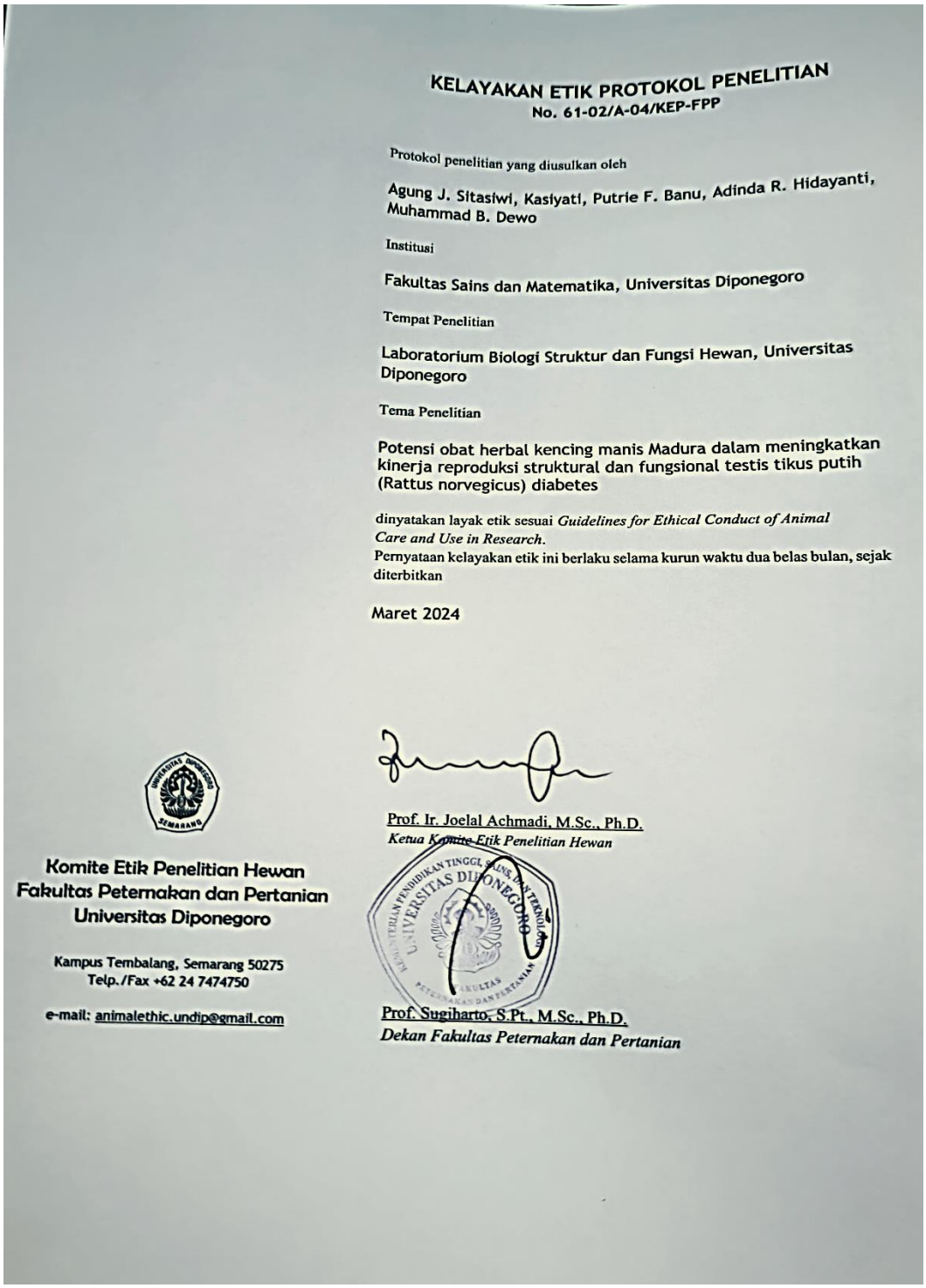
ATTACHMENT

Attachment 1. Ethical clearance



KELAYAKAN ETIK PROTOKOL PENELITIAN
No. 61-02/A-04/KEP-FPP

Protokol penelitian yang diusulkan oleh

Agung J. Sitasiwi, Kasiyati, Putrie F. Banu, Adinda R. Hidayanti, Muhammad B. Dewo

