Variance		358,260	
	Std. Deviation		18,9277	
	Minimum		182,0	
	Maximum		250,0	
	Range		68,0	
	Interquartile Range		30,0	
	Skewness		-,192	,427
	Kurtosis		-,938	,833
GD_awal	Mean		86,30	1,313
	95% Confidence Interval for Mean	Lower Bound	83,61	
		Upper Bound	88,99	
	5% Trimmed Mean		85,74	
	Median		85,00	
	Variance		51,734	
	Std. Deviation		7,193	
	Minimum		77	
	Maximum		106	
	Range		29	
	Interquartile Range		7	
	Skewness		1,342	,427
	Kurtosis		2,028	,833
vol.urin_awal	Mean		20,5367	,85213
	95% Confidence Interval for Mean	Lower Bound	18,7939	
		Upper Bound	22,2795	
	5% Trimmed Mean		20,3259	
	Median		20,4000	
	Variance		21,784	
	Std. Deviation		4,66731	
	Minimum		13,20	
	Maximum		32,00	
	Range		18,80	
	Interquartile Range		7,10	
	Skewness		,542	,427
	Kurtosis		-,097	,833

### Tests of Normality

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
BB_awal	,094	30	,200(*)	,962	30	,350
GD_awal	,187	30	,009	,874	30	,002
vol.urin_awal	,142	30	,124	,950	30	,170

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

a Lilliefors Significance Correction

## Oneway

### ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
BB_awal	Between Groups	9476,329	4	2369,082	64,857	,000
	Within Groups	913,198	25	36,528		
	Total	10389,527	29			
vol.urin_awal	Between Groups	88,321	4	22,080	1,016	,418
	Within Groups	543,408	25	21,736		
	Total	631,730	29			

## Kruskal-Wallis Test

### Ranks

Kelompok		N	Mean Rank
GD_awal	X1	6	16,83
	X2	6	10,58
	X3	6	10,58
	X4	6	12,00
	Total	24	

### Test Statistics(a,b)

GD_awal	
Chi-Square	3,190
Df	3
Asymp. Sig.	,363

a Kruskal Wallis Test

b Grouping Variable: Kelompok

**Means****Report**

Kelompok		BB_stz	GD_stz	vol.urin_stz	kadar albumin urin induksi stz
K (+)	Mean	182,133	498,00	19,5667	168,1667
	Std. Deviation	23,7343	60,511	6,05629	84,32418
	Median	175,550	488,50	18,4000	195,0000
	Minimum	160,0	415	13,20	62,00
	Maximum	217,9	600	30,00	250,00
X1	Mean	219,367	497,83	22,4000	2,5000
	Std. Deviation	38,2277	193,340	4,12602	2,73861
	Median	203,600	574,00	24,0000	2,5000
	Minimum	180,5	108	15,00	,00
	Maximum	269,6	600	25,60	5,00
X2	Mean	213,000	561,33	22,2667	5,0000
	Std. Deviation	23,5040	39,848	5,85104	,00000
	Median	223,050	563,00	21,7000	5,0000
	Minimum	173,4	495	16,00	5,00
	Maximum	233,0	600	32,00	5,00
X3	Mean	221,183	484,83	17,8333	29,3333
	Std. Deviation	47,8957	193,445	3,32846	16,58513
	Median	207,300	548,00	16,4000	29,5000
	Minimum	178,4	98	14,20	8,00
	Maximum	315,2	600	22,00	52,00
X4	Mean	206,183	500,17	20,6167	39,0000
	Std. Deviation	53,8576	194,124	3,10897	30,35787
	Median	196,650	600,00	20,7000	26,0000
	Minimum	149,8	115	17,20	18,00
	Maximum	309,0	600	25,60	99,00
Total	Mean	208,373	508,43	20,5367	48,8000
	Std. Deviation	39,3292	145,097	4,66731	72,93994
	Median	201,750	549,00	20,4000	21,0000
	Minimum	149,8	98	13,20	,00
	Maximum	315,2	600	32,00	250,00

**Explore****Descriptives**

	Statistic	Std. Error
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BB_stz	Mean		208,373	7,1805	
	95% Confidence Interval for Mean	Lower Bound	193,688		
		Upper Bound	223,059		
	5% Trimmed Mean		205,619		
	Median		201,750		
	Variance		1546,790		
	Std. Deviation		39,3292		
	Minimum		149,8		
	Maximum		315,2		
	Range		165,4		
	Interquartile Range		42,4		
	Skewness		1,252	,427	
	Kurtosis		1,735	,833	
	GD_stz	Mean		508,43	26,491
		95% Confidence Interval for Mean	Lower Bound	454,25	
Upper Bound			562,61		
5% Trimmed Mean			525,96		
Median			549,00		
Variance			21053,220		
Std. Deviation			145,097		
Minimum			98		
Maximum			600		
Range			502		
Interquartile Range			107		
Skewness			-2,238	,427	
Kurtosis			4,226	,833	
vol.urin_stz		Mean		20,5367	,85213
		95% Confidence Interval for Mean	Lower Bound	18,7939	
	Upper Bound		22,2795		
	5% Trimmed Mean		20,3259		
	Median		20,4000		
	Variance		21,784		
	Std. Deviation		4,66731		
	Minimum		13,20		
	Maximum		32,00		
	Range		18,80		
	Interquartile Range		7,10		
	Skewness		,542	,427	
	Kurtosis		-,097	,833	
	kadar albumin	Mean		48,8000	13,31695

