

APPENDIX 1
ETHICAL CLEARANCE

APPENDIX 2

PROCEDURE FOR PREPARING OF LIVER HISTOLOGY SLIDES

Overview: Histology involves the use of a set of techniques to examine the morphology, architecture and composition of tissues. The liver tissue samples was first dissected and fixed. Then the liver tissue samples was embedded in paraffin wax. After the tissue has been sliced, sections were mounted on a slide and then the sections were stained in preparation for examination by light microscope (Olympus BX61) to identification of hepatic steatosis, hepatic inflammation and Mallory bodies via Hematoxylin Eosin staining. There were eight stages in the preparation of liver histology slides as following:

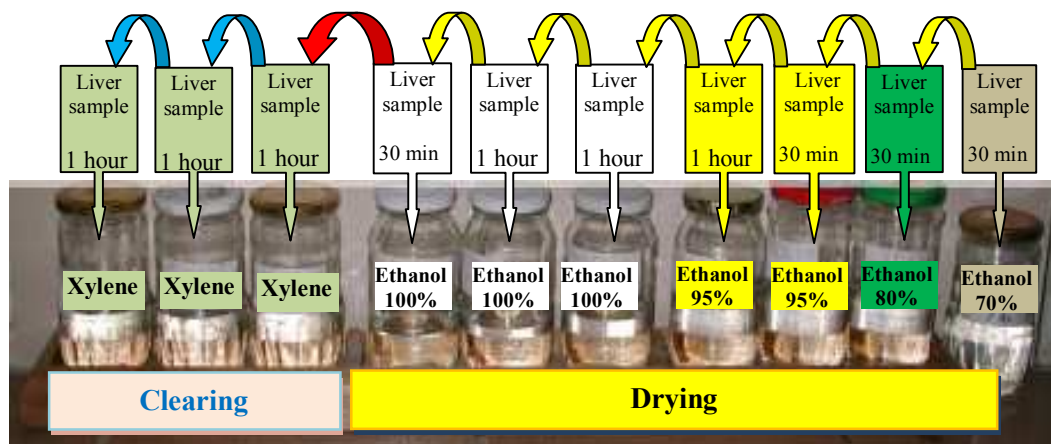
Fixation: it is performed to preserve the biological structures (both chemically and structurally) of the liver tissues in as natural a state as possible and prevent autolysis and putrefaction. This requires a chemical fixative that can stabilise the proteins, nucleic acids and mucosubstances of the liver tissues by making them insoluble. This process provides rigidity to the liver tissue, making it easier to section.



The liver tissues samples were fixed in a Common chemical fixative, which was 10% formaldehyde (10%formalin). The liver tissue samples are

immersed in the amount of formaldehyde equivalent to 15-20 times of the sample size and the duration of fixation was less than 48 hours. Once fixation has been completed, the liver tissue samples was embedded prior to sectioning.

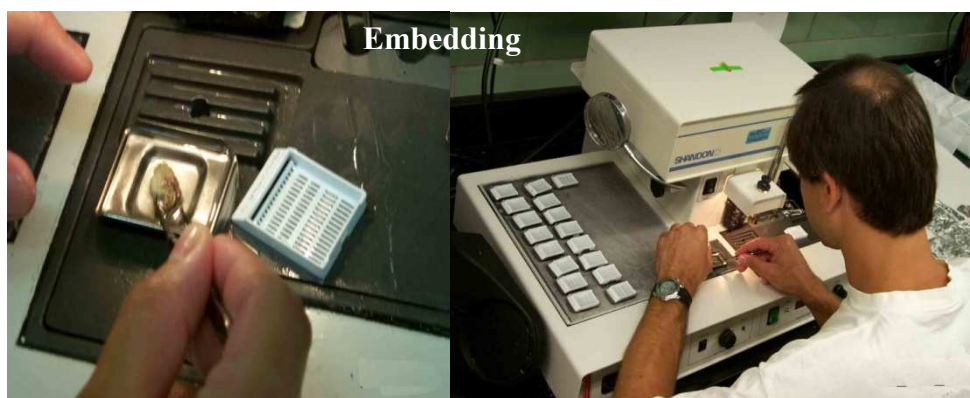
Dehydrated: liver Tissue samples processing was done to remove water from the liver tissues, replacing such water with a medium that solidifies, setting very hard and so allowing extremely thin sections to be sliced.



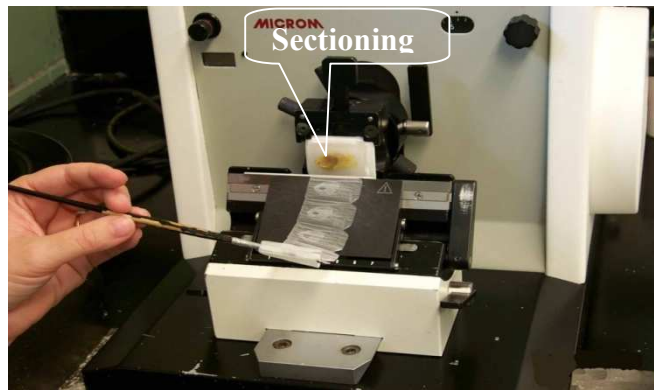
This process was done by using graded ethanol solutions as follows (70%, 80%, 95%, 95%, 100%, 100%, 100%) respectively, leaving the liver tissue samples in each solution for a sufficient period for replaced the water with alcohol. And despite the fact that paraffin is not soluble in alcohol, therefore the alcohol replaces with the paraffin solvent has capable to soluble with paraffin.

Clearing: Xylene solution used, usually, to clean the tissue mass by passing through graded xylene solutions, that ultimately lead to replacement of alcohol with xylene and then the liver tissue mass were becoming ready-to-Embed.

Embedding: Before sectioning, liver tissue samples were embedded in paraffin wax. After a short time in the liquid paraffin, the liver tissue samples placed into a mold with more paraffin. The wax was allowed to solidify, forming a block that can be held in a microtome. This step was allowed the liver tissues to be cut easily by a microtome.



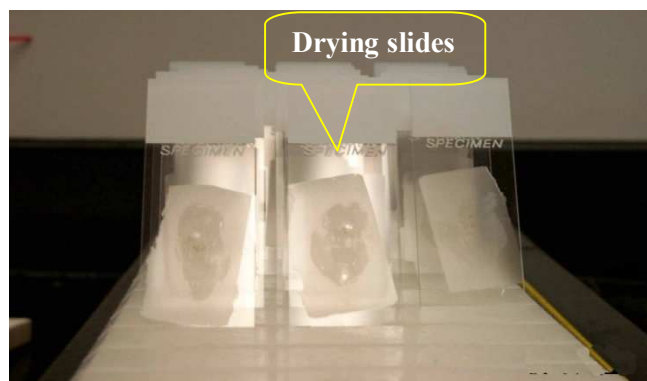
Sectioning: Liver tissue samples embedded in paraffin were mounted in the microtome. The method used to actually cut sections from the hardened block of tissue depends on the type of microscopy that will be used to observe it and hence the thickness of sample required. The liver tissue samples will be examined by using light microscopy, thereby liver tissue sections were cut at 5 μ m with rotary microtome. After sectioning, the slices were placed on a slide.



