

Appendix 1

A. Procedure for preparing histopathology slides.

The liver removed and stored immediately in buffered formalin 10 % for histopathological examination. The tissue fixed for at least 48 hours in buffered formalin 10%, the tissue then cut and put in cassette and this process done next :

1. Improvement fixation: formalin 10% 0-3 hour
2. Dehydration : alcohol 70% 30 minute
 : Alcohol 96% 30 minute
 : Alcohol 100% 30 minute
 : Alcohol 100% 1 hour
 : Alcohol 100% 1 hour
 : Alcohol 100% 1 hour
 : Alcohol 100% /xylol 30minute
3. Clearing with : Xylol 1 hour
 : Xylol 2 hour
4. Impregnation (tem <math><60^0\text{c}</math>)
 : paraffin liquid 2.5 hour
 : paraffin liquid 4 hour
5. embedding in block paraffin and give label
6. Cutting the block using microtome with diameter 3-4 micron

7. Put the tissue in floating bath and stick it to the slide that already covered with albumin-glycerin (1:1) and then give the name that already in the block related.

B. Procedure for H&E staining, Harris method.

1. Deparaffinize sections, 2 changes of xylene, and 10 minutes each.
2. Re-hydrate in 2 changes of absolute alcohol, 5 minutes each.
3. 95% alcohol for 2 minutes and 70% alcohol for 2 minutes.
4. Wash briefly in distilled water.
5. Stain in Harris hematoxylin solution for 8 minutes.
6. Wash in running tap water for 5 minutes.
7. Differentiate in 1% acid alcohol for 30 seconds.
8. Wash running tap water for 1 minute.
9. Bluing in 0.2% ammonia water or saturated lithium carbonate solution for 30 seconds to 1 minute.
10. Wash in running tap water for 5 minutes.
11. Rinse in 95% alcohol, 10 dips.
12. Counter stain in eosin-phloxine B solution (or eosin Y solution) for 30 seconds to 1 minute.
13. Dehydrate through 95% alcohol, 2 changes of absolute alcohol, and 5 minutes each.
14. Clear in 2 changes of xylene, 5 minutes each.
15. Mount with xylene based mounting medium

C. Procedure for immunohistochemistry staining.

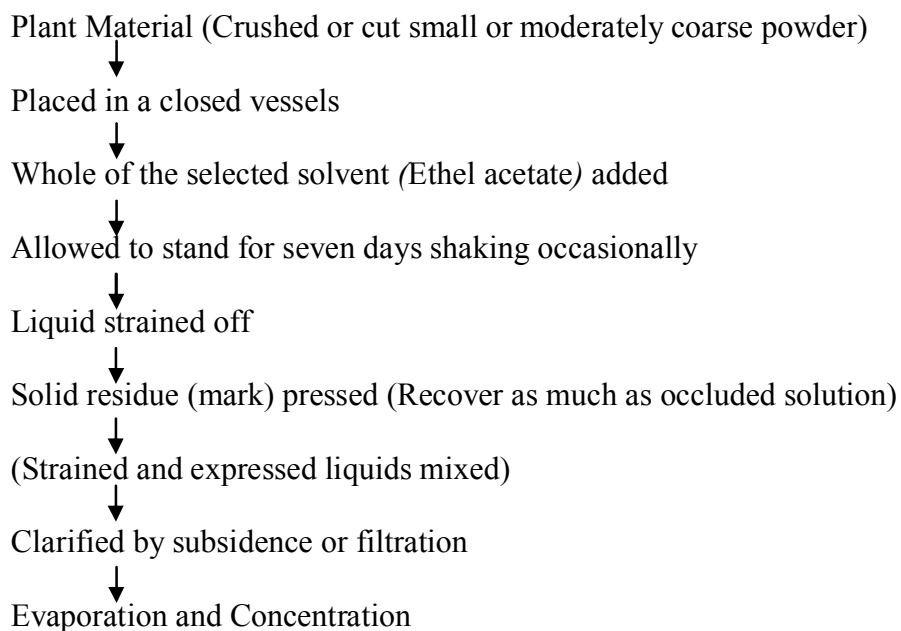
1. Fix tissue sections in formalin 10% and embed in paraffin blocks according to standard procedures.
2. Clean glass slides with 95% ethanol; treat with subbing solution and air dry. Or use pre-treated slides.
3. Cut 4–6 micron thick tissue sections, and apply to slides.
Deparaffinize in xylenes using three changes for 5 minutes each.
Hydrate sections gradually through graded alcohols: wash in 100% ethanol twice for 10 minutes each, then 95% ethanol twice for 10 minutes each. Wash in demonized H₂O for 1 minute with stirring.
Aspirate excess liquid from slides
4.
 - Antigen unmasking may be performed at this point. Certain antigenic determinants are masked by formalin fixation and paraffin embedding and may be exposed by Pepsin: Incubate sections for 10–20 minutes in 0.1% pepsin in 0.01 N HCl at room temperature. Wash slides several times in deionized H₂O. Aspirate excess liquid
5. from slides.
 - For immunoperoxidase staining of tissue sections, will use, ABC Staining Systems ,The ABC Staining Systems utilize preformed avidin-biotinylated horseradish peroxidase complex as a detection reagent.⁵²