urin induksi stz	95% Confidence Interval for Mean	Lower Bound	21,5638	
		Upper Bound	76,0362	
	5% Trimmed Mean		40,4815	
	Median		21,0000	
	Variance		5320,234	
	Std. Deviation		72,93994	
	Minimum		,00	
	Maximum		250,00	
	Range		250,00	
	Interquartile Range		49,50	
	Skewness		1,948	,427
	Kurtosis		2,680	,833

#### Tests of Normality

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
BB_stz	,148	30	,091	,894	30	,006
GD_stz	,286	30	,000	,632	30	,000
vol.urin_stz	,142	30	,124	,950	30	,170
kadar albumin	,270	30	,000	,662	30	,000
urin induksi stz	,270	30	,000	,662	30	,000

a. Lilliefors Significance Correction

#### Kruskal-Wallis Test

##### Ranks

	Kelompok	N	Mean Rank
BB_stz	X1	6	12,83

	X2	6	13,83
	X3	6	13,50
	X4	6	9,83
	Total	24	
GD_stz	X1	6	12,83
	X2	6	12,50
	X3	6	10,83
	X4	6	13,83
	Total	24	
kadar albumin urin induksi stz	X1	6	5,00
	X2	6	8,00
	X3	6	18,17
	X4	6	18,83
	Total	24	

#### Test Statistics(a,b)

	BB_stz	GD_stz	kadar albumin urin induksi stz
Chi-Square	1,200	,619	18,872
Df	3	3	3
Asymp. Sig.	,753	,892	,000

a Kruskal Wallis Test

b Grouping Variable: Kelompok

#### Oneway

#### ANOVA

vol.urin\_stz

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	88,321	4	22,080	1,016	,418
Within Groups	543,408	25	21,736		
Total	631,730	29			

#### Means

#### Report

Kelompok		BB akhir	berat ginjal	GD akhir	vol.urin akhir	Kadar albumin urin	Score VEGF
K (+)	Mean	191,683	2,0667	327,33	62,500	49,9050	2,5017
	Std. Deviation	26,7448	,19664	104,047	30,1247	17,04396	,04665
	Median	187,100	2,1000	300,50	57,500	52,9100	2,5200
	Minimum	158,4	1,80	203	30,0	30,07	2,43
	Maximum	227,3	2,30	475	105,0	67,30	2,55
X1	Mean	208,083	2,5500	417,33	92,333	26,9717	2,3683
	Std. Deviation	43,9261	,50892	79,957	6,6533	28,29910	,07026
	Median	202,100	2,4000	406,00	93,500	14,6500	2,3550
	Minimum	149,0	2,10	319	82,0	5,50	2,30
	Maximum	271,5	3,30	531	100,0	80,60	2,46
X2	Mean	198,417	2,2167	427,67	73,833	16,3100	2,2200
	Std. Deviation	20,8616	,09832	93,628	17,8372	5,60621	,06870
	Median	200,850	2,2000	405,00	72,000	14,2750	2,2200
	Minimum	158,4	2,10	344	55,0	10,60	2,13
	Maximum	215,2	2,40	589	97,0	25,61	2,33
X3	Mean	197,300	2,0667	462,83	62,167	38,9167	1,7583
	Std. Deviation	18,0692	,20656	48,996	30,1889	37,61595	,05419
	Median	193,250	2,1000	481,00	73,500	15,9600	1,7650
	Minimum	173,2	1,70	395	20,0	12,60	1,67
	Maximum	226,5	2,30	506	96,0	88,60	1,82
X4	Mean	186,667	2,2667	389,83	52,833	92,2200	1,6817
	Std. Deviation	20,3107	,15055	56,109	32,2578	56,08410	,06242
	Median	188,300	2,3000	387,00	46,500	96,8500	1,6800
	Minimum	152,0	2,00	330	25,0	13,21	1,60
	Maximum	209,8	2,40	457	113,0	158,09	1,75
Total	Mean	196,430	2,2333	405,00	68,733	44,8647	2,1060
	Std. Deviation	26,6815	,31110	86,961	27,3016	41,12588	,33882
	Median	195,150	2,2000	413,50	72,000	27,8400	2,2200
	Minimum	149,0	1,70	203	20,0	5,50	1,60
	Maximum	271,5	3,30	589	113,0	158,09	2,55