Institusi

Fakultas Sains dan Matematika, Universitas Diponegoro

Tempat Penelitian

Laboratorium Biologi Struktur dan Fungsi Hewan, Universitas Diponegoro

Tema Penelitian

Potensi obat herbal kencing manis Madura dalam meningkatkan kinerja reproduksi struktural dan fungsional testis tikus putih (Rattus norvegicus) diabetes

dinyatakan layak etik sesuai *Guidelines for Ethical Conduct of Animal Care and Use in Research.*
Pernyataan kelayakan etik ini berlaku selama kurun waktu dua belas bulan, sejak diterbitkan

Maret 2024

Prof. Ir. Joelal Achmadi, M.Sc., Ph.D.
Ketua Komite Etik Penelitian Hewan

Prof. Sugiharto, S.Pt., M.Sc., Ph.D.
Dekan Fakultas Peternakan dan Pertanian

Komite Etik Penelitian Hewan
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Universitas Diponegoro

Kampus Tembalang, Semarang 50275
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e-mail: animalethic.undip@gmail.com

Attachment 2. Research animal certificate

SEKOLAH TINGGI ILMU FARMASI YAYASAN PHARMASI SEMARANG

Jalan Letnan Jendral Sarwo Edie Wibowo Km. 1 Plamongansari - Pucanggading - Semarang - 50193
Telepon : 024 - 6706147 ; 6725272 ; Faksimile : 024 - 6706148
Email : stifar_yaphar@yahoo.com
Website : www.stifar.ac.id

SURAT KETERANGAN
Nomor : 001/YP-NE/SRT-HWN/V/2024

Yang bertandatangan di bawah ini :

Nama : Yuliana Purwaningsih, M.Sc.
Selaku : Kepala Pusat Laboratorium STIFAR Yayasan Pharmasi Semarang

Menerangkan bahwa :

Nama : Cindy Syaharani Ruriasri
NIM : 24020122420012
Institusi : Universitas Diponegoro
Judul peneltian : Potensi Jamu "Kencing Manis" Madura terhadap Struktur dan Fungsi Limpa Tikus Diabetes Melitus

Telah melakukan pembelian Tikus putih galur Wistar jenis kelamin jantan sebanyak 40 ekor dalam keadaan sehat dengan taksonomi sebagai berikut:

Kingdom : Animalia
Filum : Chordata
Class : Mamalia
Ordo : Rodentia
Famili : Muridae
Genus : Ratus
Species : Rattus norvegicus (Natawidjaya,1983)

Demikian surat keterangan ini dibuat untuk dapat digunakan seperlunya.

Semarang, 3 Mei 2024
Kepala Pusat Laboratorium STIFAR,

Yuliana purwaningsih, M.Sc.
NIY. 40217100

Attachment 3. Results of structural analysis reproductive organ of white rats (*Rattus norvegicus*) treated with *Madura kencing manis* herbal remedies.

3.1. Analysis of relative testicular weight data and gonadosomatic index in white rats

a. Test of normality

Tests of Normality

	Treatment	Kolmogorov-Smirnov[a] Statistic	df	Sig.	Shapiro-Wilk Statistic	df	Sig.
Relative Testicular Weight	P0	.174	5	.200*	.947	5	.714
	P1	.309	5	.133	.829	5	.137
	P2	.211	5	.200*	.953	5	.759
	P3	.199	5	.200*	.941	5	.673
	P4	.352	5	.042	.746	5	.027
	P5	.260	2	.			
Gonadosomatic Index	P0	.355	5	.039	.714	5	.013
	P1	.286	5	.200*	.872	5	.276
	P2	.270	5	.200*	.864	5	.241
	P3	.234	5	.200*	.939	5	.660
	P4	.242	5	.200*	.863	5	.241
	P5	.260	2	.			

