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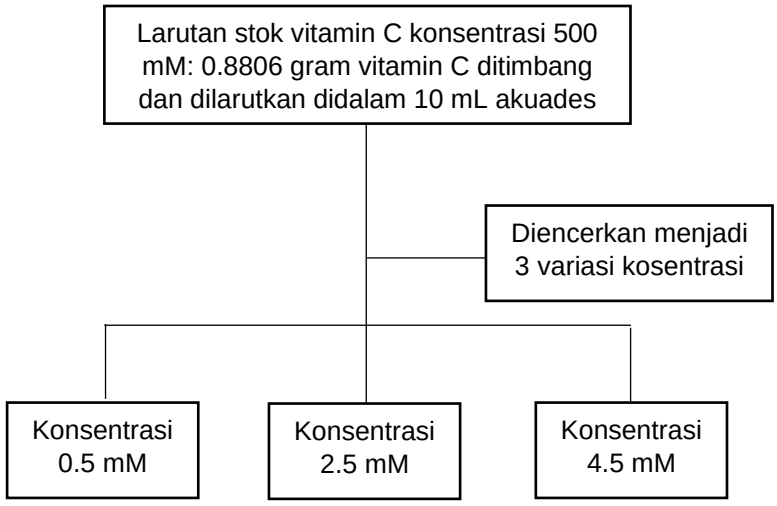
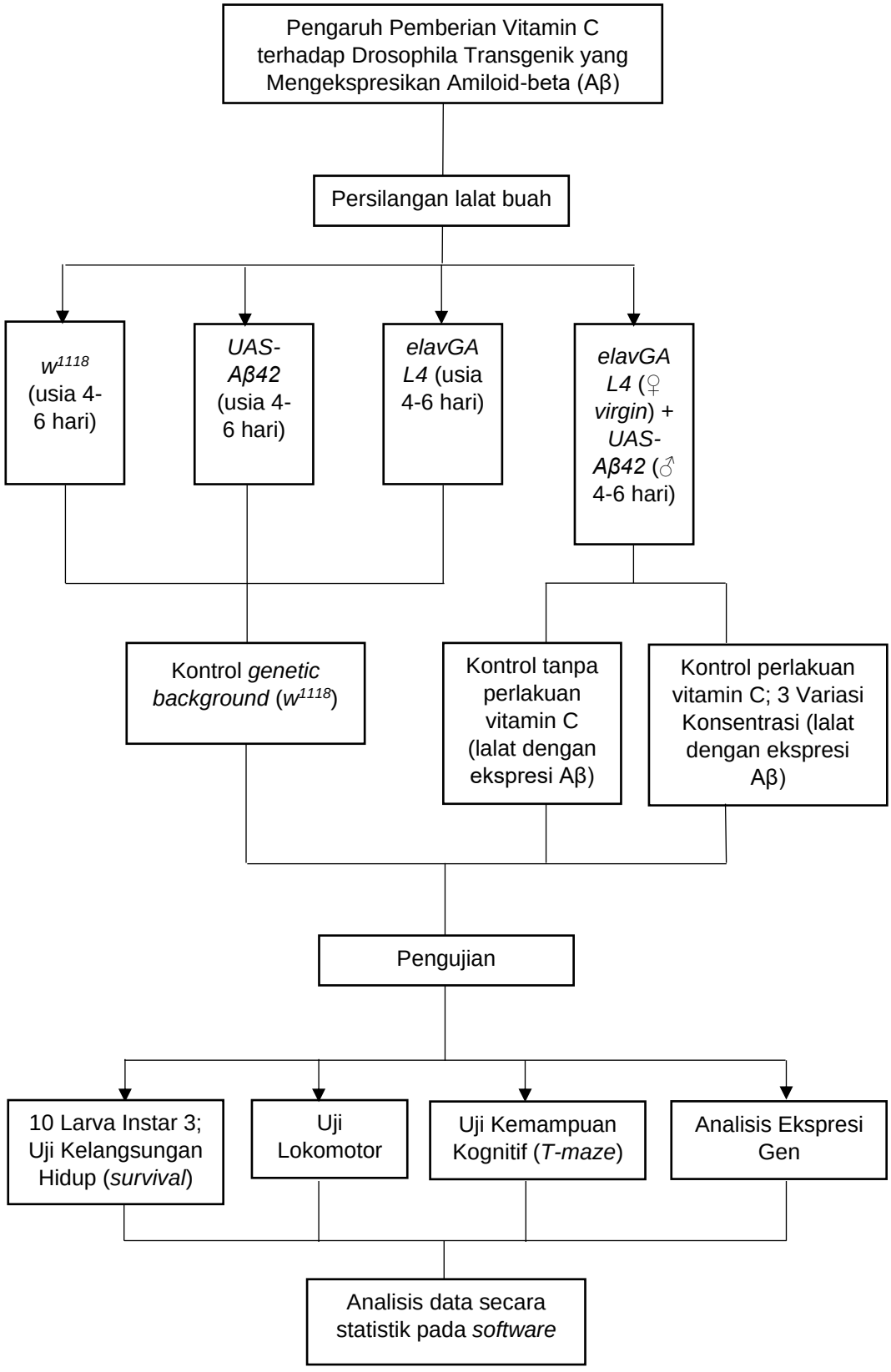
LAMPIRAN