c. Allred score.

Table: Allred Score⁵⁰

Proportion Score (PS)		Intensity Score (IS)	
Value	Significance	Value	Significance
0	none	0	none
1	<1%	1	weak
2	1- 10%	2	intermediate
3	10- 33%	3	strong
4	33- 66%		
5	> 66%		

D.Maceration process of Extraction Nigella Sativa seeds



Appendix 2

groups	First pathologist result		Second pathologist result	
groups of rats	liver tissue damage	TNF alpha expression	liver tissue damage b	TNF alpha expression b
control group	severe degree	3 allred (7,8)	severe degree	3 allred (7,8)
control group	severe degree	3 allred (7,8)	severe degree	3 allred (7,8)
control group	moderate degree	3 allred (7,8)	moderate degree	3 allred (7,8)
control group	severe degree	3 allred (7,8)	severe degree	3 allred (7,8)
control group	severe degree	2 allred (5,6)	moderate degree	2 allred (5,6)
control group	severe degree	3 allred (7,8)	severe degree	3 allred (7,8)
group 1	moderate degree	2 allred (5,6)	moderate degree	2 allred (5,6)
group 1	moderate degree	2 allred (5,6)	moderate degree	3 allred (7,8)
group 1	moderate degree	2 allred (5,6)	moderate degree	2 allred (5,6)
group 1	moderate degree	2 allred (5,6)	moderate degree	2 allred (5,6)

	degree		degree	
group 1	severe degree	3 allred (7,8)	severe degree	3 allred (7,8)
group 1	severe degree	2 allred (5,6)	moderate degree	2 allred (5,6)
group 2	mild degree	1 allred (2,3,4)	mild degree	1 allred (2,3,4)
group 2	moderate degree	1 allred (2,3,4)	mild degree	1 allred (2,3,4)
group 2	mild degree	2 allred (5,6)	mild degree	2 allred (5,6)
group 2	mild degree	1 allred (2,3,4)	mild degree	1 allred (2,3,4)
group 2	mild degree	2 allred (5,6)	mild degree	2 allred (5,6)
group 2	mild degree	1 allred (2,3,4)	mild degree	1 allred (2,3,4)
group 3	mild degree	0 allred (0 ascore)	normal	0 allred (0 ascore)
group 3	mild degree	0 allred (0 ascore)	mild degree	0 allred (0 ascore)
group 3	normal	1 allred (2,3,4)	normal	1 allred (2,3,4)
group 3	normal	0 allred (0 ascore)	normal	0 allred (0 ascore)
group 3	normal	0 allred (0 ascore)	normal	0 allred (0 ascore)
group 3	mild degree	0 allred (0 ascore)	mild degree	0 allred (0 ascore)

Appendix 3

groups of rats

Case Processing Summary

groups of rats	Cases		
	Valid		Missing
	N	Percent	N
liver tissue damage control group	6	100.0%	0
group 1	6	100.0%	0
group 2	6	100.0%	0
group 3	6	100.0%	0

Case Processing Summary

groups of rats	Cases		
	Missing	Total	
	Percent	N	Percent

liver tissue damage control group	.0%	6	100.0%
group 1	.0%	6	100.0%
group 2	.0%	6	100.0%
group 3	.0%	6	100.0%

Descriptives

groups of rats			Statistic	Std. Error
liver tissue damage	control group	Mean	2.83	.167
		95% Confidence Interval for Mean		
		Lower Bound	2.40	
		Upper Bound	3.26	
		5% Trimmed Mean	2.87	