*. This is a lower bound of the true significance.
a. Lilliefors Significance Correction

b. Test of homogeneity

Test of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
Relative Testicular Weight	Based on Mean	1.733	5	21	.171
	Based on Median	.690	5	21	.637
	Based on Median and with adjusted df	.690	5	11.355	.641
	Based on trimmed mean	1.646	5	21	.192
Gonadosomatic Index	Based on Mean	.808	5	21	.557
	Based on Median	.177	5	21	.968
	Based on Median and with adjusted df	.177	5	15.050	.967
	Based on trimmed mean	.766	5	21	.585

c. Test of Anova

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Relative Testicular Weight	Between Groups	.317	5	.063	1.736	.170
	Within Groups	.767	21	.037		
	Total	1.084	26			
Gonadosomatic Index	Between Groups	.144	5	.029	2.284	.083
	Within Groups	.265	21	.013		
	Total	.408	26			

3.2. Analysis of seminiferous tubules diameter data in rats treatment

a. Test of normality

Tests of Normality

	Treatment	Kolmogorov-Smirnov[a] Statistic	df	Sig.	Shapiro-Wilk Statistic	df	Sig.
TS1	P0	.140	5	.200*	.998	5	.998
	p1	.261	5	.200*	.882	5	.317
	P2	.380	5	.017	.778	5	.053
	P3	.271	5	.200*	.921	5	.534
	P4	.241	5	.200*	.902	5	.421

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

a. Lilliefors Significance Correction

b. Test of homogeneity

Test of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
TS1	Based on Mean	2.723	4	20	.059
	Based on Median	.934	4	20	.465
	Based on Median and with adjusted df	.934	4	5.609	.507
	Based on trimmed mean	2.225	4	20	.103

c. Test of Anova

ANOVA

TS1

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	4	.000	.548	.702
Within Groups	.000	20	.000		
Total	.000	24			

3.3. Analysis of spermatogenesis stages

a. Test of normality

Tests of Normality[c]

	Treatment	Kolmogorov-Smirnov[a] Statistic	df	Sig.	Shapiro-Wilk Statistic	df	Sig.
Spermatogenesis_changes	P0	.300	5	.161	.883	5	.325
	P1	.261	5	.200*	.859	5	.223
	P2	.197	5	.200*	.943	5	.685
	P3	.213	5	.200*	.963	5	.826
	P4	.179	5	.200*	.984	5	.955

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

a. Lilliefors Significance Correction

c. There are no valid cases for Spermatogenesis_changes when Treatment = 5.000. Statistics cannot be computed for this level.

b. Test of Homogenity

Test of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
Spermatogenesis_changes	Based on Mean	1.087	4	20	.389
	Based on Median	.742	4	20	.574
	Based on Median and with adjusted df	.742	4	16.471	.577
	Based on trimmed mean	1.048	4	20	.407

c. Test of ANOVA

ANOVA

Spermatogenesis_changes

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	14.000	4	3.500	.897	.484
Within Groups	78.000	20	3.900		
Total	92.000	24			

3.4. Analysis of seminiferous tubule damage percentage data

a. Test of normality

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Treatment	Statistic	df	Sig.	Statistic	df	Sig.
Percentage_Damage	P0	.298	5	.169	.825	5	.127
	P1	.344	5	.053	.791	5	.068
	P2	.363	5	.030	.758	5	.035
	P3	.270	5	.200*	.917	5	.513
	P4	.251	5	.200*	.862	5	.234
	P5	.260	2	.			