Lampiran 1. Preparasi Sampel



Lampiran 2. Skema Perlakuan Uji dan Analisis Data



Lampiran 3. Perhitungan variasi konsentrasi vitamin C

a. Pembuatan larutan stok vitamin C 500 mM dalam 10 mL

M $= \frac{\text{massa (gram)}}{\text{mr}} \text{x} \frac{1000}{\text{v}}$

0.5 M $= \frac{\text{massa (gram)}}{171.12} \text{x} \frac{1000}{10}$

Massa (gram) =0.8806 gram

Jadi, untuk larutan stok Vitamin C 0.5 M dalam 10 mL akuades diperlukan 0.8806 gram vitamin C yang akan ditimbang. Untuk konsentrasi 0.5 M setara dengan 500 mM.

b. Pengenceran berdasarkan variasi konsentrasi dalam pakan perlakuan

Berikut adalah perhitungan untuk konsentrasi variasi vitamin C yang terkandung di dalam 25 mL pakan normal dengan persamaan:

$$V_1N_1 = V_2N_2$$

- **0.5 mM**

  500 mM x V_1 = 0.5 mM x 25 mL

  V_1 = 0,025 ml

  V_1 = 25 µL

- **2.5 mM**

  500 mM x V_1 = 2.5 mM x 25 mL

  V_1 = 0,125 ml

  V_1 = 125 µL

- **4.5 mM**

  500 mM x V_1 = 4.5 mM x 25 mL

  V_1 = 0,225 ml

  V_1 = 225 µL

Lampiran 4. Data Statistik

Tabel 2. *Log-rank* uji survival

GAL4/UAS vs Kontrol *Genetic Background* (W^{1118})	
Log-rank (Mantel-Cox) test	
Chi square	11,74
df	3
P value	0,0083
P value summary	**
Are the survival curves sig different?	Yes
Vit. C 0.5 mM vs GAL4/UAS	
Log-rank (Mantel-Cox) test	
Chi square	8,214
df	1
P value	0,0042
P value summary	**
Are the survival curves sig different?	Yes
Vit. C 2.5 mM vs GAL4/UAS	
Log-rank (Mantel-Cox) test	
Chi square	8,44
df	1
P value	0,0037
P value summary	**
Are the survival curves sig different?	Yes
Vit. C 4.5 mM vs GAL4/UAS	
Log-rank (Mantel-Cox) test	
Chi square	6,598
df	1
P value	0,0102
P value summary	*
Are the survival curves sig different?	Yes

Tabel 3. Hasil analisis uji lokomotor

Two-way ANOVA - Ordinary					
Alpha	0,05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	14,27	0,0023	**	Yes	
Row Factor	30,53	<0,0001	****	Yes	
Column Factor	27,02	<0,0001	****	Yes	
Tukey's multiple comparisons test	**Predicted (LS) mean diff,**	**95,00% CI of diff,**	**Below threshold?**	**Summary**	**Adjusted P Value**
Hari ke-1					
K+ vs. 0.5 mM	-8	-27,17 to 11,17	No	ns	0,6688
K+ vs. 2.5 mM	-4	-23,17 to 15,17	No	ns	0,9403
K+ vs. 4.5 mM	-8	-27,17 to 11,17	No	ns	0,6688
0.5 mM vs. 2.5 mM	4	-15,17 to 23,17	No	ns	0,9403
0.5 mM vs. 4.5 mM	0	-19,17 to 19,17	No	ns	>0,9999
2.5 mM vs. 4.5 mM	-4	-23,17 to 15,17	No	ns	0,9403
Hari ke-15					
K+ vs. 0.5 mM	-7,867	-27,60 to 11,86	No	ns	0,7109
K+ vs. 2.5 mM	-10,67	-30,40 to 9,064	No	ns	0,4778
K+ vs. 4.5 mM	-17,67	-37,40 to 2,064	No	ns	0,0935
0.5 mM vs. 2.5 mM	-2,8	-19,89 to 14,29	No	ns	0,9714
0.5 mM vs. 4.5 mM	-9,8	-26,89 to 7,287	No	ns	0,4261
2.5 mM vs. 4.5 mM	-7	-24,09 to 10,09	No	ns	0,6934
Hari ke-35					
K+ vs. 0.5 mM	-44,4	-66,54 to -22,26	Yes	****	<0,0001
K+ vs. 2.5 mM	-40,9	-61,23 to -20,57	Yes	****	<0,0001
K+ vs. 4.5 mM	-41,65	-61,98 to -21,32	Yes	****	<0,0001
0.5 mM vs. 2.5 mM	3,5	-19,65 to 26,65	No	ns	0,9758
0.5 mM vs. 4.5 mM	2,75	-20,40 to 25,90	No	ns	0,988
2.5 mM vs. 4.5 mM	-0,75	-22,18 to 20,68	No	ns	0,9997