	Median	3.00	
	Variance	.167	
	Std. Deviation	.408	
	Minimum	2	
	Maximum	3	
	Range	1	
	Interquartile Range	0	
	Skewness	-2.449	.845
	Kurtosis	6.000	1.741
group 1	Mean	2.33	.211
	95% Confidence Interval for Mean	Lower Bound	1.79
		Upper Bound	2.88

	5%	2.31	
	Trimmed		
	Mean		
	Median	2.00	
	Variance	.267	
	Std.	.516	
	Deviation		
	Minimum	2	
	Maximum	3	
	Range	1	
	Interquartil	1	
	e Range		
	Skewness	.968	.845
	Kurtosis	-1.875	1.741
group 2	Mean	1.17	.167

	95% Confidence	Lower	.74	
	Interval for Mean	Bound		
		Upper	1.60	
		Bound		
		5%	1.13	
		Trimmed		
		Mean		
		Median	1.00	
		Variance	.167	
		Std.	.408	
		Deviation		
		Minimum	1	
		Maximum	2	
		Range	1	
		Interquartil e Range	0	
	Skewness	2.449	.845	

	Kurtosis	6.000	1.741
group 3	Mean	.50	.224
95% Confidence Interval for Mean	Lower Bound	-.07	
	Upper Bound	1.07	
	5% Trimmed Mean	.50	
	Median	.50	
	Variance	.300	
	Std. Deviation	.548	
	Minimum	0	
	Maximum	1	
	Range	1	

	Interquartile Range	1	
	Skewness	.000	.845
	Kurtosis	-3.333	1.741

Tests of Normality

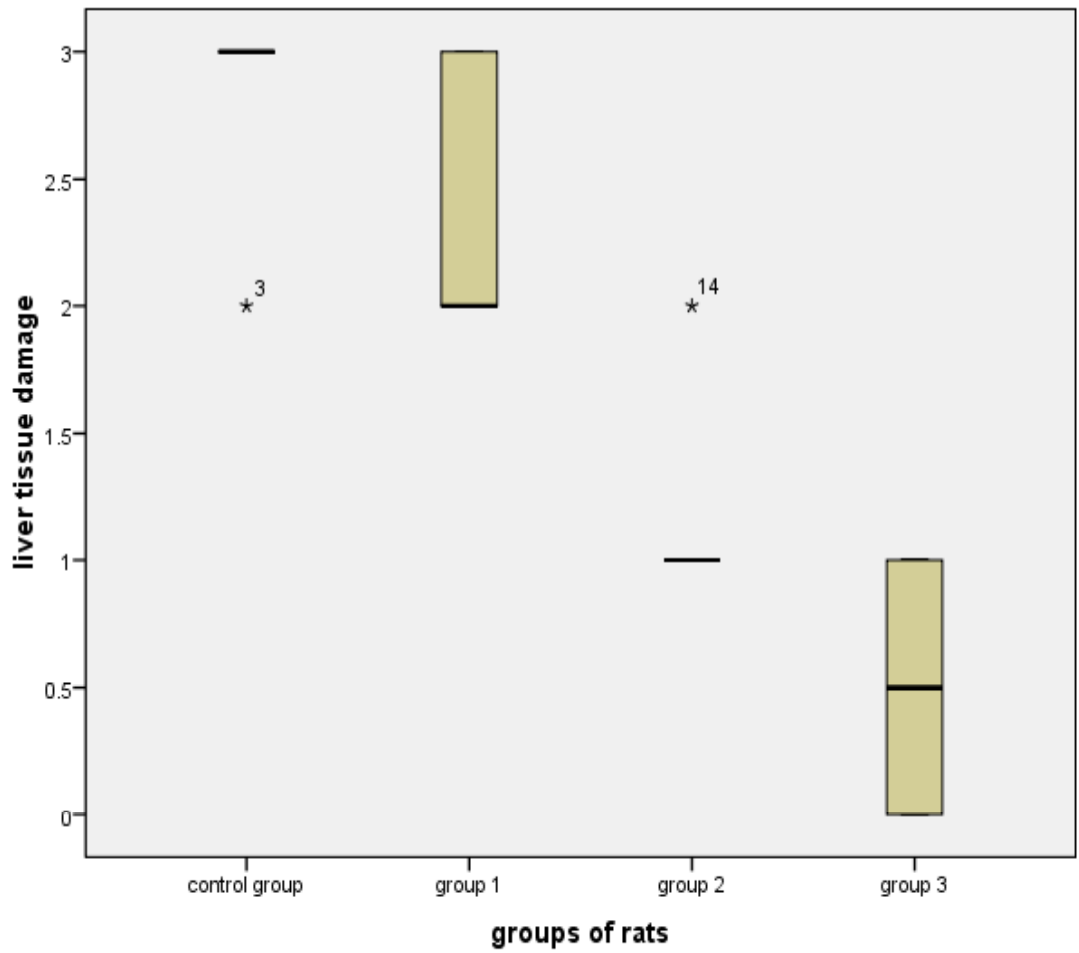
groups of rats	Kolmogorov-Smirnov ^a	
	Statistic	df
liver tissue damage control group	.492	6
group 1	.407	6
group 2	.492	6
group 3	.319	6

