

**APPENDIX**

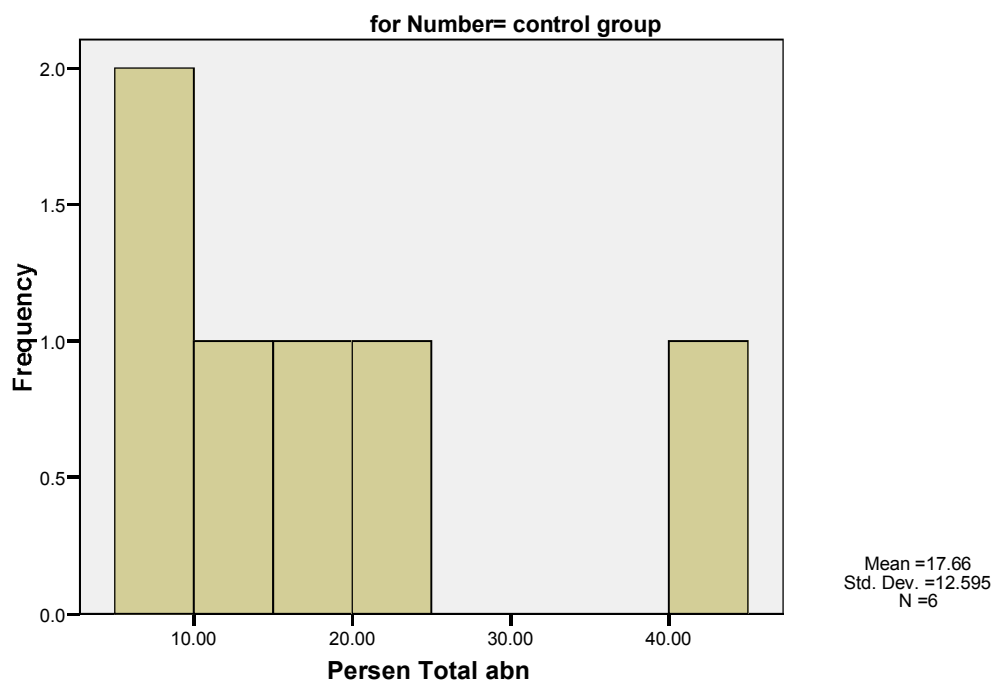
**Appendix 2. HE Staining Examination Result: Distribution of of BALB/c mice nucleus liver cells changes in percents between control group and intervention groups.**

**Descriptives**

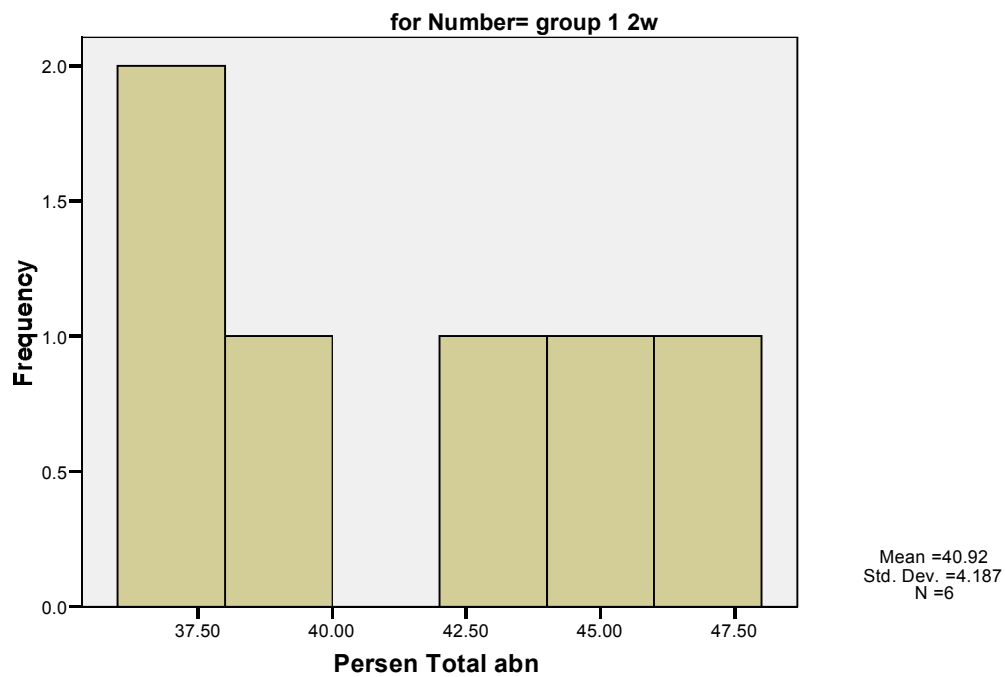
Groups			Statistic	Std. Error			
Persen Total abn	control group	Mean	17.6550	5.14192			
		95% Confidence Interval for Mean	Lower Bound	4.4373			
			Upper Bound	30.8727			
		5% Trimmed Mean	16.9483				
		Median	14.3550				
		Variance	158.636				
		Std. Deviation	12.59507				
		Minimum	7.33				
		Maximum	40.70				
		Range	33.37				
		Interquartile Range	18.55				
		Skewness	1.492	.845			
		Kurtosis	2.234	1.741			
		group 1 2w		Mean	40.9250	1.70954	
				95% Confidence Interval for Mean	Lower Bound	36.5305	
					Upper Bound	45.3195	
				5% Trimmed Mean	40.8883		
Median	40.3900						
Variance	17.535						
Std. Deviation	4.18750						
Minimum	36.04						
Maximum	46.47						
Range	10.43						
Interquartile Range	8.05						
Skewness	.246			.845			
Kurtosis	-1.930			1.741			
group 2 4m				Mean	51.9400	1.21465	
				95% Confidence Interval for Mean	Lower Bound	48.8176	
					Upper Bound	55.0624	
				5% Trimmed Mean	52.0028		
		Median	52.1700				