Explore

Descriptives

			Statistic	Std. Error
BB_akhir	Mean		196,430	4,8714
	95% Confidence Interval for Mean	Lower Bound	186,467	
		Upper Bound	206,393	
	5% Trimmed Mean		195,409	
	Median		195,150	
	Variance		711,904	
	Std. Deviation		26,6815	
	Minimum		149,0	
	Maximum		271,5	
	Range		122,5	
	Interquartile Range		35,5	
	Skewness		,495	,427
	Kurtosis		1,010	,833
	berat ginjal	Mean		2,2333
95% Confidence Interval for Mean		Lower Bound	2,1172	
		Upper Bound	2,3495	
5% Trimmed Mean			2,2074	
Median			2,2000	
Variance			,097	
Std. Deviation			,31110	
Minimum			1,70	
Maximum			3,30	
Range			1,60	
Interquartile Range			,20	
Skewness			1,728	,427
Kurtosis			4,812	,833
GD_akhir		Mean		405,00
	95% Confidence Interval for Mean	Lower Bound	372,53	
		Upper Bound	437,47	
	5% Trimmed Mean		406,06	
	Median		413,50	
	Variance		7562,276	
	Std. Deviation		86,961	
	Minimum		203	
	Maximum		589	
	Range		386	
	Interquartile Range		127	
	Skewness		-,196	,427
	Kurtosis		-,137	,833

vol.urin_akhir	Mean		68,733	4,9846
	95% Confidence Interval for Mean	Lower Bound	58,539	
		Upper Bound	78,928	
	5% Trimmed Mean		69,037	
	Median		72,000	
	Variance		745,375	
	Std. Deviation		27,3016	
	Minimum		20,0	
	Maximum		113,0	
	Range		93,0	
	Interquartile Range		45,5	
	Skewness		-,303	,427
	Kurtosis		-1,151	,833
	Kadar albumin urin	Mean		44,8647
95% Confidence Interval for Mean		Lower Bound	29,5080	
		Upper Bound	60,2213	
5% Trimmed Mean			40,9481	
Median			27,8400	
Variance			1691,338	
Std. Deviation			41,12588	
Minimum			5,50	
Maximum			158,09	
Range			152,59	
Interquartile Range			57,27	
Skewness			1,325	,427
Kurtosis			1,119	,833
Score VEGF		Mean		2,1060
	95% Confidence Interval for Mean	Lower Bound	1,9795	
		Upper Bound	2,2325	
	5% Trimmed Mean		2,1093	
	Median		2,2200	
	Variance		,115	
	Std. Deviation		,33882	
	Minimum		1,60	
	Maximum		2,55	
	Range		,95	
	Interquartile Range		,68	
	Skewness		-,210	,427
	Kurtosis		-1,683	,833

### Tests of Normality

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
BB_akhir	,087	30	,200(*)	,971	30	,567
berat ginjal	,215	30	,001	,833	30	,000
GD_akhir	,102	30	,200(*)	,986	30	,959
vol.urin_akhir	,126	30	,200(*)	,940	30	,092
Kadar albumin urin	,192	30	,007	,819	30	,000
Score VEGF	,201	30	,003	,865	30	,001

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

a Lilliefors Significance Correction

### Oneway

#### ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
BB_akhir	Between Groups	1550,145	4	387,536	,507	,731
	Within Groups	19095,058	25	763,802		
	Total	20645,203	29			
GD_akhir	Between Groups	61636,333	4	15409,083	2,443	,073
	Within Groups	157669,667	25	6306,787		
	Total	219306,000	29			
vol.urin_akhir	Between Groups	5506,533	4	1376,633	2,136	,106
	Within Groups	16109,333	25	644,373		
	Total	21615,867	29			

### Kruskal-Wallis Test

#### Ranks

	Kelompok	N	Mean Rank
berat ginjal	X1	6	15,50
	X2	6	12,33
	X3	6	7,42
	X4	6	14,75
	Total	24	

#### Test Statistics(a,b)

	berat ginjal
Chi-Square	4,976
Df	3
Asymp. Sig.	,174

a Kruskal Wallis Test

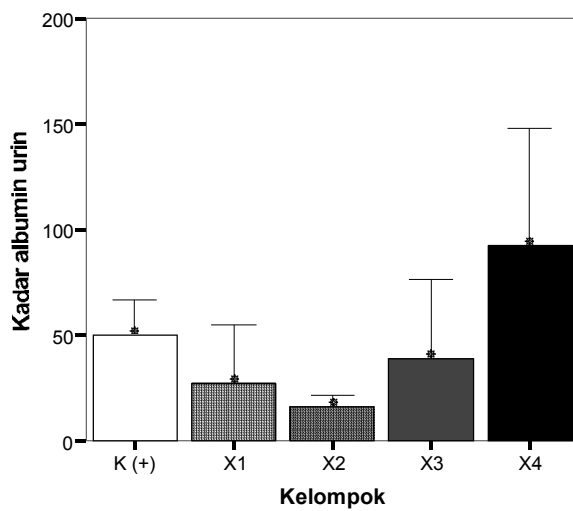
b Grouping Variable: Kelompok

### Means

#### Report

Kadar albumin urin

kelp_perlk	Mean	Std. Deviation	Median	Minimum	Maximum
MC_10mg/dL (X1)	26,9717	28,29910	14,6500	5,50	80,60
MC_20mg/dL (X2)	16,3100	5,60621	14,2750	10,60	25,61
MC_40mg/dL (X3)	38,9167	37,61595	15,9600	12,60	88,60
MC_80mg/dL (X4)	92,2200	56,08410	96,8500	13,21	158,09
STZ_noMC (K+)	49,9050	17,04396	52,9100	30,07	67,30
Total	44,8647	41,12588	27,8400	5,50	158,09