a. Lilliefors Significance Correction

Tests of Normality

groups of rats	Kolmogorov-Smirnov ^a	Shapiro-Wilk		
	Sig.	Statistic	df	Sig.
liver tissue damage control group	.000	.496	6	.000
group 1	.002	.640	6	.001
group 2	.000	.496	6	.000
group 3	.056	.683	6	.004

a. Lilliefors Significance Correction



Case Processing Summary

groups of rats	Cases		
	Valid		Missing
	N	Percent	N

TNF alpha expression	control group	6	100.0%	0
	group 1	6	100.0%	0
	group 2	6	100.0%	0
	group 3	6	100.0%	0

Case Processing Summary

		Cases		
		Missing	Total	
		Percent	N	Percent
TNF alpha expression	control group	.0%	6	100.0%
	group 1	.0%	6	100.0%
	group 2	.0%	6	100.0%
	group 3	.0%	6	100.0%

Descriptives

groups of rats			Statistic	Std. Error
TNF alpha expression	control group	Mean	2.83	.167
		95% Confidence Interval for Mean		
		Lower Bound	2.40	
		Upper Bound	3.26	
		5% Trimmed Mean	2.87	
		Median	3.00	
		Variance	.167	
		Std. Deviation	.408	

	Minimum	2	
	Maximum	3	
	Range	1	
	Interquartile Range	0	
	Skewness	-2.449	.845
	Kurtosis	6.000	1.741
group 1	Mean	2.17	.167
	95% Confidence Interval for Mean	Lower Bound	1.74
		Upper Bound	2.60

5%	2.13
Trimmed Mean	
Median	2.00
Variance	.167
Std. Deviation	.408
Minimum	2
Maximum	3
Range	1
Interquartile Range	0

	Skewness	2.449	.845
	Kurtosis	6.000	1.741
group 2	Mean	1.33	.211
	95% Confidence Interval for Mean	Lower Bound	.79
		Upper Bound	1.88
	5% Trimmed Mean	1.31	
	Median	1.00	
	Variance	.267	
	Std. Deviation	.516	

	Minimum	1	
	Maximum	2	
	Range	1	
	Interquartile Range	1	
	Skewness	.968	.845
	Kurtosis	-1.875	1.741
group 3	Mean	.17	.167
	95% Confidence Interval for Mean	Lower Bound	-.26
		Upper Bound	.60

5%	.13
Trimmed Mean	
Median	.00
Variance	.167
Standard Deviation	.408
Minimum	0
Maximum	1
Range	1
Interquartile Range	0