Mounting: After several slices of the paraffin-embedded tissue have been sectioned, the slices are removed from the blade and floated atop a warm water bath to smooth out the sample.

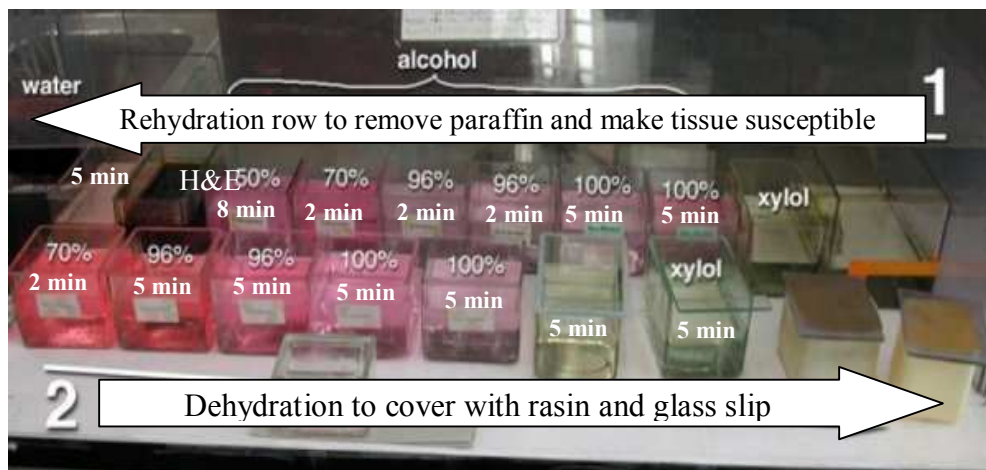


After the slides were dried, they are placed in an oven to "bake" the paraffin. The oven is warm and helps the section of tissue adhere to the slide.



Staining: Finally, the mounted sections are treated with an appropriate histology stain. One especially common stain is Hematoxylin and Eosin (H&E), colors the nuclei dark blue (hematoxylin) and the remaining cell components pink (eosin). For staining, slides are put into a staining rack and then manually processed through the staining row. After finishing, slides are dried in incubator before mounting the cover slip. For "blueing", running fresh tap water is needed. The staining is performed in this order:-

Heat slides in incubator for 15min to liquify paraffin, and then the slides was immersed in Xylene, alcohol 100%, alcohol 100%, alcohol 95%, alcohol 95%, alcohol 70%, alcohol 50%, Stains: Hematoxylin Eosin staining, alcohol 70%, alcohol 95%, alcohol 95%, alcohol 100%, alcohol 100%, xylene and ultimately the slides was dried in oven and then mount cover slip respectively.



Above; (1) rehydration row to remove paraffin and make tissue susceptible for dye. (2) dehydration to cover with rasin and glass slip.

APPENDIX 3

HEPATIC TISSUE CHANGES SCORING, GRADING AND ITS EXAMINATION RESULTS BY TWO PATHOLOGISTS

Hepatic tissue changes scoring and grading system

Histological diagnosis of hepatic steatosis by NASH activity score			Histological diagnosis of hepatic inflammation by Knodell scoring system		
The degree of liver steatosis	% Range of liver steatosis	score	The degree of Porta inflammation	Score	The degree of Mallory bodies
None	<5%	0	Non porta inflammation	0	Present
Mild	5-33%	1	Mild(sprinkling of inflammatory cells in < 1/3 of portal tracts) .	1	Non present
Moderate	33-66%	2	Moderate (increase inflammatory cells in 1/3-2/3 of portal tracts).	3	
Severe	>66%	3	Marked (dense packingof inflammatory cells in >2/3 of portal tracts) .	4	

Results Of Hepatic Tissue Changes Examination By Two Pathologists

Groups	First pathologist result			Second pathologist result		
Treatment of groups	The degree of liver steatosis	The degree of Portal inflammation	The presence of Mallory bodies	The degree of liver steatosis	The degree of Portal inflammation	The presence of Mallory bodies
Control group	Sever	Marked	None present	Moderate	Marked	Present
Control group	Sever	Moderate	Present	Severe	Moderate	Present
Control group	Moderate	Marked	Present	Moderate	Marked	Present
Control group	Sever	Marked	None present	Severe	Marked	Present
Control group	Sever	Marked	Present	Severe	Marked	Present
Control group	Sever	Moderate	Present	Severe	Marked	Present
Group 1	Moderate	Moderate	None present	Severe	Moderate	Present
Group 1	Sever	Moderate	None present	Moderate	Mild	None present
Group 1	Sever	Moderate	Present	Severe	Moderate	Present
Group 1	Moderate	Moderate	None present	Moderate	Moderate	Present
Group 1	Moderate	Moderate	Present	Moderate	Moderate	Present
Group 1	Sever	Marked	None present	Severe	Moderate	Present
Group 2	Mild	Moderate	None present	Mild	Mild	Present
Group 2	Mild	Moderate	Present	Mild	Moderate	Present
Group 2	Moderate	Mild	None present	Moderate	Mild	None present
Group 2	Mild	Mild	None present	Mild	Mild	None present
Group 2	Moderate	Moderate	None present	Moderate	Mild	None present
Group 2	Moderate	Mild	None present	Moderate	Mild	None present
Group 3	Mild	Mild	None present	Mild	None	None present
Group 3	Mild	Mild	None present	None	Mild	None present
Group 3	Mild	Mild	None present	Mild	Mild	None present
Group 3	None	None	None present	None	None	None present
Group 3	Mild	Mild	None present	Mild	Mild	None present
Group 3	Mild	None	None present	Mild	None	None present

Results Of Hepatic Tissue Changes Examination By Two Pathologists

Treatment of groups	First pathologist result			Second pathologist result		
	The degree of liver steatosis	The degree of Portal inflammation	The presence of Mallory bodies	The degree of liver steatosis	The degree of Portal inflammation	The presence of Mallory bodies
Control group	3	4	1	2	4	0
Control group	3	3	0	3	3	0
Control group	2	4	0	2	4	0
Control group	3	4	1	3	4	0
Control group	3	4	0	3	4	0
Control group	3	4	0	3	3	0
Group 1	2	3	1	3	3	0
Group 1	3	1	1	2	3	1
Group 1	3	3	0	3	3	0
Group 1	2	3	1	2	3	0
Group 1	2	3	0	2	3	0
Group 1	3	3	1	3	4	0
Group 2	1	1	1	1	3	0
Group 2	1	3	0	1	3	0
Group 2	2	1	1	2	1	1
Group 2	1	1	1	1	1	1
Group 2	2	1	1	2	3	1
Group 2	2	1	1	2	1	1
Group 3	1	0	1	1	1	1
Group 3	1	1	1	0	1	1
Group 3	1	1	1	1	1	1
Group 3	0	0	1	0	0	1
Group 3	1	1	1	1	1	1
Group 3	1	0	1	1	0	1

APPENDIX 4

THE INTER-PATHOLOGISTS AGREEMENT

The inter-pathologists agreement for diagnosis of hepatic steatosis .