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

a. Lilliefors Significance Correction

b. Test of homogeneity

Test of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
Percentage_Damage	Based on Mean	.237	5	21	.942
	Based on Median	.071	5	21	.996
	Based on Median and with adjusted df	.071	5	17.372	.996
	Based on trimmed mean	.227	5	21	.947

c. Test of Anova

ANOVA

Percentage_Damage

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	23819.307	5	4763.861	19.305	.000
Within Groups	5182.100	21	246.767		
Total	29001.407	26			

d. Test of Duncan

Homogeneous Subsets

Percentage_Damage

	Treatment	N	Subset for alpha = 0.05		
			1	2	3
Duncan[a,b]	P0	5	14.80		
	P5	2	37.50		
	P1	5		62.00	
	P4	5		82.00	82.00
	P2	5			90.00
	P3	5			97.20
	Sig.		.054	.086	.209

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

3.5. Analysis average body weight of rats in initial condition, during treatment, and after treatment

Table L3.1. Average body weight of rats in initial condition, during treatment, and after treatment.

Treatment	initial condition ($\bar{x}$ ± SD)	condition during treatment ($\bar{x}$ ± SD)	condition after treatment ($\bar{x}$ ± SD)
P0	223.79 ± 31.28	273.92 ± 28.87	225.32 ± 37.48
P1	218.90 ± 27.10	276.61 ± 33.22	263.85 ± 30.12
P2	206.17 ± 12.39	191.57 ± 25.10	198.29 ± 35.25
P3	214.38 ± 23.95	227.81 ± 23.58	223.18 ± 38.63
P4	214 ± 18.61	241.02 ± 49.44	214.02 ± 17.31

3.6. Analysis results of blood glucose levels average in DM rats after being given *Madura kencing manis* herbal remedies

Table L3.2. Results of blood glucose levels day 0,3, and 7.

Treatment	TO ($\bar{x}$ ± SD)	T3 ($\bar{x}$ ± SD)	T7 ($\bar{x}$ ± SD)
P0	109 ± 21.989	109.2 ± 14.721	81 ± 27.613
P1	101.6 ± 6.656	394 ± 128.573	260.6 ± 128.179
P2	65.8 ± 24.386	403.2 ± 53.462	317 ± 174.356
P3	89.4 ± 16.861	366.4 ± 71.416	259.2 ± 140.389
P4	93.8 ± 8.526	439.4 ± 142.476	315.2 ± 121.627

Table L3.3. Blood glucose levels average day 10 and 20.

Treatment	T10 ($\bar{x}$ ± SD)	T20 ($\bar{x}$ ± SD)
P0	92.75 ± 29.781	112 ± 18.385
P1	311 ± 153.17	245 ± 174.846
P2	301.2 ± 132.984	156.5 ± 13.435
P3	311.8 ± 141.141	301.5 ± 160.791
P4	309.2 ± 143.646	239 ± 172.534

Attachment 4. Results of functional analysis of the testes of white rats (*Rattus norvegicus*) treated with *Madura kencing manis* herbal remedies.

4.1. Analysis SOD Level

a. Test of normality

One-Sample Kolmogorov-Smirnov Test

		Unstandardized Residual
N		26
Normal Parameters[a,b]	Mean	.0000000
	Std. Deviation	.27379439
Most Extreme Differences	Absolute	.134
	Positive	.134
	Negative	-.099
Test Statistic		.134
Asymp. Sig. (2-tailed)		.200[c,d]

a. Test distribution is Normal.
b. Calculated from data.
c. Lilliefors Significance Correction.
d. This is a lower bound of the true significance.

b. Test of homogenity

Tests of Normality

	Perlakuan	Kolmogorov-Smirnov[a] Statistic	df	Sig.	Shapiro-Wilk Statistic	df	Sig.
SOD	Kontrol uji	.251	5	.200*	.913	5	.487
	Metformin	.157	5	.200*	.980	5	.933
	Jamu madura 5 g	.347	5	.049	.803	5	.085
	Jamu madura 10 g	.228	5	.200*	.950	5	.736
	Jamu madura 15 g	.180	6	.200*	.937	6	.636