### Interactive Graph

#### Explore

#### Descriptives

	Statistic	Std. Error



Kadar albumin urin	Mean		44,8647	7,50852
	95% Confidence Interval for Mean	Lower Bound	29,5080	
		Upper Bound	60,2213	
	5% Trimmed Mean		40,9481	
	Median		27,8400	
	Variance		1691,338	
	Std. Deviation		41,12588	
	Minimum		5,50	
	Maximum		158,09	
	Range		152,59	
	Interquartile Range		57,27	
	Skewness		1,325	,427
	Kurtosis		1,119	,833

#### Tests of Normality

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Kadar albumin urin	,192	30	,007	,819	30	,000

a. Lilliefors Significance Correction

#### Kruskal-Wallis Test

##### Rank

	Kelompok	N	Mean Rank
Kadar albumin urin	MC_10mg/dL	6	10,42
	MC_20mg/dL	6	9,25
	MC_40mg/dL	6	15,00
	MC_80mg/dL	6	23,17
	STZ_noMC	6	19,67
	Total	30	

#### Test Statistics(a,b)

	Kadar albumin urin
Chi-Square	10,941
df	4
Asymp. Sig.	,027

a. Kruskal Wallis Test

b. Grouping Variable: Kelompok

#### Mann-Whitney Test

##### Ranks

	Kelompok	N	Mean Rank	Sum of Ranks
Kadar albumin urin	MC_10mg/dL	6	4,83	29,00
	STZ_noMC	6	8,17	49,00
	Total	12		

**Test Statistics(b)**

	Kadar albumin urin
Mann-Whitney U	8,000
Wilcoxon W	29,000
Z	-1,601
Asymp. Sig. (2-tailed)	,109
Exact Sig. [2*(1-tailed Sig.)]	,132(a)

a Not corrected for ties.

b Grouping Variable: Kelompok

**Mann-Whitney Test****Ranks**

	Kelompok	N	Mean Rank	Sum of Ranks
Kadar albumin urin	MC_20mg/dL	6	3,50	21,00
	STZ_noMC	6	9,50	57,00
	Total	12		

**Test Statistics(b)**

	Kadar albumin urin
Mann-Whitney U	,000
Wilcoxon W	21,000
Z	-2,882
Asymp. Sig. (2-tailed)	,004
Exact Sig. [2*(1-tailed Sig.)]	,002(a)

a Not corrected for ties.

b Grouping Variable: Kelompok

**Mann-Whitney Test****Ranks**

	Kelompok	N	Mean Rank	Sum of Ranks
Kadar albumin urin	MC_40mg/dL	6	5,50	33,00
	STZ_noMC	6	7,50	45,00
	Total	12		

#### Test Statistics(b)

	Kadar albumin urin
Mann-Whitney U	12,000
Wilcoxon W	33,000
Z	-,961
Asymp. Sig. (2-tailed)	,337
Exact Sig. [2*(1-tailed Sig.)]	,394(a)

a Not corrected for ties.

b Grouping Variable: Kelompok

### Mann-Whitney Test

#### Ranks

	Kelompok	N	Mean Rank	Sum of Ranks
Kadar albumin urin	MC_80mg/dL	6	8,00	48,00
	STZ_noMC	6	5,00	30,00
	Total	12		

#### Test Statistics(b)

	Kadar albumin urin
Mann-Whitney U	9,000
Wilcoxon W	30,000
Z	-1,441
Asymp. Sig. (2-tailed)	,150
Exact Sig. [2*(1-tailed Sig.)]	,180(a)

a Not corrected for ties.

b Grouping Variable: Kelompok

### Mann-Whitney Test

#### Ranks

	Kelompok	N	Mean Rank	Sum of Ranks
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Kadar albumin urin	MC_10mg/dL	6	6,42	38,50
	MC_20mg/dL	6	6,58	39,50
	Total	12		

**Test Statistics(b)**

	Kadar albumin urin
Mann-Whitney U	17,500
Wilcoxon W	38,500
Z	-,080
Asymp. Sig. (2-tailed)	,936
Exact Sig. [2*(1-tailed Sig.)]	,937(a)

a Not corrected for ties.

b Grouping Variable: Kelompok

**Mann-Whitney Test****Ranks**

	Kelompok	N	Mean Rank	Sum of Ranks
Kadar albumin urin	MC_10mg/dL	6	5,50	33,00
	MC_40mg/dL	6	7,50	45,00
	Total	12		

**Test Statistics(b)**

	Kadar albumin urin
Mann-Whitney U	12,000
Wilcoxon W	33,000
Z	-,961
Asymp. Sig. (2-tailed)	,337
Exact Sig. [2*(1-tailed Sig.)]	,394(a)

a Not corrected for ties.

b Grouping Variable: Kelompok

**Mann-Whitney Test****Ranks**

	Kelompok	N	Mean Rank	Sum of Ranks
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Kadar albumin urin	MC_10mg/dL	6	4,17	25,00
	MC_80mg/dL	6	8,83	53,00
	Total	12		