Tabel 4. Hasil analisis uji peningkatan Kognitif (*T-maze*)

Two-way ANOVA - Ordinary					
Alpha	0,05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	14,77	0,0082	**	Yes	
Row Factor	14,62	<0,0001	****	Yes	
Column Factor	23,49	<0,0001	****	Yes	
Tukey's multiple comparisons test	**Predicted (LS) mean diff,**	**95,00% CI of diff,**	**Below threshold?**	**Summary**	**Adjusted P Value**
Hari Ke-1					
K+ vs. 0.5 mM	-1	-23,70 to 21,70	No	ns	0,9993
K+ vs. 2.5 mM	-5	-27,70 to 17,70	No	ns	0,9293
K+ vs. 4.5 mM	-9	-31,70 to 13,70	No	ns	0,6985
0.5 mM vs. 2.5 mM	-4	-25,41 to 17,41	No	ns	0,955
0.5 mM vs. 4.5 mM	-8	-29,41 to 13,41	No	ns	0,7349
2.5 mM vs. 4.5 mM	-4	-25,41 to 17,41	No	ns	0,955
Hari ke-15					
K+ vs. 0.5 mM	-18,4	-46,63 to 9,827	No	ns	0,3173
K+ vs. 2.5 mM	-20,6	-48,83 to 7,627	No	ns	0,2244
K+ vs. 4.5 mM	-23,2	-51,43 to 5,027	No	ns	0,1414
0.5 mM vs. 2.5 mM	-2,2	-30,43 to 26,03	No	ns	0,9968
0.5 mM vs. 4.5 mM	-4,8	-33,03 to 23,43	No	ns	0,9688
2.5 mM vs. 4.5 mM	-2,6	-30,83 to 25,63	No	ns	0,9948
Hari Ke-35					
K+ vs. 0.5 mM	-71,5	-100,8 to -42,19	Yes	****	<0,0001
K+ vs. 2.5 mM	-71,5	-100,8 to -42,19	Yes	****	<0,0001
K+ vs. 4.5 mM	-71,75	-101,1 to -42,44	Yes	****	<0,0001
0.5 mM vs. 2.5 mM	0	-23,93 to 23,93	No	ns	>0,9999
0.5 mM vs. 4.5 mM	-0,25	-24,18 to 23,68	No	ns	>0,9999
2.5 mM vs. 4.5 mM	-0,25	-24,18 to 23,68	No	ns	>0,9999

Tabel 5. Hasil Analisis Ekspresi Gen *Aβ42* pada Usia ke-15 vs ke-35

ANOVA summary					
F	212,4				
P value	0,0006				
P value summary	***				
Significant diff. among means (P < 0.05)?	Yes				
R squared	0,993				
Dunnett's multiple comparisons test	**Mean Diff,**	**95,00% CI of diff,**	**Below threshold?**	**Summary**	**Adjusted P Value**
15 vs. 35	-0,01175	-0,01429 to -0,009211	Yes	***	0,0006
Test details	**Mean 1**	**Mean 2**	**Mean Diff,**	**SE of diff,**	**n1**
15 vs. 35	0,0026	0,01435	-0,01175	0,000657	2

Tabel 6. Hasil Analisis Ekspresi Gen *Aβ42* pada *UAS*/*GAL4* vs *GAL4*, *UAS-Aβ42*, dan w^{1118} pada Hari Ke-35