*. This is a lower bound of the true significance.
a. Lilliefors Significance Correction

c. Test of Anova

ANOVA

TS1

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	4	.000	.548	.702
Within Groups	.000	20	.000		
Total	.000	24			

d. The results of scoring the stages of spermatogenesis are based on the Johnsen criteria

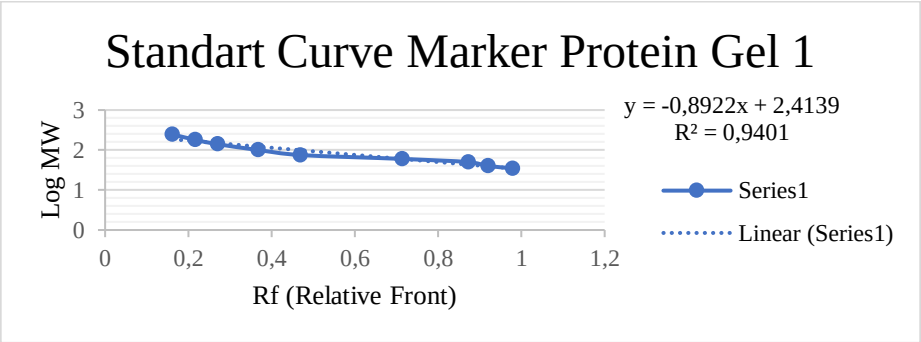
Table L4.1. The stages of spermatogenesis are based on the Johnsen criteria.

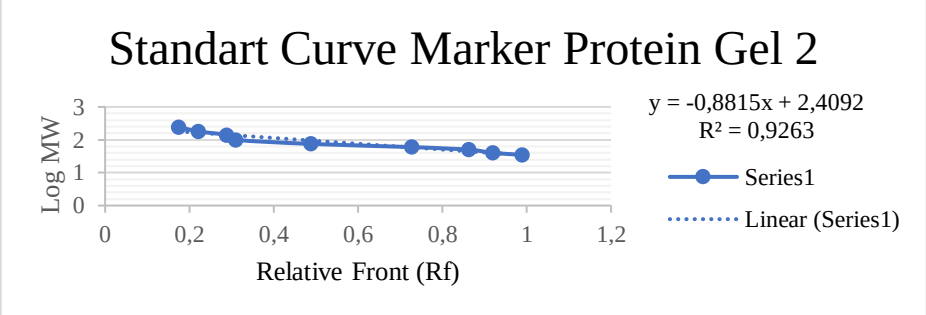
Scoring	Treatment Group				
	P0	P1	P2	P3	P4
10					
9	v				
8					
7		v	v	v	
6					v
5					
4					
3					
2					
1					

V:sample is detected

4.2. Analysis of protein molecular weight

a. Standart curve of protein marker gel 1 and gel 2





b. Log mw and rf values of protein marker gel 1 and gel 2

Table L4.2. Log mw and rf values of protein marker gel 1.

No	MW (kDa)	Log MW	Run (cm)	Band (cm)	Rf
1.	245	2,389166084	5,73	0,93	0,162304
2.	180	2,255272505	5,73	1,24	0,216405
3.	140	2,146128036	5,73	1,55	0,270506
4.	100	2	5,73	2,11	0,368237
5.	75	1,875061263	5,73	2,69	0,469459
6.	60	1,77815125	5,73	4,09	0,713787
7.	50	1,698970004	5,73	5	0,8726
8.	40	1,602059991	5,73	5,27	0,919721
9.	35	1,544068044	5,73	5,61	0,979058

Table L4.3. Log mw and rf values of protein marker gel 2.

No	MW (kDa)	Log MW	Run (cm)	Band (cm)	Rf
1.	245	2,389166	6	1,05	0,175
2.	180	2,255273	6	1,33	0,221667
3.	140	2,146128	6	1,73	0,288333
4.	100	2	6	1,86	0,31
5.	75	1,875061	6	2,93	0,488333
6.	60	1,778151	6	4,37	0,728333
7.	50	1,69897	6	5,18	0,863333
8.	40	1,60206	6	5,52	0,92
9.	35	1,544068	6	5,94	0,99

c. Stacking of protein molecular weight of testis

Table L4.4. Protein molecular weight of testis.