**Test Statistics(b)**

	Kadar albumin urin
Mann-Whitney U	4,000
Wilcoxon W	25,000
Z	-2,242
Asymp. Sig. (2-tailed)	,025
Exact Sig. [2*(1-tailed Sig.)]	,026(a)

a Not corrected for ties.

b Grouping Variable: Kelompok

**Mann-Whitney Test****Ranks**

	Kelompok	N	Mean Rank	Sum of Ranks
Kadar albumin urin	MC_20mg/dL	6	5,50	33,00
	MC_40mg/dL	6	7,50	45,00
	Total	12		

**Test Statistics(b)**

	Kadar albumin urin
Mann-Whitney U	12,000
Wilcoxon W	33,000
Z	-,961
Asymp. Sig. (2-tailed)	,337
Exact Sig. [2*(1-tailed Sig.)]	,394(a)

a Not corrected for ties.

b Grouping Variable: Kelompok

**Mann-Whitney Test****Ranks**

	Kelompok	N	Mean Rank	Sum of Ranks
--	----------	---	-----------	--------------

Kadar albumin urin	MC_20mg/dL	6	4,17	25,00
	MC_80mg/dL	6	8,83	53,00
	Total	12		

**Test Statistics(b)**

	Kadar albumin urin
Mann-Whitney U	4,000
Wilcoxon W	25,000
Z	-2,242
Asymp. Sig. (2-tailed)	,025
Exact Sig. [2*(1-tailed Sig.)]	,026(a)

a Not corrected for ties.

b Grouping Variable: Kelompok

**Mann-Whitney Test****Ranks**

	Kelompok	N	Mean Rank	Sum of Ranks
Kadar albumin urin	MC_40mg/dL	6	5,00	30,00
	MC_80mg/dL	6	8,00	48,00
	Total	12		

**Test Statistics(b)**

	Kadar albumin urin
Mann-Whitney U	9,000
Wilcoxon W	30,000
Z	-1,441
Asymp. Sig. (2-tailed)	,150
Exact Sig. [2*(1-tailed Sig.)]	,180(a)

a Not corrected for ties.

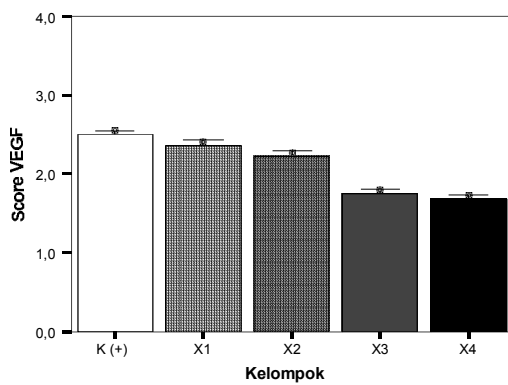
b Grouping Variable: Kelompok

**Means****Report**

Score VEGF

Kelompok	Mean	Std. Deviation	Median	Minimum	Maximum
----------	------	----------------	--------	---------	---------

MC_10mg/dL (X1)	2,3683	,07026	2,3550	2,30	2,46
MC_20mg/dL (X2)	2,2200	,06870	2,2200	2,13	2,33
MC_40mg/dL (X3)	1,7583	,05419	1,7650	1,67	1,82
MC_80mg/dL (X4)	1,6817	,06242	1,6800	1,60	1,75
STZ_noMC (K+)	2,5017	,04665	2,5200	2,43	2,55
Total	2,1060	,33882	2,2200	1,60	2,55



**Interactive Graph**  
**Explore**

**Descriptives**

			Statistic	Std. Error
Score VEGF	Mean		2,1060	,06186
	95% Confidence Interval for Mean	Lower Bound	1,9795	
		Upper Bound	2,2325	
	5% Trimmed Mean		2,1093	
	Median		2,2200	
	Variance		,115	
	Std. Deviation		,33882	
	Minimum		1,60	
	Maximum		2,55	
	Range		,95	
	Interquartile Range		,68	
	Skewness		-,210	,427
	Kurtosis		-1,683	,833

**Tests of Normality**

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.

Score VEGF	,201	30	,003	,865	30	,001
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a Lilliefors Significance Correction

### Kruskal-Wallis Test

#### Ranks

	Kelompok	N	Mean Rank
Score VEGF	MC_10mg/dL	6	21,33
	MC_20mg/dL	6	16,00
	MC_40mg/dL	6	8,50
	MC_80mg/dL	6	4,50
	STZ_noMC	6	27,17
	Total	30	

#### Test Statistics(a,b)

	Score VEGF
Chi-Square	26,406
df	4
Asymp. Sig.	,000

a Kruskal Wallis Test

b Grouping Variable: Kelompok

### Mann-Whitney Test

#### Ranks

	Kelompok	N	Mean Rank	Sum of Ranks
Score VEGF	MC_10mg/dL	6	3,83	23,00
	STZ_noMC	6	9,17	55,00
	Total	12		