	Variance		8.852	
	Std. Deviation		2.97528	
	Minimum		46.88	
	Maximum		55.87	
	Range		8.99	
	Interquartile Range		4.00	
	Skewness		-.751	.845
	Kurtosis		1.787	1.741
group 3 6w	Mean		61.2200	.59471
	95% Confidence Interval for Mean	Lower Bound	59.6912	
		Upper Bound	62.7488	
	5% Trimmed Mean		61.1794	
	Median		60.7950	
	Variance		2.122	
	Std. Deviation		1.45674	
	Minimum		59.72	
	Maximum		63.45	
	Range		3.73	
	Interquartile Range		2.69	
	Skewness		.747	.845
	Kurtosis		-.964	1.741
group 4 8w	Mean		60.7683	3.60177
	95% Confidence Interval for Mean	Lower Bound	51.5097	
		Upper Bound	70.0270	
	5% Trimmed Mean		61.0609	
	Median		63.3950	
	Variance		77.836	
	Std. Deviation		8.82250	
	Minimum		45.84	
	Maximum		70.43	
	Range		24.59	
	Interquartile Range		13.85	
	Skewness		-1.021	.845
	Kurtosis		.700	1.741

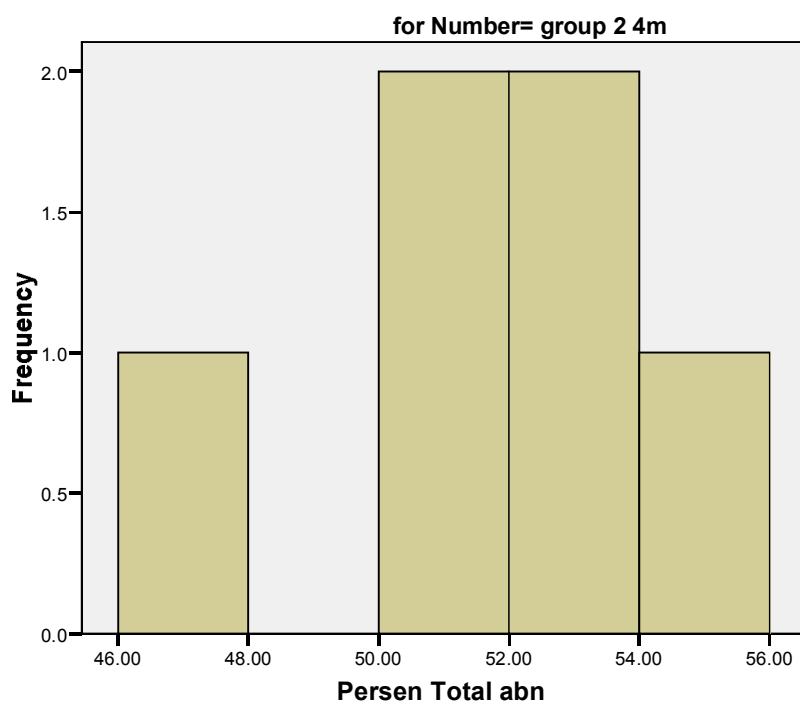
### Histogram



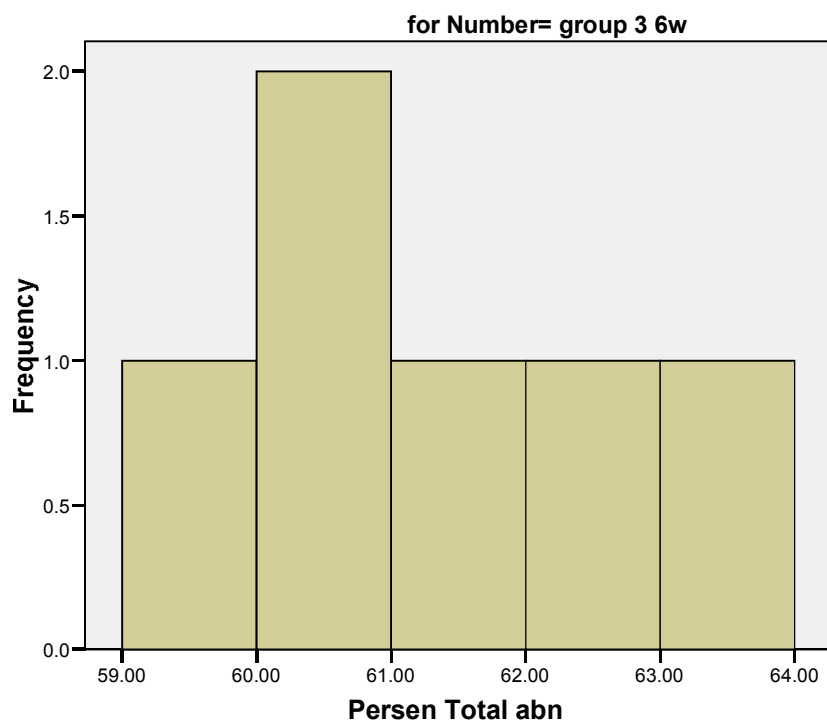
### Histogram



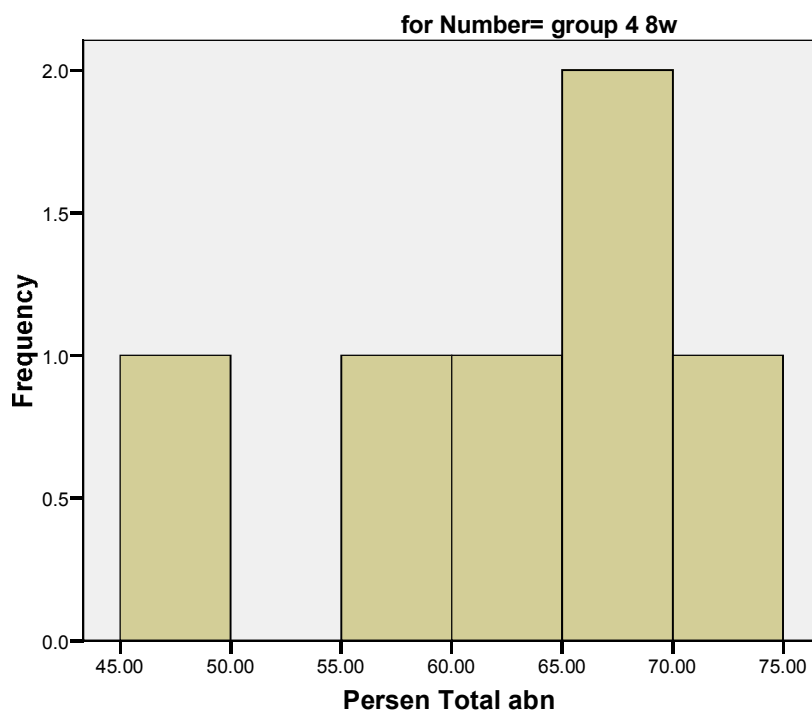
### Histogram



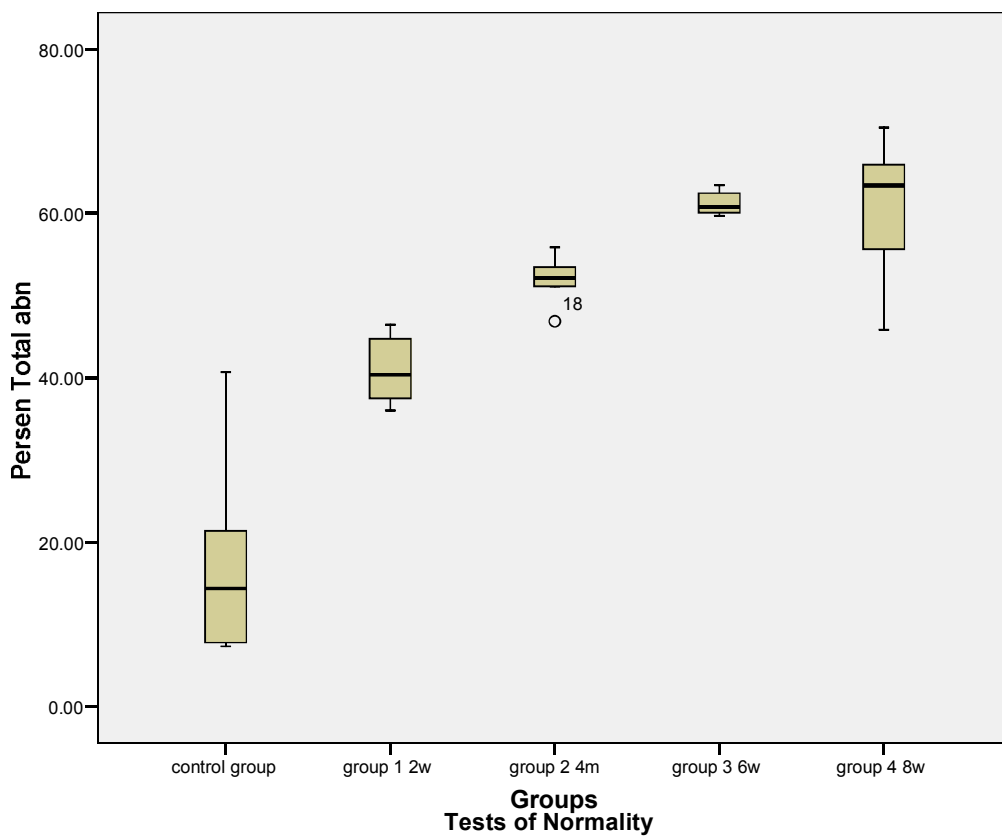
### Histogram



### Histogram



### Box Plot control groups and four intervention groups



Groups		Kolmogorov-Smirnov(a)			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Persen Total abn	control group	.216	6	.200(*)	.844	6	.139
	group 1 2w	.207	6	.200(*)	.934	6	.615
	group 2 4m	.223	6	.200(*)	.950	6	.743
	group 3 6w	.209	6	.200(*)	.918	6	.493
	group 4 8w	.204	6	.200(*)	.929	6	.569