	Molecular Weight (kDa)											
No	GEL 1						GEL 2					
	M	P0	P1	P2	P3	P4	M(2)	P0	P1	P2	P3	P4
1	245	205,5	187,2	193,9	194,6	180,0	245	98,0	192,8	188,0	174,6	160,6
2	180	192,9	170,9	180,4	174,3	170,0	180	86,4	179,5	146,0	160,5	129,6
3	140	178,3	151,7	173,0	157,9	158,9	140	77,6	169,6	114,6	130,3	122,4
4	100	162,9	119,9	156,2	118,8	144,9	100	70,4	155,6	107,0	123,0	111,8
5	75	134,2	110,6	128,6	96,7	112,3	75	60,1	126,0	82,5	98,1	96,4
6	60	123,0	96,8	119,6	85,3	104,9	60	58,6	118,3	70,6	89,9	87,3
7	50	98,6	73,4	94,7	81,5	81,9	50		96,1	63,7	80,1	82,2
8	40	86,4	65,4	84,2	78,7	18,1	40		86,1	56,8	76,0	78,3
9	35	83,3	59,3	77,0	65,5	66,4	35		81,1	52,1	70,9	73,9
10		79,4	53,6	65,1	60,1	62,1			75,5	46,0	61,6	70,4
11		74,0	48,4	59,1	54,8	43,7			69,7		48,4	60,2
12		66,4	39,6		46,2				58,1			47,0
13		60,1			34,3				45,8			
14		56,7										
15		46,8										
16		36,3										

Attachment 5. Research documentation of the potential of *Madura kencing manis* herbal remedies in improving testicular structural and functional reproductive performance of diabetic white rats (*Rattus norvegicus*).

5.1. Documentation research of The Potential of *Madura kencing manis* herbal remedies in improving testicular structural and functional reproductive performance of diabetic White rats (*Rattus norvegicus*).

Figure L.5.1. Cage preparation.

Figure L.5.2. Herb infusion.

Figure L.5.3. Streptozotocin induction on white rat.

Figure L.5.4. Administration of *Madura kencing manis* HR.

Figure L.5.5. Blood glucose measurement.

Figure L.5.6. Water preparation

Figure L.5.7. Anaesthesia process of mice.

Figure L.5.8. Dissection of white test animals.

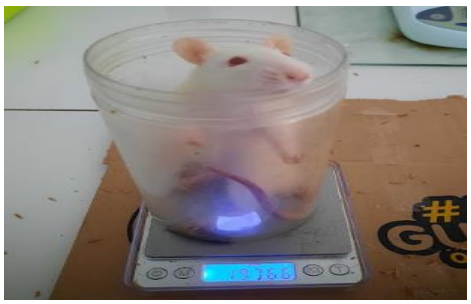


Figure L.5.9. Rat body weighting.

Figure L.5.10. Weighting testes

L.5.11. Testicular organs

Figure L.5.12. Insertion of organs into 10% BNF solutions.

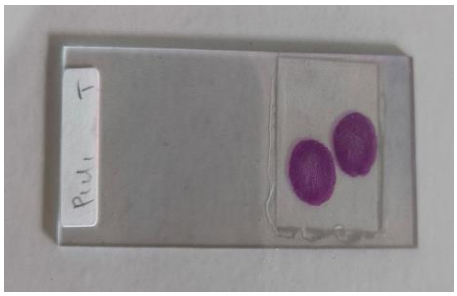


Figure L.5.13. Testicular histology preparate.

5.2. Documentation of procedure measurement molecular weight protein using SDS-PAGE

a. Procedural of protein extraction

Figure L.5.14. Protein sentrifugation.

Figure L.5.15. Protein extraction into gel acrylamide SDS – PAGE.