Crosstabs

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Diagnosis by pathologist 1 * Diagnosis by pathologist 2	24	51.1%	23	48.9%	47	100.0%

Diagnosis by pathologist 1 * Diagnosis by pathologist 2 Crosstabulation

			Diagnosis by pathologist 2				Total
			None	Mild	Moderate	Severe	
Diagnosis by pathologist 1 None	Count		1	0	0	0	1
	Expected Count		.1	.3	.3	.3	1.0
Mild	Count		1	7	0	0	8
	Expected Count		.7	2.3	2.7	2.3	8.0
Moderate	Count		0	0	6	1	7
	Expected Count		.6	2.0	2.3	2.0	7.0
Sever	Count		0	0	2	6	8
	Expected Count		.7	2.3	2.7	2.3	8.0
Total	Count		2	7	8	7	24
	Expected Count		2.0	7.0	8.0	7.0	24.0

Symmetric Measures

	Value	Asymp. Std. Error ^a	Approx. T ^b	Approx. Sig.
Measure of Agreement Kappa	.764	.107	5.903	.000
N of Valid Cases	24			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

The inter-pathologists agreement for diagnosis of hepatic inflammation .

Crosstabs

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Diagnosis by pathologist 1 * Diagnosis by pathologist 2	24	51.1%	23	48.9%	47	100.0%

Diagnosis by pathologist 1 * Diagnosis by pathologist 2 Crosstabulation

			Diagnosis by pathologist 2				Total
			None	Mild	Moderate	Marked	
Diagnosis by pathologist 1	None	Count	2	1	0	0	3
		Expected Count	.3	.9	1.3	.6	3.0
	Mild	Count	0	6	3	0	9
		Expected Count	.8	2.6	3.8	1.9	9.0
	Moderate	Count	0	0	6	1	7
		Expected Count	.6	2.0	2.9	1.5	7.0
	Marked	Count	0	0	1	4	5
		Expected Count	.4	1.5	2.1	1.0	5.0
Total	Count	2	7	10	5	24	
	Expected Count	2.0	7.0	10.0	5.0	24.0	

Symmetric Measures

	Value	Asymp. Std. Error ^a	Approx. T ^b	Approx. Sig.
Measure of Agreement Kappa	.650	.124	5.278	.000
N of Valid Cases	24			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

The inter-pathologists agreement for diagnosis of Mallory bodies.

Crosstabs

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Diagnosis by pathologist 1 *	24	51.1%	23	48.9%	47	100.0%
Diagnosis by pathologist 2						

Diagnosis by pathologist 1 * Diagnosis by pathologist 2 Crosstabulation

		Diagnosis by pathologist 2		Total
		Present	None present	
Diagnosis by pathologist 1 Present	Count	6	1	7
	Expected Count	2.0	5.0	7.0
None present	Count	1	16	17
	Expected Count	5.0	12.0	17.0
Total	Count	7	17	24
	Expected Count	7.0	17.0	24.0

Symmetric Measures

	Value	Asymp. Std. Error ^a	Approx. T ^b	Approx. Sig.
Measure of Agreement Kappa	.798	.136	3.911	.000
N of Valid Cases	24			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

APPENDIX 5

Distribution of hepatic steatosis data and hepatic inflammation data in percents between control group and treatment groups

**Explore
Treatment groups**

Case Processing Summary

Treatment groups		Cases					
		Valid		Missing		Total	
		N	Percent	N	Percent	N	Percent
Hepatic steatosis	Control group	6	100.0%	0	.0%	6	100.0%
	Group 1	6	100.0%	0	.0%	6	100.0%
	Group 2	6	100.0%	0	.0%	6	100.0%
	Group 3	6	100.0%	0	.0%	6	100.0%

Descriptives

Treatment groups			Statistic	Std. Error
Hepatic steatosis	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		2.50	.224
	95% Confidence Interval for Mean	Lower Bound	1.93	
		Upper Bound	3.07	
	5% Trimmed Mean		2.50	
	Median		2.50	
	Variance		.300	
	Std. Deviation		.548	
	Minimum		2	
	Maximum		3	
	Range		1	
	Interquartile Range		1	
	Skewness		.000	.845
	Kurtosis		-3.333-	1.741
	Group 2	Mean		1.50
95% Confidence Interval for Mean		Lower Bound	.93	
		Upper Bound	2.07	
5% Trimmed Mean			1.50	
Median			1.50	
Variance			.300	
Std. Deviation			.548	
Minimum			1	
Maximum			2	
Range			1	
Interquartile Range			1	
Skewness			.000	.845
Kurtosis			-3.333-	1.741
Group 3		Mean		.83
	95% Confidence Interval for Mean	Lower Bound	.40	
		Upper Bound	1.26	
	5% Trimmed Mean		.87	

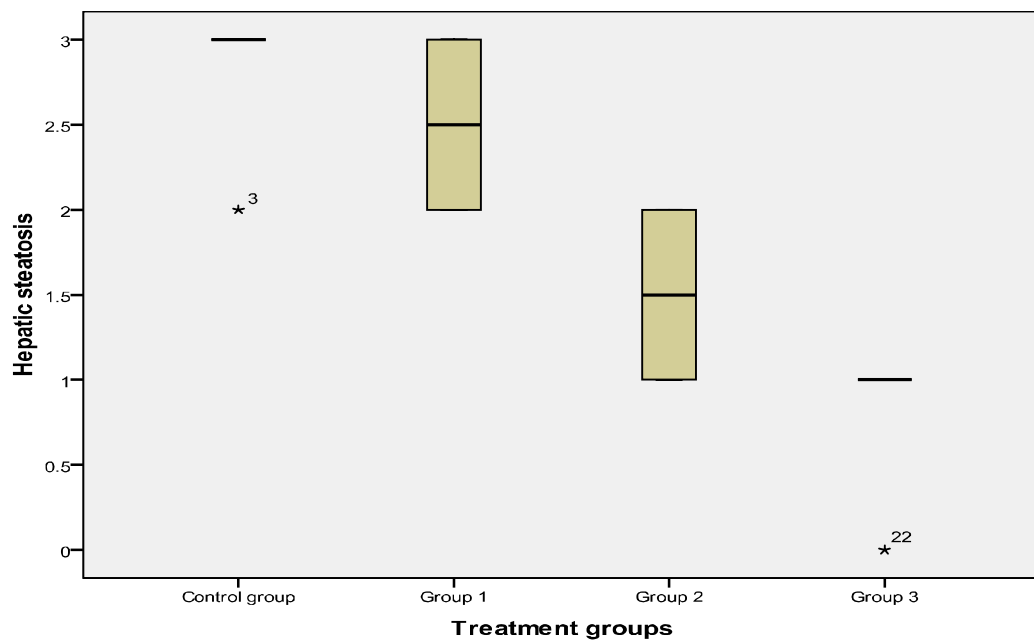
Median	1.00	
Variance	.167	
Std. Deviation	.408	
Minimum	0	
Maximum	1	
Range	1	
Interquartile Range	0	
Skewness	-2.449	.845
Kurtosis	6.000	1.741