	Skewness	2.449	.845
	ss		
	Kurtosis	6.000	1.741
	s		

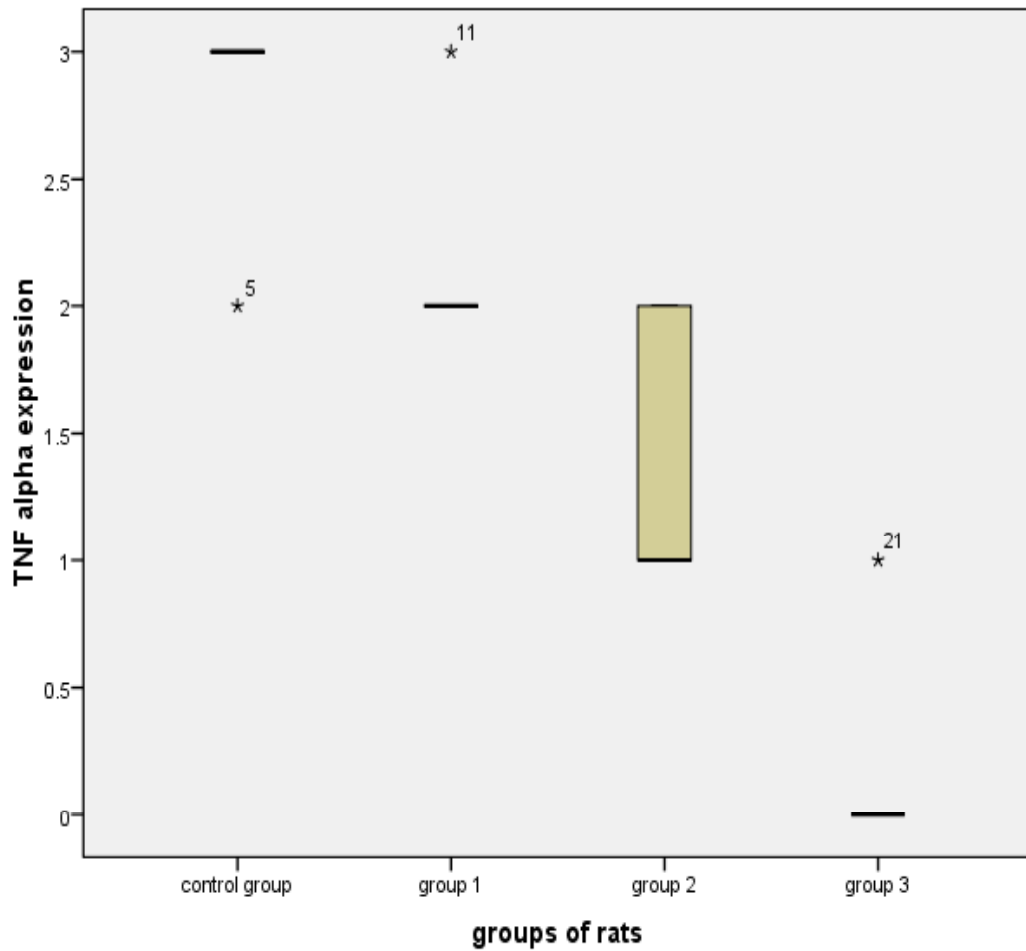
Tests of Normality

		Kolmogorov-Smirnov ^a	
		Statistic	df
TNF alpha expression	control group	.492	6
	group 1	.492	6
	group 2	.407	6
	group 3	.492	6

a. Lilliefors Significance Correction

Tests of Normality

		Kolmogorov-Smirnov ^a	Shapiro-Wilk		
		Sig.	Statistic	df	Sig.
TNF alpha expression	control group	.000	.496	6	.000
	group 1	.000	.496	6	.000
	group 2	.002	.640	6	.001
	group 3	.000	.496	6	.000



Tests of Normality

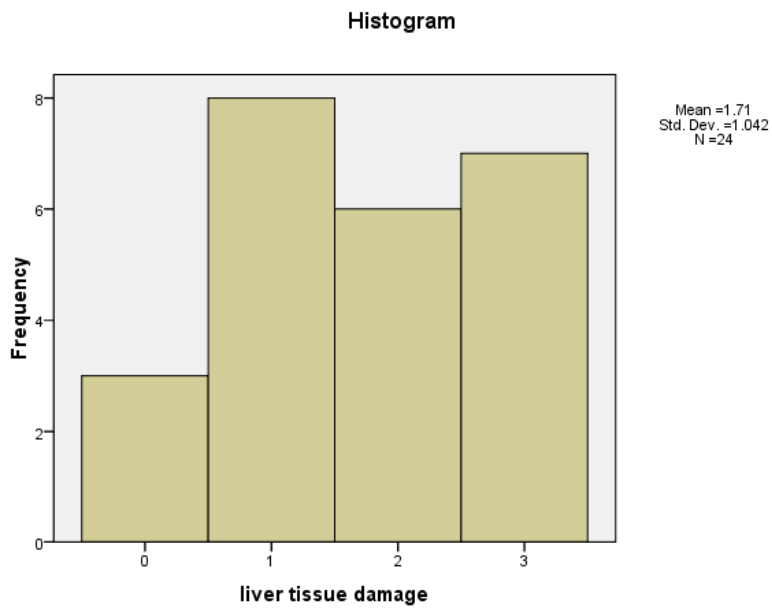
	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
liver tissue damage	.210	24	.008	.866	24	.004
TNF alpha expression	.226	24	.003	.851	24	.002