### Kruskal-Wallis Test

Among all groups

#### Test Statistics(a,b)

	Persen Total abn
Chi-Square	24.202
df	4
Asymp. Sig.	.000

a Kruskal Wallis Test

b Grouping Variable: Groups

### Post Hoc Test

Mann-Whitney test

Between control group and group 1

#### Test Statistics(b)

	Persen Total abn
Mann-Whitney U	3.000
Wilcoxon W	24.000
Z	-2.402
Asymp. Sig. (2-tailed)	.016
Exact Sig. [2*(1-tailed Sig.)]	.015(a)

a Not corrected for ties.

b Grouping Variable: Groups

Between control group and group 2

**Test Statistics(b)**

	Persen Total abn
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: Groups

Between control group and group 3

**Test Statistics(b)**

	Persen Total abn
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: Groups

Between control group and group 4

**Test Statistics(b)**

	Persen Total abn
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: Groups

Between group 1 and group 2

**Test Statistics(b)**

	Persen Total abn
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: Groups

Between group 1 and group 3

**Test Statistics(b)**

	Persen Total abn
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: Groups

Between group 1 and group 4

**Test Statistics(b)**

	Persen Total abn
Mann-Whitney U	1.000
Wilcoxon W	22.000
Z	-2.722
Asymp. Sig. (2-tailed)	.006
Exact Sig. [2*(1-tailed Sig.)]	.004(a)

a Not corrected for ties.

b Grouping Variable: Groups

Between group 2 and group 3

**Test Statistics(b)**

	Persen Total abn
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: Groups

Between group 2 and group 4

**Test Statistics(b)**

	Persen Total abn
Mann-Whitney U	7.000
Wilcoxon W	28.000
Z	-1.761
Asymp. Sig. (2-tailed)	.078
Exact Sig. [2*(1-tailed Sig.)]	.093(a)

a Not corrected for ties.

b Grouping Variable: Groups

Between group 3 and group 4

**Test Statistics(b)**

	Persen Total abn
Mann-Whitney U	14.000
Wilcoxon W	35.000
Z	-.641
Asymp. Sig. (2-tailed)	.522
Exact Sig. [2*(1-tailed Sig.)]	.589(a)

a Not corrected for ties.

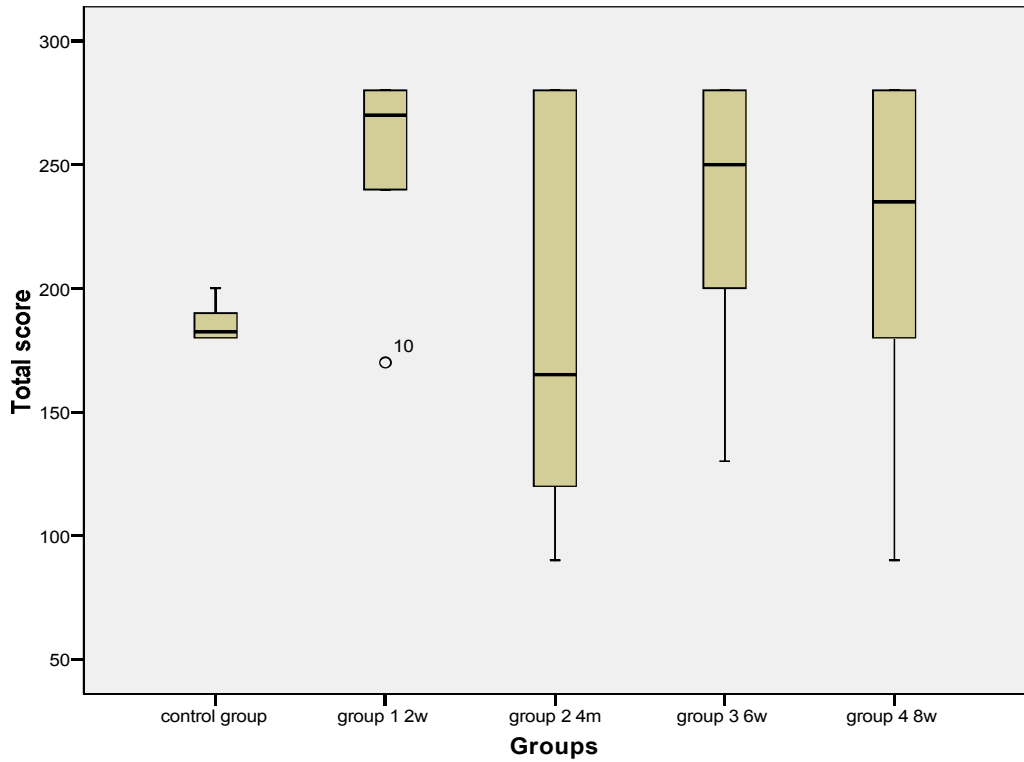
b Grouping Variable: Groups

**Appendix 3. Immunohistochemistry Staining Examination Result:  
Distribution of of BALB/c mice cytocrome P450 expression in percents  
between control group and intervention groups.**