Tests of Normality

Treatment groups		Kolmogorov-Smirnov ^a			Shapiro-Wilk		
		Statistic	Df	Sig.	Statistic	df	Sig.
Hepatic steatosis	Control group	.492	6	.000	.496	6	.000
	Group 1	.319	6	.056	.683	6	.004
	Group 2	.319	6	.056	.683	6	.004
	Group 3	.492	6	.000	.496	6	.000

a. Lilliefors Significance Correction

Detrended Normal Q-Q Plot of Hepatic steatosis



Explore

Treatment groups

Case Processing Summary

Treatment groups		Cases					
		Valid		Missing		Total	
		N	Percent	N	Percent	N	Percent
Hepatic inflammation	Control group	6	100.0%	0	.0%	6	100.0%
	Group 1	6	100.0%	0	.0%	6	100.0%
	Group 2	6	100.0%	0	.0%	6	100.0%
	Group 3	6	100.0%	0	.0%	6	100.0%

Descriptives

Treatment groups			Statistic	Std. Error
Hepatic inflammation	Control group	Mean	3.83	.167
		95% Confidence Interval for Mean	Lower Bound 3.40	Upper Bound 4.26
		5% Trimmed Mean	3.87	
		Median	4.00	
		Variance	.167	
		Std. Deviation	.408	
		Minimum	3	
		Maximum	4	
		Range	1	
		Interquartile Range	0	
		Skewness	-2.449	.845
		Kurtosis	6.000	1.741
		Group 1	Mean	2.67
95% Confidence Interval for Mean	Lower Bound 1.81		Upper Bound 3.52	
5% Trimmed Mean	2.74			

	Median		3.00	
	Variance		.667	
	Std. Deviation		.816	
	Minimum		1	
	Maximum		3	
	Range		2	
	Interquartile Range		0	
	Skewness		-2.449-	.845
	Kurtosis		6.000	1.741
Group 2	Mean		1.33	.333
	95% Confidence Interval for Mean	Lower Bound	.48	
		Upper Bound	2.19	
	5% Trimmed Mean		1.26	
	Median		1.00	
	Variance		.667	
	Std. Deviation		.816	
	Minimum		1	
	Maximum		3	
	Range		2	
	Interquartile 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	

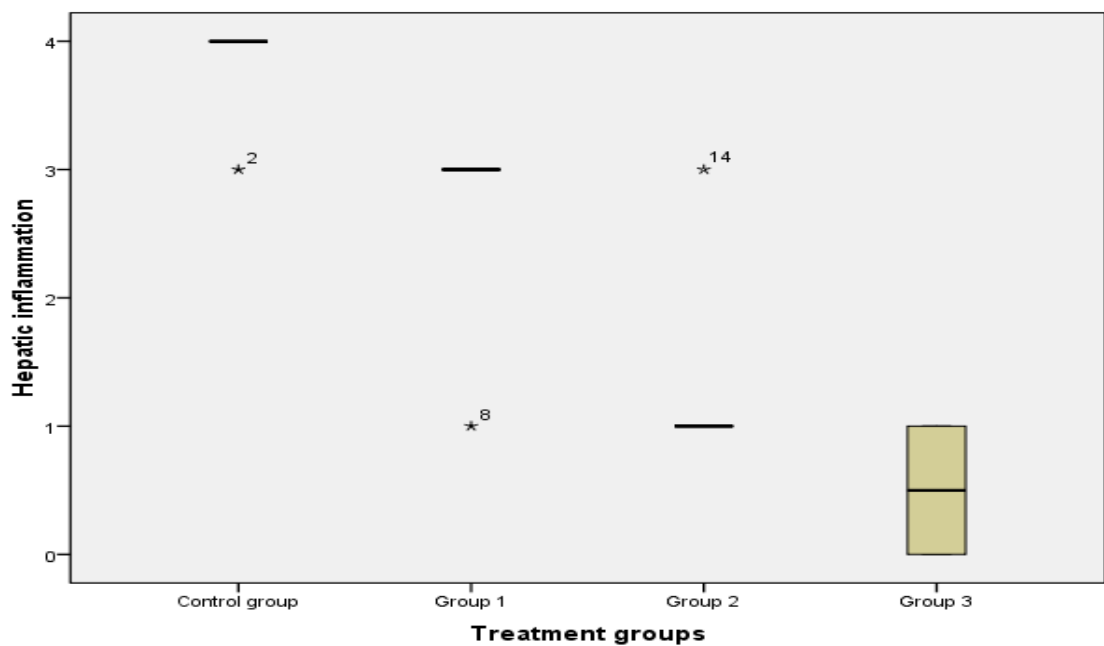
Variance	.300	
Std. Deviation	.548	
Minimum	0	
Maximum	1	
Range	1	
Interquartile Range	1	
Skewness	.000	.845
Kurtosis	-3.333-	1.741

Tests of Normality

Treatment groups	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
	Statistic	Df	Sig.	Statistic	df	Sig.
Hepatic inflammation Control group	.492	6	.000	.496	6	.000
Group 1	.492	6	.000	.496	6	.000
Group 2	.492	6	.000	.496	6	.000
Group 3	.319	6	.056	.683	6	.004

a. Lilliefors Significance Correction

Detrended Normal Q-Q Plot of Hepatic inflammation



Explore

Case Processing Summary

Treatment groups		Cases					
		Valid		Missing		Total	
		N	Percent	N	Percent	N	Percent
Mallory bodies	Control group	6	100.0%	0	.0%	6	100.0%
	Group 1	6	100.0%	0	.0%	6	100.0%
	Group 2	6	100.0%	0	.0%	6	100.0%
	Group 3	6	100.0%	0	.0%	6	100.0%