ANOVA summary					
F	231,1				
P value	<0,0001				
P value summary	****				
Significant diff. among means (P < 0.05)?	Yes				
R squared	0,9943				
Tukey's multiple comparisons test	**Mean Diff,**	**95,00% CI of diff,**	**Below threshold?**	**Summary**	**Adjusted P Value**
W^{1118} vs. UAS-Aβ42	0,000999	-0,001480 to 0,003478	No	ns	0,4521
W^{1118} vs. GAL4	0,000995	-0,001484 to 0,003474	No	ns	0,4549
W^{1118} vs. GAL4/UAS	-0,0124	-0,01487 to -0,009916	Yes	***	0,0001
UAS-Aβ42 vs. GAL4	-4E-06	-0,002483 to 0,002475	No	ns	>0,9999
UAS-Aβ42 vs. GAL4/UAS	-0,01339	-0,01587 to -0,01091	Yes	***	0,0001
GAL4 vs. GAL4/UAS	-0,01339	-0,01587 to -0,01091	Yes	***	0,0001

Tabel 7. Hasil Analisis Ekspresi Gen *Aβ42* pada *UAS/GAL4* setelah Perlakuan Vitamin C pada Hari Ke-35

ANOVA summary					
F	44,81				
P value	0,0016				
P value summary	**				
Significant diff. among means (P < 0.05)?	Yes				
R squared	0,9711				
Dunnett's multiple comparisons test	**Mean Diff,**	**95,00% CI of diff,**	**Below threshold?**	**Summary**	**Adjusted P Value**
GAL4/UAS vs. VitC 0.5	-0,00165	-0,006811 to 0,003511	No	ns	0,573
GAL4/UAS vs. VitC 2.5	0	-0,005161 to 0,005161	No	ns	>0,9999
GAL4/UAS vs. VitC 4.5	0,01287	0,007704 to 0,01803	Yes	**	0,0019
VitC 4,5 Vs GAL4/UAS					
Unpaired t test					
P value	0,0037				
P value summary	**				
Significantly different (P < 0.05)?	Yes				
One- or two-tailed P value?	Two-tailed				
t, df	t=16,37, df=2				

Tabel 8. Hasil Analisis Ekspresi Gen *sod1* dan *sod2* pada *GAL4/UAS* pada Hari Ke-35

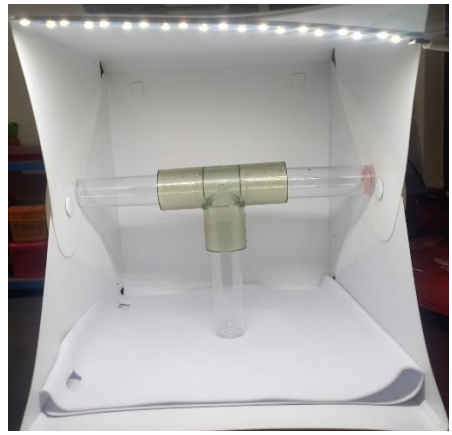
Gen *sod1*						
ANOVA summary						
F	3,153					
P value	0,1482					
P value summary	ns					
Significant diff. among means (P < 0.05)?	No					
R squared	0,7028					
Dunnett's multiple comparisons test	**Mean Diff,**	**95,00% CI of diff,**	**Below threshold?**	**Summary**	**Adjusted P Value**	**A-?**
GAL4/UAS vs. VitC 0.5	-2,37	-6,594 to 1,854	No	ns	0,2337	B
GAL4/UAS vs. VitC 2.5	-2,25	-6,474 to 1,974	No	ns	0,2604	C
GAL4/UAS vs. VitC 4.5	-3,5	-7,724 to 0,7238	No	ns	0,0879	D
Gen *sod2*						
ANOVA summary						
F	1,214					
P value	0,4128					
P value summary	ns					
Significant diff. among means (P < 0.05)?	No					
R squared	0,4765					
Dunnett's multiple comparisons test	**Mean Diff,**	**95,00% CI of diff,**	**Below threshold?**	**Summary**	**Adjusted P Value**	**A-?**
GAL4/UAS vs. VitC 0.5	0,305	-0,8724 to 1,482	No	ns	0,6955	B
GAL4/UAS vs. VitC 2.5	0,4	-0,7774 to 1,577	No	ns	0,5349	C
GAL4/UAS vs. VitC 4.5	-0,14	-1,317 to 1,037	No	ns	0,9462	D

Lampiran 5. Dokumentasi Penelitian

Gambar 17. Pembuatan pakan lalat normal dan pakan vitamin C

Gambar 18. Penyiapan hewan uji

Gambar 19. Uji Lokomotor



Gambar 20. Uji peningkatan kognitif (*T-maze*)

Gambar 21. Isolasi RNA

Gambar 22. Proses analisis dengan instrument RT-qPCR