**Descriptives**

Groups				Statistic	Std. Error
Total score	control group	Mean		185.83	3.270
		95% Confidence Interval for Mean	Lower Bound	177.43	
			Upper Bound	194.24	
		5% Trimmed Mean		185.37	
		Median		182.50	
		Variance		64.167	
		Std. Deviation		8.010	
		Minimum		180	
		Maximum		200	
		Range		20	
		Interquartile Range		13	
		Skewness		1.354	.845
		Kurtosis		1.240	1.741
	group 1 2w	Mean		251.67	17.591
		95% Confidence Interval for Mean	Lower Bound	206.45	
			Upper Bound	296.89	
		5% Trimmed Mean		254.63	
		Median		270.00	
		Variance		1856.667	
		Std. Deviation		43.089	
Minimum			170		
Maximum			280		
Range			110		
Interquartile Range			58		
Skewness			-1.790	.845	
Kurtosis			3.180	1.741	
group 2 4m	Mean		183.33	32.931	
	95% Confidence Interval for Mean	Lower Bound	98.68		
		Upper Bound	267.98		
	5% Trimmed Mean		183.15		
	Median		165.00		
	Variance		6506.667		
	Std. Deviation		80.664		
	Minimum		90		

	Maximum		280	
	Range		190	
	Interquartile Range		168	
	Skewness		.402	.845
	Kurtosis		-1.821	1.741
group 3 6w	Mean		231.67	24.822
	95% Confidence Interval for Mean	Lower Bound	167.86	
		Upper Bound	295.47	
	5% Trimmed Mean		234.63	
	Median		250.00	
	Variance		3696.667	
	Std. Deviation		60.800	
	Minimum		130	
	Maximum		280	
	Range		150	
	Interquartile Range		98	
	Skewness		-.995	.845
	Kurtosis		.114	1.741
group 4 8w	Mean		216.67	30.840
	95% Confidence Interval for Mean	Lower Bound	137.39	
		Upper Bound	295.94	
	5% Trimmed Mean		220.19	
	Median		235.00	
	Variance		5706.667	
	Std. Deviation		75.542	
	Minimum		90	
	Maximum		280	
	Range		190	
	Interquartile Range		123	
	Skewness		-.993	.845
	Kurtosis		.189	1.741



**Tests of Normality**

Groups		Kolmogorov-Smirnov(a)			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Positive staining	control group	.319	6	.056	.683	6	.004
	group 1 2w	.267	6	.200(*)	.809	6	.070
	group 2 4m	.294	6	.114	.843	6	.138
	group 3 6w	.265	6	.200(*)	.799	6	.058
	group 4 8w	.277	6	.165	.825	6	.097