Descriptives^a

Treatment groups			Statistic	Std. Error	
Mallory bodies	Control group	Mean	.33	.211	
		95% Confidence Interval for Mean	Lower Bound	-.21	
			Upper Bound	.88	
		5% Trimmed Mean	.31		
		Median	.00		
		Variance	.267		
		Std. Deviation	.516		
		Minimum	0		
		Maximum	1		
		Range	1		
		Interquartile Range	1		
		Skewness	.968	.845	
		Kurtosis	-1.875	1.741	
Group 1	Mean	.67	.211		
	95% Confidence Interval for Mean	Lower Bound	.12		
		Upper Bound	1.21		
	5% Trimmed Mean	.69			
	Median	1.00			
	Variance	.267			

	Std. Deviation		.516	
	Minimum		0	
	Maximum		1	
	Range		1	
	Interquartile Range		1	
	Skewness		-.968-	.845
	Kurtosis		-1.875-	1.741
Group 2	Mean		.83	.167
	95% Confidence Interval for Mean	Lower Bound	.40	
		Upper Bound	1.26	
	5% Trimmed Mean		.87	
	Median		1.00	
	Variance		.167	
	Std. Deviation		.408	
	Minimum		0	
	Maximum		1	
	Range		1	
	Interquartile Range		0	
	Skewness		-2.449-	.845
	Kurtosis		6.000	1.741

a. Mallory bodies is constant when Treatment groups = Group 3. It has been omitted.

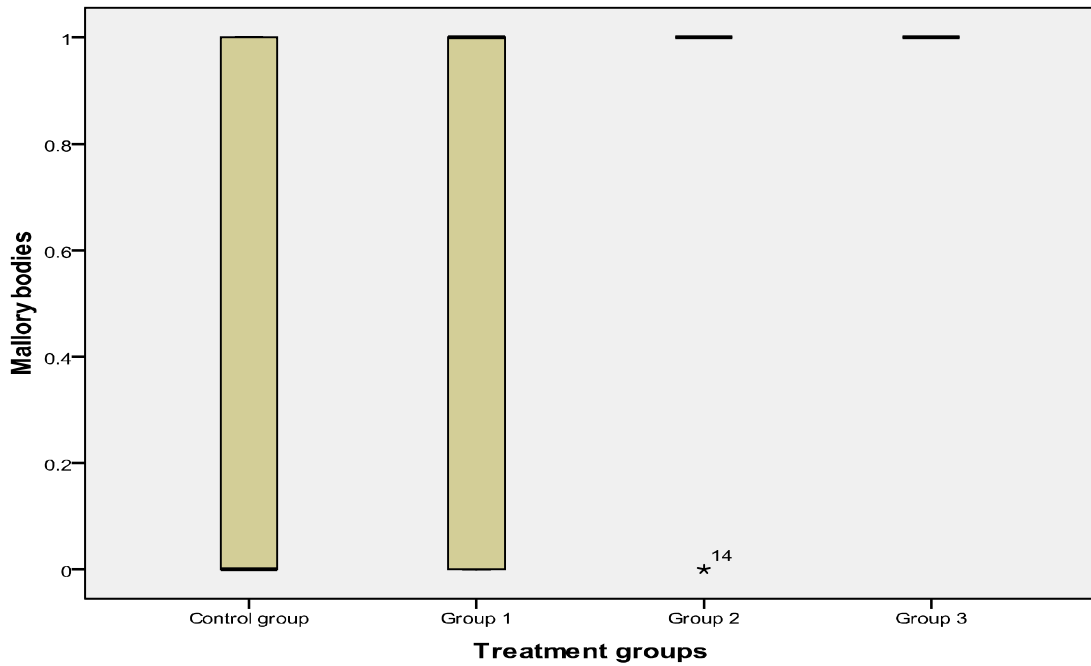
Tests of Normality^b

Treatment groups		Kolmogorov-Smirnov ^a			Shapiro-Wilk		
		Statistic	Df	Sig.	Statistic	Df	Sig.
Mallory bodies	Control group	.407	6	.002	.640	6	.001
	Group 1	.407	6	.002	.640	6	.001
	Group 2	.492	6	.000	.496	6	.000

a. Lilliefors Significance Correction

b. Mallory bodies is constant when Treatment groups = Group 3. It has been omitted.

Detrended Normal Q-Q Plots of Mallory bodies



Explore

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Hepatic steatosis	24	100.0%	0	.0%	24	100.0%
Hepatic inflammation	24	100.0%	0	.0%	24	100.0%
Mallory bodies	24	100.0%	0	.0%	24	100.0%

Descriptives

		Statistic	Std. Error	
Hepatic steatosis	Mean	1.92	.190	
	95% Confidence Interval for Mean	Lower Bound	1.52	
		Upper Bound	2.31	
	5% Trimmed Mean	1.95		
	Median	2.00		
	Variance	.862		
	Std. Deviation	.929		

	Minimum		0	
	Maximum		3	
	Range		3	
	Interquartile Range		2	
	Skewness		-.179-	.472
	Kurtosis		-1.145-	.918
Hepatic inflammation	Mean		2.38	.275
	95% Confidence Interval for Mean	Lower Bound	1.81	
		Upper Bound	2.94	
	5% Trimmed Mean		2.42	
	Median		3.00	
	Variance		1.810	
	Std. Deviation		1.345	
	Minimum		0	
	Maximum		4	
	Range		4	
	Interquartile Range		2	
	Skewness		-.411-	.472
	Kurtosis		-1.279-	.918
	Mallory bodies	Mean		.71
95% Confidence Interval for Mean		Lower Bound	.51	
		Upper Bound	.90	
5% Trimmed Mean			.73	
Median			1.00	
Variance			.216	
Std. Deviation			.464	
Minimum			0	
Maximum			1	
Range			1	
Interquartile Range			1	
Skewness			-.979-	.472

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Hepatic steatosis	24	100.0%	0	.0%	24	100.0%
Hepatic inflammation	24	100.0%	0	.0%	24	100.0%
Kurtosis					-1.145-	.918