#### Test Statistics(b)

	Score VEGF
Mann-Whitney U	2,000
Wilcoxon W	23,000
Z	-2,580
Asymp. Sig. (2-tailed)	,010
Exact Sig. [2*(1-tailed Sig.)]	,009(a)

a Not corrected for ties.

b Grouping Variable: Kelompok

### Mann-Whitney Test

#### Ranks

	Kelompok	N	Mean Rank	Sum of Ranks
--	----------	---	-----------	--------------



Score VEGF	MC_20mg/dL	6	3,50	21,00
	STZ_noMC	6	9,50	57,00
	Total	12		

#### Test Statistics(b)

	Score VEGF
Mann-Whitney U	,000
Wilcoxon W	21,000
Z	-2,892
Asymp. Sig. (2-tailed)	,004
Exact Sig. [2*(1-tailed Sig.)]	,002(a)

a Not corrected for ties.

b Grouping Variable: Kelompok

#### Mann-Whitney Test

##### Ranks

	Kelompok	N	Mean Rank	Sum of Ranks
Score VEGF	MC_40mg/dL	6	3,50	21,00
	STZ_noMC	6	9,50	57,00
	Total	12		

#### Test Statistics(b)

	Score VEGF
Mann-Whitney U	,000
Wilcoxon W	21,000
Z	-2,887
Asymp. Sig. (2-tailed)	,004
Exact Sig. [2*(1-tailed Sig.)]	,002(a)

a Not corrected for ties.

b Grouping Variable: Kelompok

#### Mann-Whitney Test

##### Ranks

	Kelompok	N	Mean Rank	Sum of Ranks
--	----------	---	-----------	--------------

Score VEGF	MC_80mg/dL	6	3,50	21,00
	STZ_noMC	6	9,50	57,00
	Total	12		

**Test Statistics(b)**

	Score VEGF
Mann-Whitney U	,000
Wilcoxon W	21,000
Z	-2,892
Asymp. Sig. (2-tailed)	,004
Exact Sig. [2*(1-tailed Sig.)]	,002(a)

a Not corrected for ties.

b Grouping Variable: Kelompok

**Mann-Whitney Test****Ranks**

	Kelompok	N	Mean Rank	Sum of Ranks
Score VEGF	MC_10mg/dL	6	9,00	54,00
	MC_20mg/dL	6	4,00	24,00
	Total	12		

**Test Statistics(b)**

	Score VEGF
Mann-Whitney U	3,000
Wilcoxon W	24,000
Z	-2,410
Asymp. Sig. (2-tailed)	,016
Exact Sig. [2*(1-tailed Sig.)]	,015(a)

a Not corrected for ties.

b Grouping Variable: Kelompok

**Mann-Whitney Test****Ranks**

	Kelompok	N	Mean Rank	Sum of Ranks
Score VEGF	MC_10mg/dL	6	9,50	57,00
	MC_40mg/dL	6	3,50	21,00
	Total	12		

**Test Statistics(b)**

	Score VEGF
Mann-Whitney U	,000

Wilcoxon W	21,000
Z	-2,887
Asymp. Sig. (2-tailed)	,004
Exact Sig. [2*(1-tailed Sig.)]	,002(a)

a Not corrected for ties.

b Grouping Variable: Kelompok

### Mann-Whitney Test

#### Ranks

	Kelompok	N	Mean Rank	Sum of Ranks
Score VEGF	MC_10mg/dL	6	9,50	57,00
	MC_80mg/dL	6	3,50	21,00
	Total	12		

#### Test Statistics(b)

	Score VEGF
Mann-Whitney U	,000
Wilcoxon W	21,000
Z	-2,892
Asymp. Sig. (2-tailed)	,004
Exact Sig. [2*(1-tailed Sig.)]	,002(a)

a Not corrected for ties.

b Grouping Variable: Kelompok

### Mann-Whitney Test

#### Ranks

	Kelompok	N	Mean Rank	Sum of Ranks
Score VEGF	MC_20mg/dL	6	9,50	57,00
	MC_40mg/dL	6	3,50	21,00
	Total	12		

#### Test Statistics(b)

	Score VEGF
Mann-Whitney U	,000

Wilcoxon W	21,000
Z	-2,887
Asymp. Sig. (2-tailed)	,004
Exact Sig. [2*(1-tailed Sig.)]	,002(a)

a Not corrected for ties.

b Grouping Variable: Kelompok

### Mann-Whitney Test

#### Ranks

	Kelompok	N	Mean Rank	Sum of Ranks
Score VEGF	MC_20mg/dL	6	9,50	57,00
	MC_80mg/dL	6	3,50	21,00
	Total	12		

#### Test Statistics(b)

	Score VEGF
Mann-Whitney U	,000
Wilcoxon W	21,000
Z	-2,892
Asymp. Sig. (2-tailed)	,004
Exact Sig. [2*(1-tailed Sig.)]	,002(a)

a Not corrected for ties.

b Grouping Variable: Kelompok

### Mann-Whitney Test

#### Ranks

	Kelompok	N	Mean Rank	Sum of Ranks
Score VEGF	MC_40mg/dL	6	8,50	51,00
	MC_80mg/dL	6	4,50	27,00
	Total	12		