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

a Lilliefors Significance Correction

**Kruskal-Wallis**

Among groups

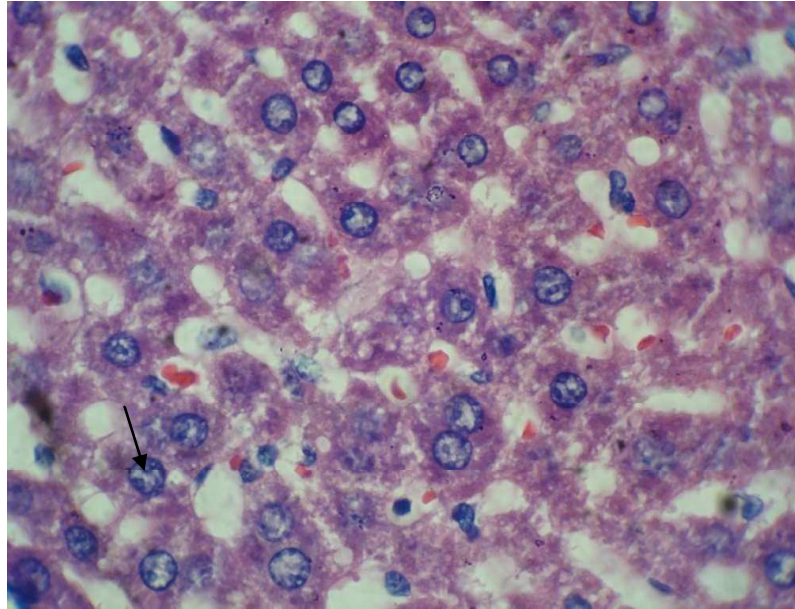
**Test Statistics(a,b)**

	Total score
Chi-Square	5.216
df	4
Asymp. Sig.	.266

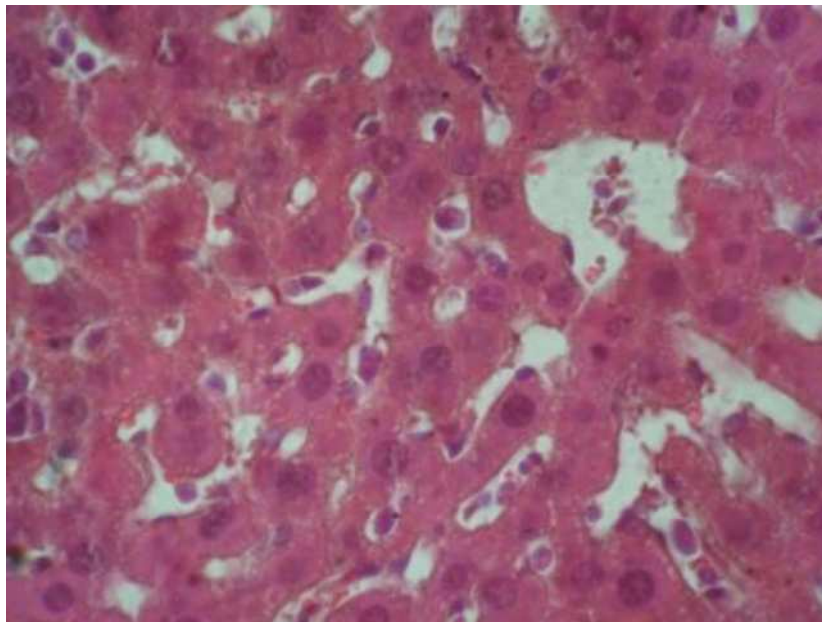
a Kruskal Wallis Test

b Grouping Variable: Groups

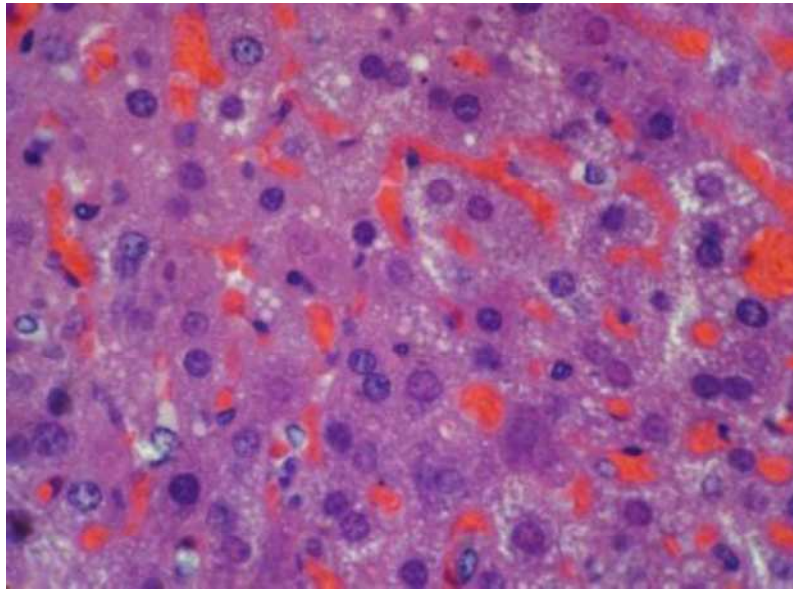
#### Appendix 4. Research Pictures



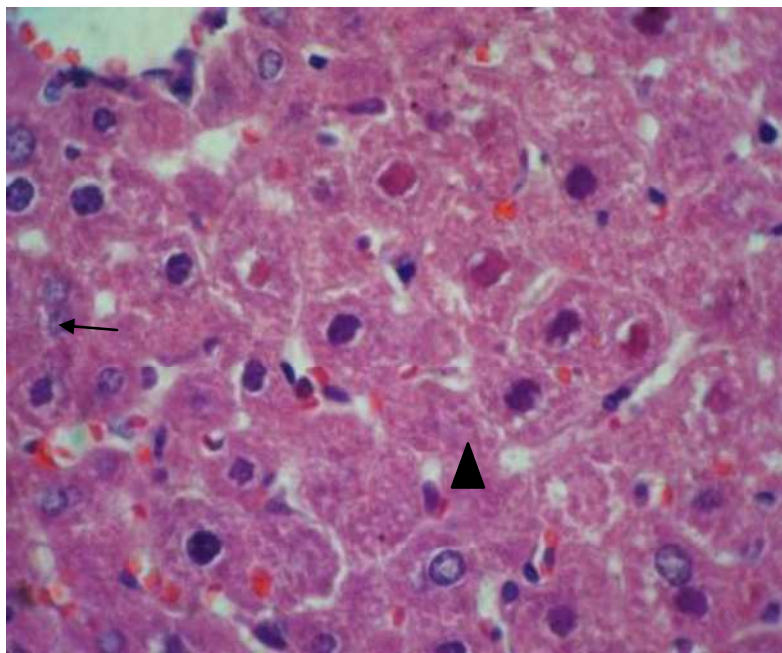
**HE staining examination of control group (1000X magnification)**  
There were cells with enlarge nucleus (arrow, the mean percentage was 17.65%)



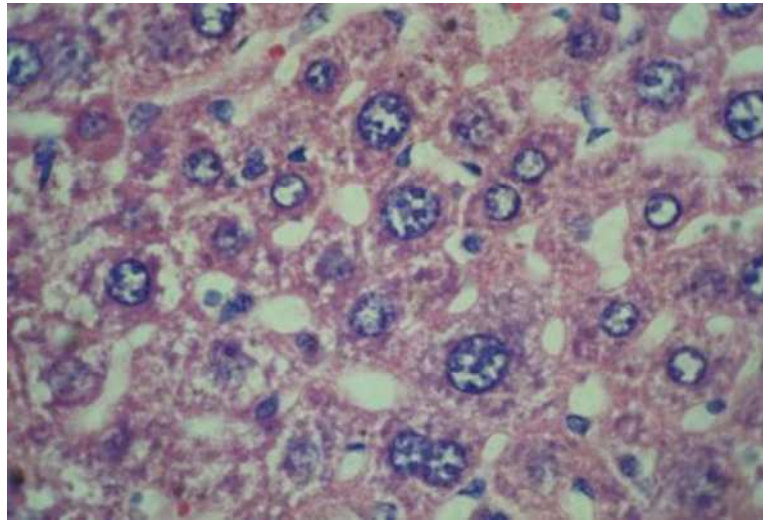
**HE staining examination of group 1 (1000X magnification)**  
The mean percentage of abnormal cells was 40.93%