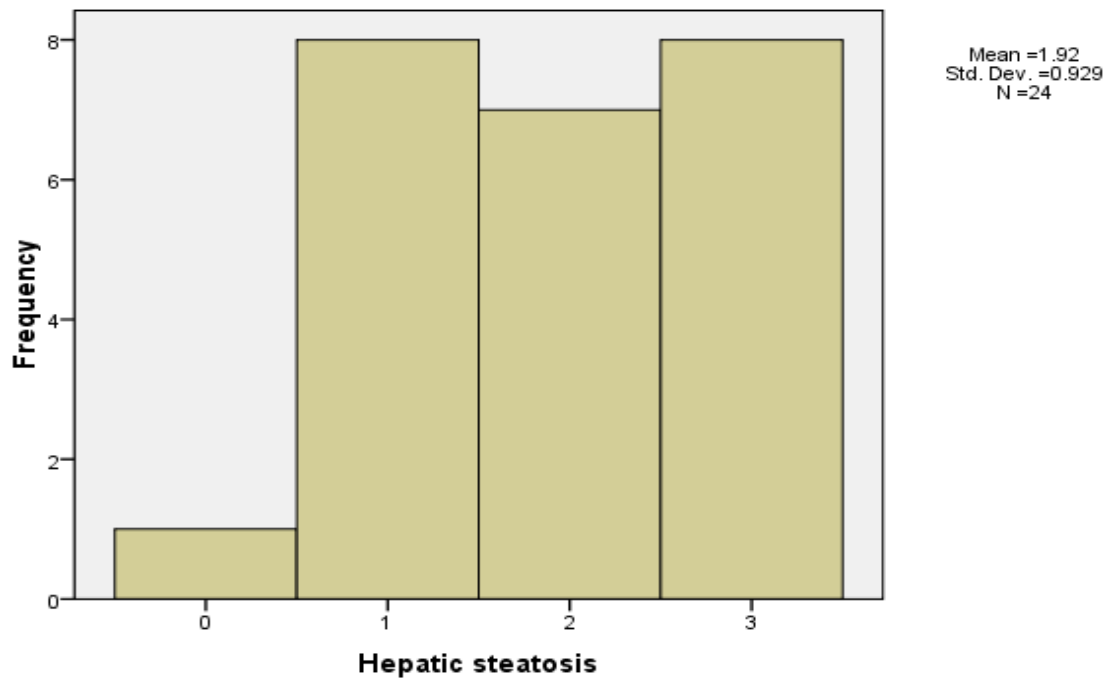
Tests of Normality

	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
	Statistic	Df	Sig.	Statistic	df	Sig.
Hepatic steatosis	.213	24	.006	.846	24	.002
Hepatic inflammation	.304	24	.000	.835	24	.001
Mallory bodies	.443	24	.000	.573	24	.000

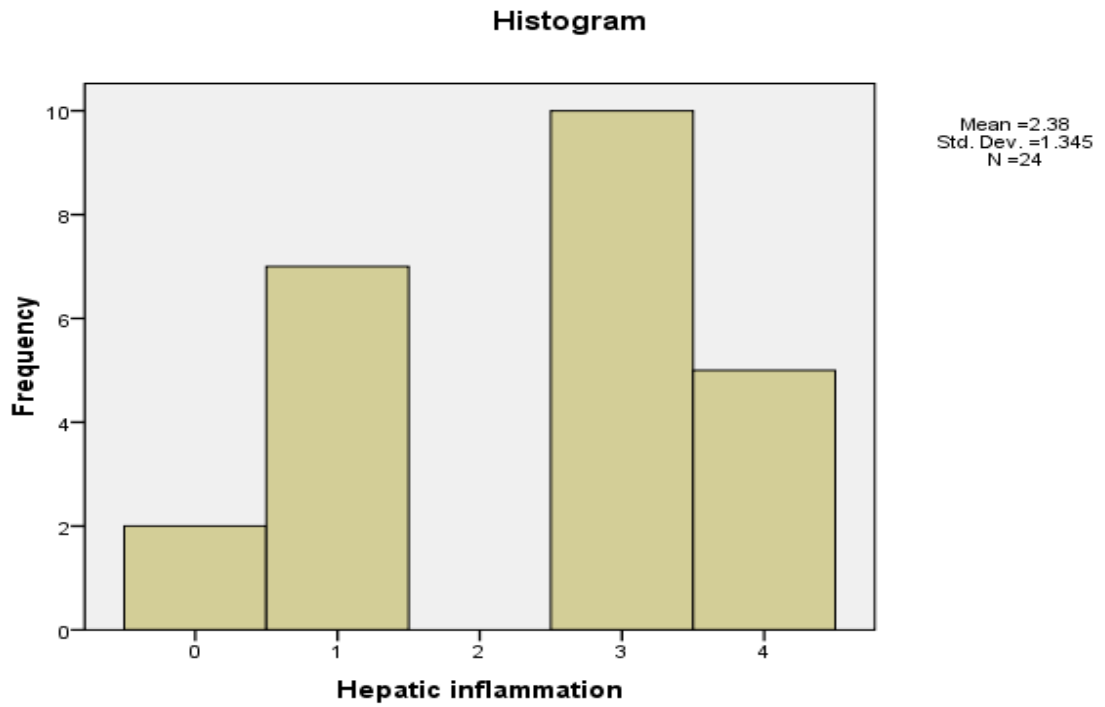
a. Lilliefors Significance Correction

Hepatic steatosis

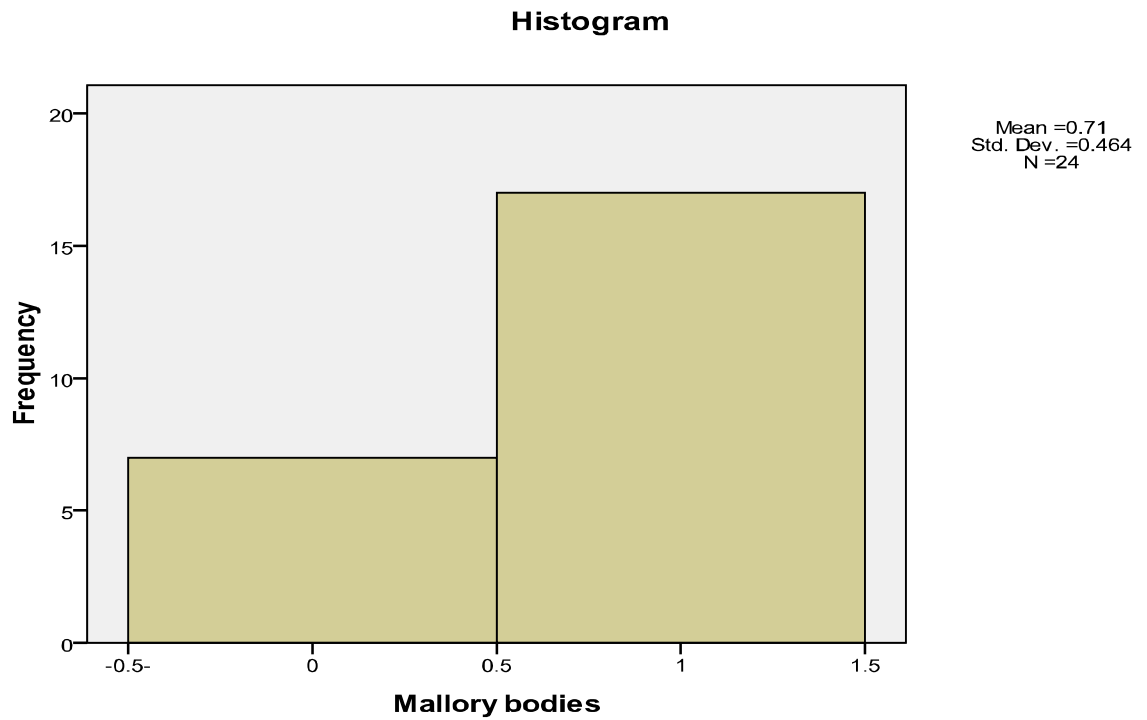
Histogram



Hepatic inflammation



Mallory bodies



NPar Tests
Kruskal-Wallis Test

Ranks

	Treatment groups	N	Mean Rank
Hepatic steatosis	Control group	6	19.25
	Group 1	6	16.75
	Group 2	6	9.25
	Group 3	6	4.75
	Total	24	
Hepatic inflammation	Control group	6	21.00
	Group 1	6	14.67
	Group 2	6	9.33
	Group 3	6	5.00
	Total	24	