Tests of Normality

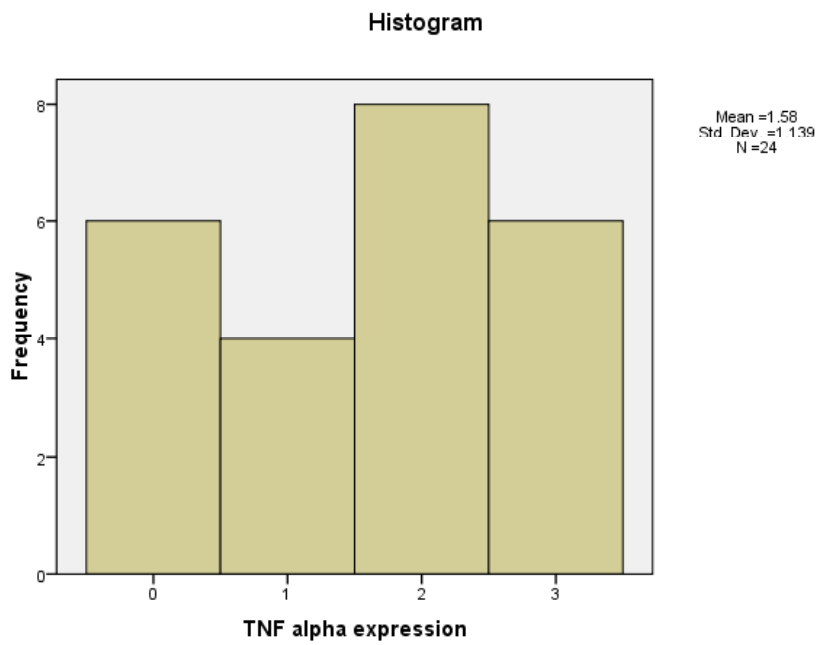
	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
liver tissue damage	.210	24	.008	.866	24	.004
TNF alpha expression	.226	24	.003	.851	24	.002

a. Lilliefors Significance Correction

liver tissue damage



TNF alpha expression



Kruskal-Wallis Test

Ranks

	groups of rats	N	Mean Rank
liver tissue damage	control group	6	19.92
	group 1	6	16.67
	group 2	6	8.67
	group 3	6	4.75
	Total	24	
TNF alpha expression	control group	6	20.33
	group 1	6	15.67
	group 2	6	10.50
	group 3	6	3.50
	Total	24	

Test Statistics^{a,b}

	liver tissue damage	TNF alpha expression
Chi-Square	19.145	20.208
df	3	3
Asymp. Sig.	.000	.000

a. Kruskal Wallis Test

b. Grouping Variable: groups of rats

Mann-Whitney Test

Ranks

groups of rats		N	Mean Rank	Sum of Ranks
liver tissue damage	control group	6	8.00	48.00
	group 1	6	5.00	30.00
	Total	12		
TNF alpha	control group	6	8.50	51.00

expression	group 1	6	4.50	27.00
	Total	12		

Test Statistics^b

	liver tissue damage	TNF alpha expression
Mann-Whitney U	9.000	6.000
Wilcoxon W	30.000	27.000
Z	-1.682	-2.211
Asymp. Sig. (2-tailed)	.093	.027
Exact Sig. [2*(1-tailed Sig.)]	.180 ^a	.065 ^a

a. Not corrected for ties.

b. Grouping Variable: groups of rats

Mann-Whitney Test

Ranks

groups of rats		N	Mean Rank	Sum of Ranks
liver tissue damage	control group	6	9.42	56.50
	group 2	6	3.58	21.50
	Total	12		
TNF alpha expression	control group	6	9.33	56.00
	group 2	6	3.67	22.00
	Total	12		

Test Statistics^b

	liver tissue damage	TNF alpha expression
Mann-Whitney U	.500	1.000
Wilcoxon W	21.500	22.000
Z	-3.028	-2.900
Asymp. Sig. (2-tailed)	.002	.004

Exact Sig. [2*(1-tailed Sig.)]	.002 ^a	.004 ^a
-----------------------------------	-------------------	-------------------

a. Not corrected for ties.

b. Grouping Variable: groups of rats

Mann-Whitney Test

Ranks

groups of rats		N	Mean Rank	Sum of Ranks
liver tissue damage	control group	6	9.50	57.00
	group 3	6	3.50	21.00
	Total	12		
TNF alpha expression	control group	6	9.50	57.00
	group 3	6	3.50	21.00
	Total	12		