**HE staining examination of group 2 (1000X magnification)**  
The mean percentage of abnormal cells was 51.94%

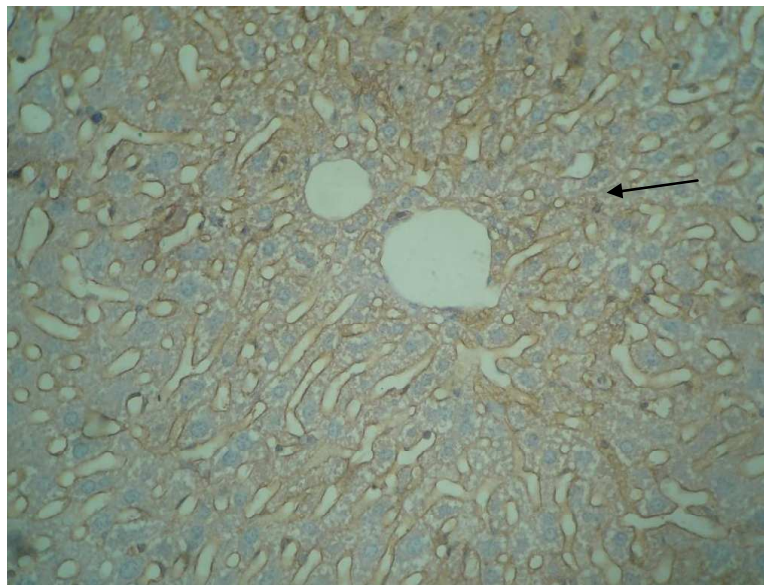


**HE staining examination of group 3 (1000X magnification)**  
The mean percentage of abnormal cells was 61.22%. Karyorhexis (arrow),  
karyolysis (arrow head)



**HE staining examination of group 4 (1000X magnification)**

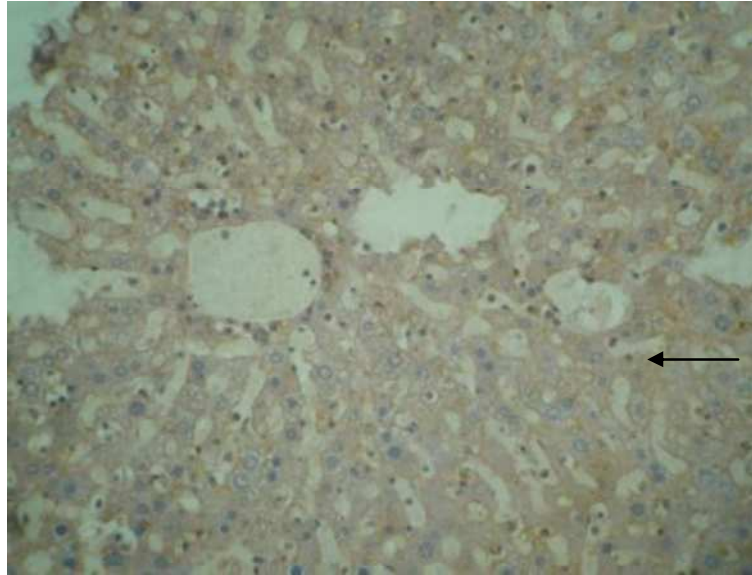
The mean percentage of abnormal cells was 60.77%



**IHC staining examination of control group**

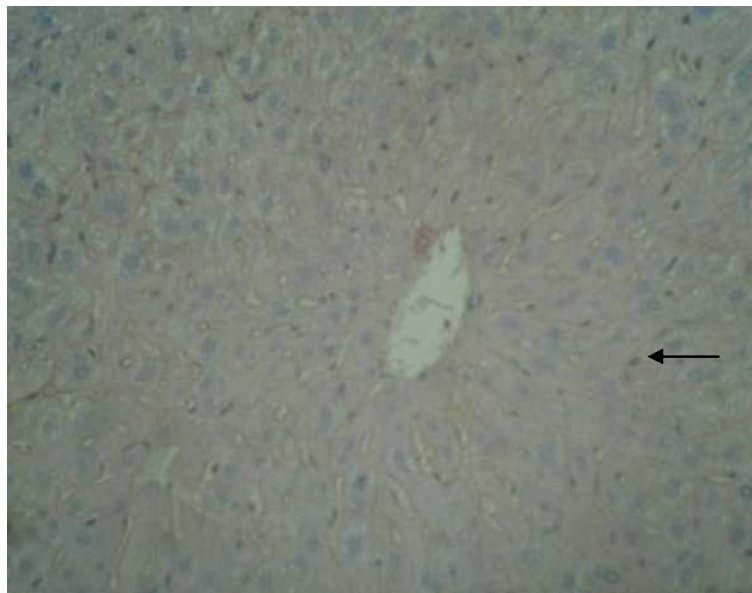
Morphology of P450 expression of the Balb c/ mice liver cells in control group, using 400X magnification. The cells were slightly brown coloured (arrow).