Test Statistics^{a,b}

	Hepatic steatosis	Hepatic inflammation
Chi-Square	17.848	18.823
df	3	3
Asymp. Sig.	.000	.000

a. Kruskal Wallis Test

b. Grouping Variable: Treatment groups

NPar Tests
Mann-Whitney Test

Ranks

	Treatment groups	N	Mean Rank	Sum of Ranks
Hepatic steatosis	Control group	6	7.50	45.00
	Group 1	6	5.50	33.00
	Total	12		
Hepatic inflammation	Control group	6	9.08	54.50
	Group 1	6	3.92	23.50
	Total	12		

Test Statistics^b

	Hepatic steatosis	Hepatic inflammation
Mann-Whitney U	12.000	2.500
Wilcoxon W	33.000	23.500
Z	-1.173-	-2.762-
Asymp. Sig. (2-tailed)	.241	.006
Exact Sig. [2*(1-tailed Sig.)]	.394 ^a	.009 ^a

a. Not corrected for ties.

b. Grouping Variable: Treatment groups

NPar Tests

Mann-Whitney Test

Ranks

		Treatment groups	N	Mean Rank	Sum of Ranks
Hepatic steatosis	Control group		6	9.25	55.50
	Group 2		6	3.75	22.50
	Total		12		
Hepatic inflammation	Control group		6	9.42	56.50
	Group 2		6	3.58	21.50
	Total		12		

Test Statistics^b

	Hepatic steatosis	Hepatic inflammation
Mann-Whitney U	1.500	.500
Wilcoxon W	22.500	21.500
Z	-2.815-	-3.028-
Asymp. Sig. (2-tailed)	.005	.002
Exact Sig. [2*(1-tailed Sig.)]	.004 ^a	.002 ^a

a. Not corrected for ties.

b. Grouping Variable: Treatment groups

NPar Tests
Mann-Whitney Test

Ranks

	Treatment groups	N	Mean Rank	Sum of Ranks
Hepatic steatosis	Control group	6	9.50	57.00
	Group 3	6	3.50	21.00
	Total	12		
Hepatic inflammation	Control group	6	9.50	57.00
	Group 3	6	3.50	21.00
	Total	12		

Test Statistics^b

	Hepatic steatosis	Hepatic inflammation
Mann-Whitney U	.000	.000
Wilcoxon W	21.000	21.000
Z	-3.108-	-3.035-
Asymp. Sig. (2-tailed)	.002	.002
Exact Sig. [2*(1-tailed Sig.)]	.002 ^a	.002 ^a

a. Not corrected for ties.

b. Grouping Variable: Treatment groups

NPar Tests
Mann-Whitney Test

Ranks

	Treatment groups	N	Mean Rank	Sum of Ranks
Hepatic steatosis	Group 1	6	8.75	52.50
	Group 2	6	4.25	25.50
	Total	12		
Hepatic inflammation	Group 1	6	8.50	51.00
	Group 2	6	4.50	27.00
	Total	12		

Test Statistics^b

	Hepatic steatosis	Hepatic inflammation
Mann-Whitney U	4.500	6.000
Wilcoxon W	25.500	27.000
Z	-2.345-	-2.211-
Asymp. Sig. (2-tailed)	.019	.027
Exact Sig. [2*(1-tailed Sig.)]	.026 ^a	.065 ^a

a. Not corrected for ties.

b. Grouping Variable: Treatment groups

NPar Tests

Mann-Whitney Test

Ranks

	Treatment groups	N	Mean Rank	Sum of Ranks
Hepatic steatosis	Group 1	6	9.50	57.00
	Group 3	6	3.50	21.00
	Total	12		
Hepatic inflammation	Group 1	6	9.25	55.50
	Group 3	6	3.75	22.50
	Total	12		

Test Statistics^b

	Hepatic steatosis	Hepatic inflammation
Mann-Whitney U	.000	1.500
Wilcoxon W	21.000	22.500
Z	-3.035-	-2.815-
Asymp. Sig. (2-tailed)	.002	.005
Exact Sig. [2*(1-tailed Sig.)]	.002 ^a	.004 ^a

a. Not corrected for ties.

b. Grouping Variable: Treatment groups

NPar Tests
Mann-Whitney Test

Ranks

Treatment groups		N	Mean Rank	Sum of Ranks
Hepatic steatosis	Group 2	6	8.25	49.50
	Group 3	6	4.75	28.50
	Total	12		
Hepatic inflammation	Group 2	6	8.25	49.50
	Group 3	6	4.75	28.50
	Total	12		

Test Statistics^b

	Hepatic steatosis	Hepatic inflammation
Mann-Whitney U	7.500	7.500
Wilcoxon W	28.500	28.500
Z	-2.021-	-2.021-
Asymp. Sig. (2-tailed)	.043	.043
Exact Sig. [2*(1-tailed Sig.)]	.093 ^a	.093 ^a

a. Not corrected for ties.

b. Grouping Variable: Treatment groups

APPENDIX 6

ASSOCIATION BETWEEN ADMINISTRATION OF *NIGELLA SATIVA* SEEDS AND MALLORY BODIES

**The Kendall's tau-b test
Crosstabs**

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Treatment groups * Mallory bodies	24	100.0%	0	.0%	24	100.0%

Treatment groups * Mallory bodies Crosstabulation

			Mallory bodies		Total
			Present	None present	
Treatment groups	Control group	Count	4	2	6
		Expected Count	1.8	4.3	6.0
	Group 1	Count	2	4	6
		Expected Count	1.8	4.3	6.0
	Group 2	Count	1	5	6
		Expected Count	1.8	4.3	6.0
	Group 3	Count	0	6	6
		Expected Count	1.8	4.3	6.0
Total		Count	7	17	24
		Expected Count	7.0	17.0	24.0

Symmetric Measures

	Value	Asymp. Std. Error ^a	Approx. T ^b	Approx. Sig.
Ordinal by Ordinal Kendall's tau-b	.487	.127	3.250	.001
N of Valid Cases	24			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

APENDIX 7

RESEARCH PICTURES



Wistar rats housed in metal cages



Three doses of *Nigella sativa* seeds extract (0.5,1,1.5 g/kg/day for 8weeks)



Dose of ethanol (4ml of 40% ethanol/day for 8 weeks)



Administration of rats with *Nigella sativa* seeds extract along with ethanol by using the intragastric feeding model (sonde tube).