#### Test Statistics(b)

	Score VEGF
Mann-Whitney U	6,000

Wilcoxon W	27,000
Z	-1,935
Asymp. Sig. (2-tailed)	,053
Exact Sig. [2*(1-tailed Sig.)]	,065(a)

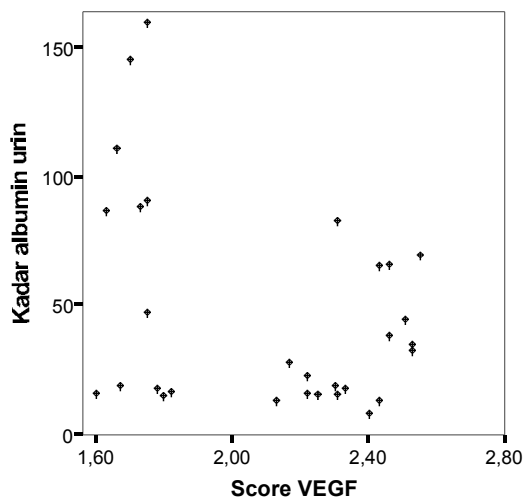
a Not corrected for ties.

b Grouping Variable: Kelompok

### Nonparametric Correlations

#### Correlations

		Kadar albumin urin	Score VEGF
Spearman's rho	Kadar albumin urin	Correlation Coefficient	1,000
		Sig. (2-tailed)	,189
		N	30
	Score VEGF	Correlation Coefficient	-,189
		Sig. (2-tailed)	,317
		N	30



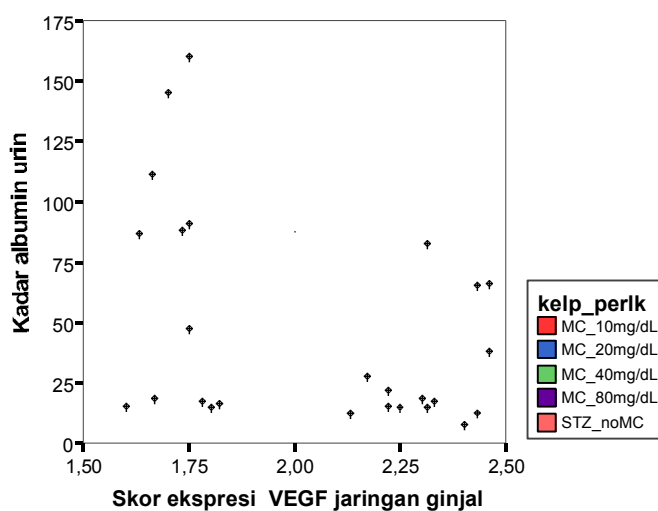
### Interactive Graph

### Nonparametric Correlations

#### Correlations

			Kadar albumin urin	Score VEGF
Spearman's rho	Kadar albumin urin	Correlation Coefficient	1,000	-,189
		Sig. (2-tailed)	.	,317
		N	30	30
	Score VEGF	Correlation Coefficient	-,189	1,000
		Sig. (2-tailed)	,317	.
		N	30	30

**Interactive Graph**



**Nonparametric Correlations: pada kelompok K(+), X1 dan X2**

**Correlations**

			Kadar albumin urin	Score VEGF
Spearman's rho	Kadar albumin urin	Correlation Coefficient	1,000	,509(*)
		Sig. (2-tailed)	.	,031
		N	18	18
	Score VEGF	Correlation Coefficient	,509(*)	1,000
		Sig. (2-tailed)	,031	.
		N	18	18

\* Correlation is significant at the 0.05 level (2-tailed).

**Nonparametric Correlations: pada kelompok X3 dan X4**

**Correlations**

			Kadar albumin urin	Score VEGF
Spearman's rho	Kadar albumin urin	Correlation Coefficient	1,000	-,246
		Sig. (2-tailed)	.	,440
		N	12	12
	Score VEGF	Correlation Coefficient	-,246	1,000
		Sig. (2-tailed)	,440	.
		N	12	12