Test Statistics^b

	liver tissue damage	TNF alpha expression
--	------------------------	-------------------------

Mann-Whitney U	.000	.000
Wilcoxon W	21.000	21.000
Z	-3.035	-3.207
Asymp. Sig. (2-tailed)	.002	.001
Exact Sig. [2*(1-tailed Sig.)]	.002 ^a	.002 ^a

a. Not corrected for ties.

b. Grouping Variable: groups of rats

Mann-Whitney Test

Ranks

	groups of rats	N	Mean Rank	Sum of Ranks
liver tissue damage	group 1	6	9.17	55.00
	group 2	6	3.83	23.00
	Total	12		
TNF alpha	group 1	6	8.67	52.00

expression	group 2	6	4.33	26.00
	Total	12		

Test Statistics^b

	liver tissue damage	TNF alpha expression
Mann-Whitney U	2.000	5.000
Wilcoxon W	23.000	26.000
Z	-2.768	-2.373
Asymp. Sig. (2-tailed)	.006	.018
Exact Sig. [2*(1-tailed Sig.)]	.009 ^a	.041 ^a

a. Not corrected for ties.

b. Grouping Variable: groups of rats

Mann-Whitney Test

Ranks

	groups of rats	N	Mean Rank	Sum of Ranks
liver tissue damage	group 1	6	9.50	57.00
	group 3	6	3.50	21.00
	Total	12		
TNF alpha expression	group 1	6	9.50	57.00
	group 3	6	3.50	21.00
	Total	12		

Test Statistics^b

	liver tissue damage	TNF alpha expression
Mann-Whitney U	.000	.000
Wilcoxon W	21.000	21.000

Z	-2.983	-3.207
Asymp. Sig. (2-tailed)	.003	.001
Exact Sig. [2*(1-tailed Sig.)]	.002 ^a	.002 ^a

a. Not corrected for ties.

b. Grouping Variable: groups of rats

Mann-Whitney Test

Ranks

groups of rats		N	Mean Rank	Sum of Ranks
liver tissue damage	group 2	6	8.25	49.50
	group 3	6	4.75	28.50
	Total	12		
TNF alpha expression	group 2	6	9.50	57.00
	group 3	6	3.50	21.00
	Total	12		

Test Statistics^b

	liver tissue damage	TNF alpha expression
Mann-Whitney U	7.500	.000
Wilcoxon W	28.500	21.000
Z	-2.021	-3.146
Asymp. Sig. (2-tailed)	.043	.002
Exact Sig. [2*(1-tailed Sig.)]	.093 ^a	.002 ^a

a. Not corrected for ties.

b. Grouping Variable: groups of rats

Reliability Statistics

Cronbach's Alpha	N of Items
.992	2

Reliability Statistics

Cronbach's Alpha	N of Items
.992	2

Test Statistics^b

	liver tissue damage	TNF alpha expression
Mann-Whitney U	7.500	.000
Wilcoxon W	28.500	21.000
Z	-2.021	-3.146
Asymp. Sig. (2-tailed)	.043	.002
Exact Sig. [2*(1-tailed Sig.)]	.093 ^a	.002 ^a

a. Not corrected for ties.

RELIABILITY /VARIABLES=tissue_damage

tissue_damage_b

Reliability Statistics

Cronbach's Alpha	N of Items
.965	2

RELIABILITY /VARIABLES=TNFalpha

TNFalpha_b

Appendix 4

Research pictures



Feeding the rats



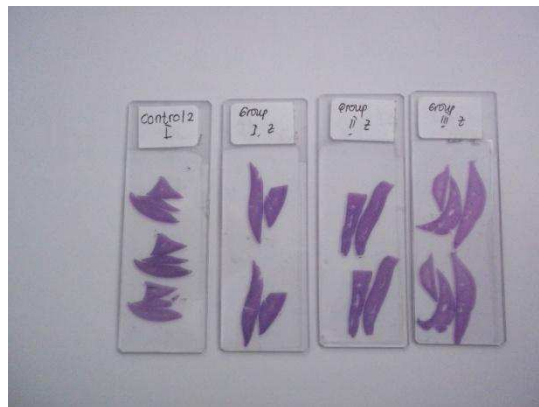
Taking the liver tissue after termination



Gross liver tissue



Tissue block



Tissue slides



Reading slides