The mean score of P450 expression was 185.83



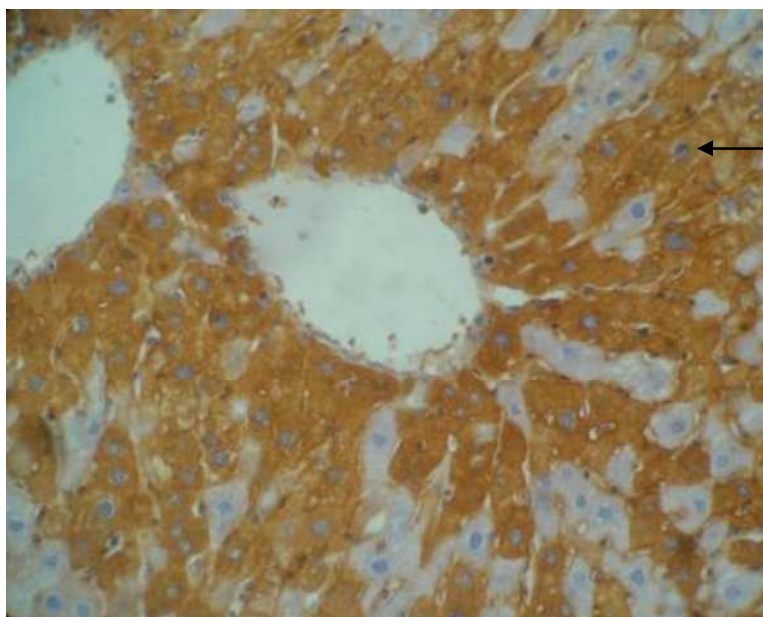
**IHC staining examination of group 1**

Morphology of P450 expression of the Balb c/ mice liver cells in group 1, using 400X magnification. The cells were mediumly brown coloured (arrow). The mean score of P450 expression was 251.67



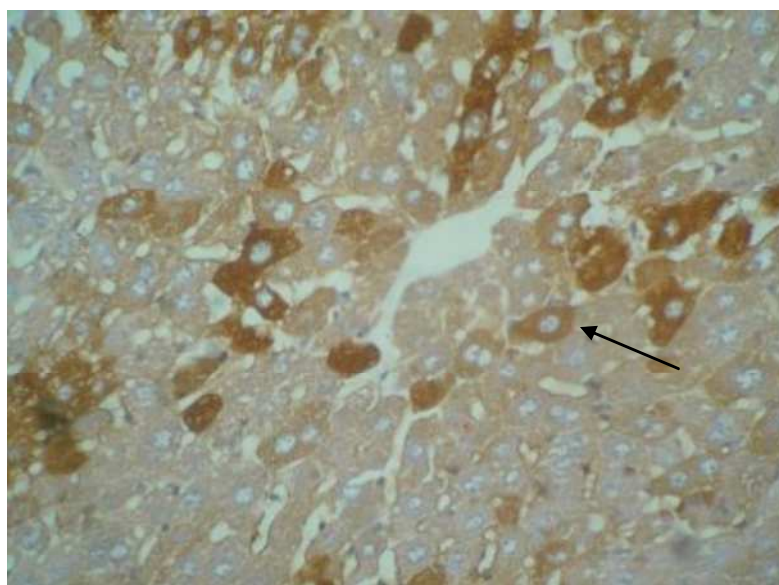
**IHC staining examination of group 2**

Morphology of P450 expression of the Balb c/ mice liver cells in group 2, using 400X magnification. The cells were slightly brown coloured (arrow). The mean score of P450 expression was 183.33



**IHC staining examination of group 3**

Morphology of P450 expression of the Balb c/ mice liver cells in group 3, using 400X magnification. The cells were intensely brown coloured (arrow).  
The mean score of P450 expression was 231.67



**IHC staining examination of group 4**

Morphology of P450 expression of the Balb c/ mice liver cells in group 4, using 400X magnification. The cells were intensely brown coloured (arrow).  
The mean score of P450 expression was 216.67



Male balb/C mice in the group



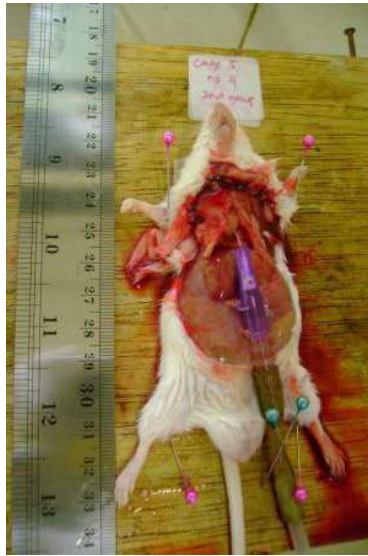
Halothane, plate and syringe



Mice on the cages



Halothane was put on a plate



Operating the mice to take its liver tissue



Liver of the Balb c/ mice



Histokinet



Microtome