	<b>KOMISI ETIK PENELITIAN KESEHATAN (KEPK)          FAKULTAS KEDOKTERAN UNIVERSITAS DIPONEGORO          DAN RSUP dr KARIADI SEMARANG</b> Sekretariat : Kantor Dekanat FK Undip Lt.3 Jl. Dr. Soetomo 18. Semarang Telp.024-8311523/Fax. 024-8446905	
<b>ETHICAL CLEARANCE</b> <b>No.100/EC/FK/RSDK/2010</b>		
Komisi Etik Penelitian Kesehatan Fakultas Kedokteran Universitas Diponegoro/ RSUP. Dr. Kariadi Semarang, setelah membaca dan menelaah Usulan Penelitian dengan judul :		
<b>PENGARUH PEMBERIAN MENGKUDU ( <i>Morinda citrifolia</i> L) TERHADAP          INDIKATOR VEGF DAN MIKROALBUMINURIA (MAU) DIABETES NEVROPATI          PADA TIKUS SPRAQUE DAWLEY YANG DIINDUKSI          STREPTOZOTOCIN (STZ)</b>		
Peneliti Utama	:	dr. Indranila KS, Sp.PK-K
Promotor	:	Prof.dr. Lisyani B Suromo,Sp.PK-K
Ko Promotor	:	1. Prof.Dr.dr.Tjahjono,Sp.PA(K)FIAC 2. Prof.Dr.dr.Suharyo HS, Sp.PD-KPTI
Anggota Peneliti	:	1. dra. Murnah, Apt 2. Arya Iswara, S.Si 3. Judiono,MPS 4. dr. Roy H, Sp.PD
Penelitian	:	Dilaksanakan di LPPU UGM Jogjakarta, Lab PA FK Undip/RSDK & Lab GAKY UNDIP Semarang
Setuju untuk dilaksanakan, dengan memperhatikan prinsip-prinsip yang dinyatakan dalam Deklarasi Helsinki 1975, dan Pedoman Nasional Etik Penelitian Kesehatan (PNEPK) Departemen Kesehatan RI 2004		
Pada laporan akhir peneliti harus melampirkan cara pemeliharaan & dekapitasi hewan coba		
Semarang, 25 Agustus 2010		
 dr. Soetomo, RAK, Sp KK(K) NIP. 19450802 197306 1 001	 Prof. Dr.dr. Tjahjono, Sp PA(K),FIAC NIP.19450514 1973081 001	Semarak, Ketua Komisi Etik Penelitian Kesehatan Fakultas Kedokteran Undip/RS. Dr. Kariadi

**Lampiran 9 : Surat Keterangan Standardisasi Pemeliharaan Hewan Coba dan Teknik Euthanasi, induksi STZ di LPPT-UGM**





**UNIVERSITAS GADJAH MADA**  
**LABORATORIUM PENELITIAN DAN PENGUJIAN TERPADU**  
**( LPPT – UGM )**  
**Bidang Layanan Penelitian Pra – Klinik dan Pengembangan Hewan Percobaan**  
**Jl. Agro Karang Malang Kampus UGM**

Telp. (0274) 7497705, FAX. ( 0274 ) 546868, e-mail: lppt\_info@mail.ugm.ac.id

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### SURAT KETERANGAN

#### **A. Standar Pemeliharaan Hewan Coba di Unit Pra-Klinik LPPT-UGM**

Secara umum, hewan dipelihara secara berkelompok di dalam kandang plastik atau kaca yang berukuran panjang 40 cm, lebar 25 cm, tinggi 17 cm. Setiap kandang dihuni oleh 6 ekor tikus.

Untuk hewan yang akan digunakan dalam suatu penelitian, diletakkan dalam kandang individu standar yang terbuat dari *stainless steel* berukuran panjang 20 cm, lebar 30 cm dan tinggi 17 cm. Pakan yang diberikan berupa pakan berbentuk pellet jenis AD II (Produksi PT. Japfa Comfeed Indonesia Tbk.) serta air minum *reverse osmosis* diberikan secara *ad libitum*. Kelembaban ruangan berkisar antara 70-75 % sedangkan temperatur berkisar antara 25-28°C. Penerimaan cahaya diatur 12 jam terang serta 12 jam gelap. Semua data tentang kondisi kandang dicatat dan dilakukan setiap hari.

Pemeriksaan terhadap parasit secara random dilakukan setiap 6 bulan sekali, sedangkan pemeriksaan terhadap beberapa jenis bakteri dilakukan 1 tahun sekali. Pemeriksaan terakhir, menunjukkan bahwa tikus dan mencit yang dipelihara di Unit Pra-klinik LPPT dinyatakan bebas dari parasit, dan bebas dari bakteri *Salmonella spp*, *Streptococcus pneumoniae*, *E coli* dan *Pasteurella spp*.

#### **B. Teknik Euthanasi**

Teknik etanasi yang akan digunakan adalah teknik anestesi overdosis dengan menggunakan ether (AVMA, 2000; Inglis 1980). Awalnya, tikus yang akan dikorbankan dimasukkan ke dalam stoples, kemudian ditutup rapat. Selanjutnya, 10 – 20 ml ether dituang ke dalam kapas dan dimasukkan ke dalam stoples yang telah dihuni tikus tersebut (hewan yang akan dikorbankan). Dua

sampai 5 menit kemudian, dilakukan pengamatan terhadap nafas, dan denyut jantung. Apabila tikus sudah tidak bernafas, tutup toples dibuka, hewan diletakkan di tempat nekropsi. Sebelum dilakukan pembedahan, dilakukan pengamatan kembali terhadap denyut jantung dan nafas untuk memastikan hewan telah benar-benar mati.

#### C. Cara Pemberian Perlakuan

Induksi hiperglikemik dikerjakan dengan melakukan injeksi streptozotocin dalam dosis 40 mg/kg BB secara intraperitoneal. Streptozotocin dilarutkan ke dalam buffer sitrat dengan PH 5,5/0,1 M

Yogyakarta, 07 April 2011

Kabid Pra - Klinik,

  
Dr. drh. Pudi Astuti, M. P

NIP : 19601012 198703 2 001

**Lampiran 10: Identifikasi *Morinda citrifolia* L**