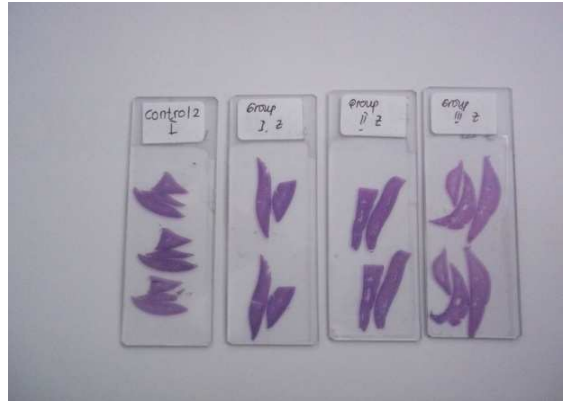
Taking the liver tissue after termination



Gross liver tissue



Liver tissue block



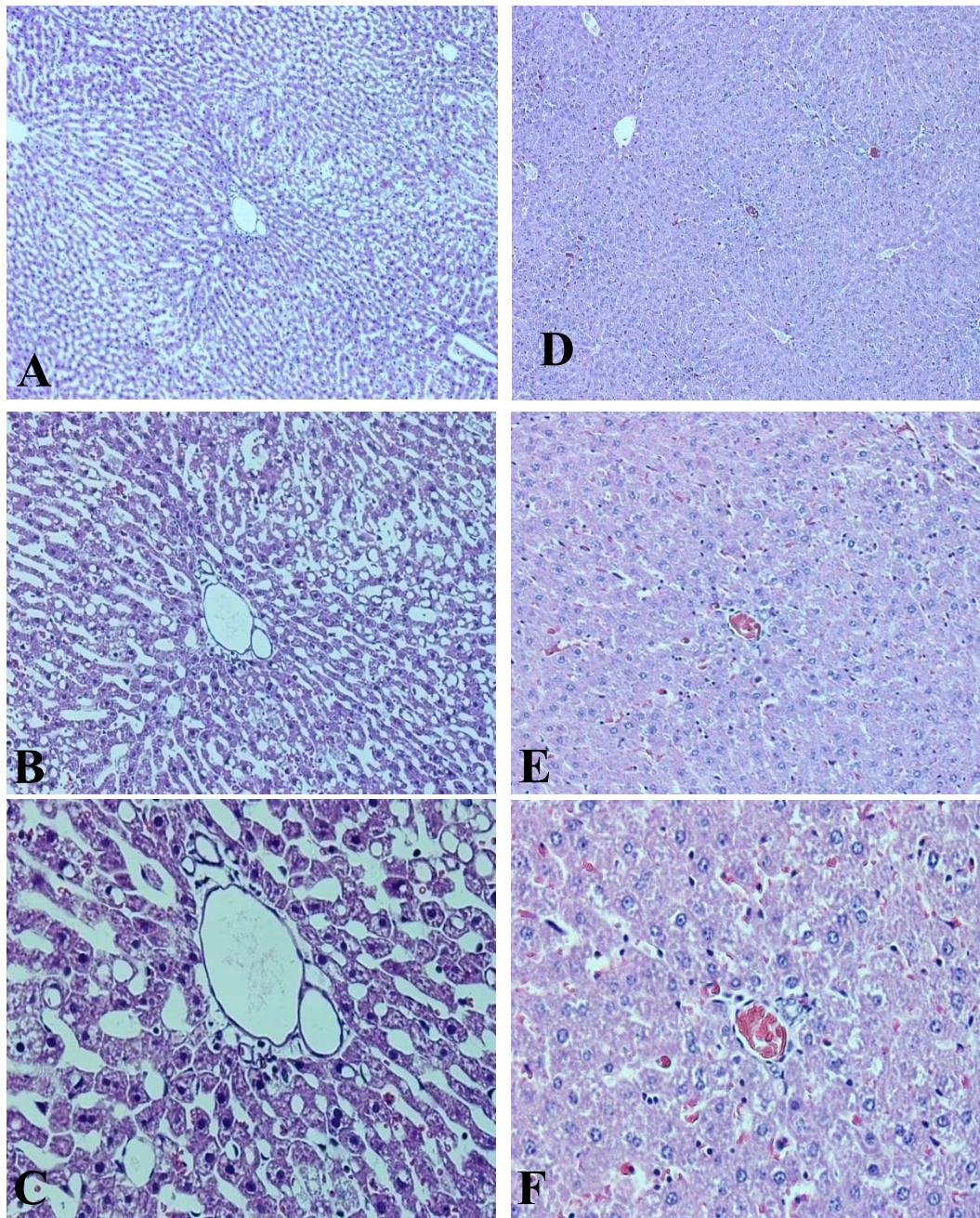
Liver tissue slides



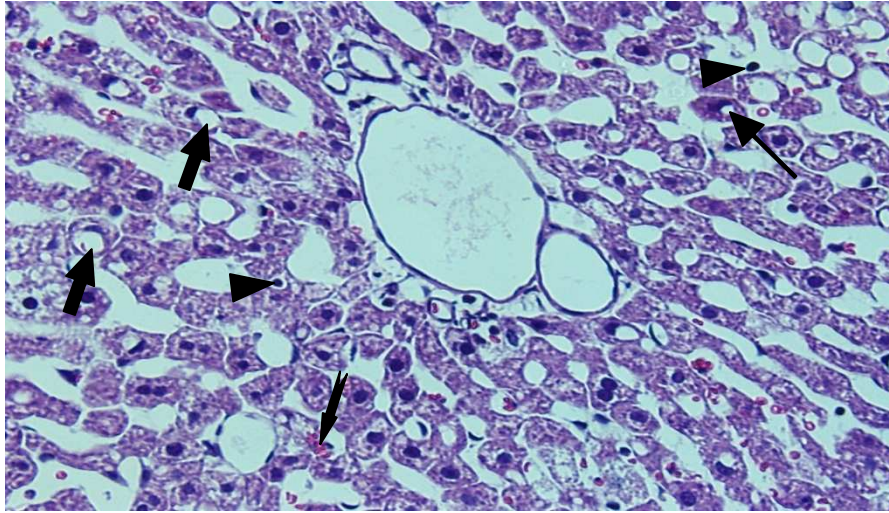
Examination of liver tissue slides by first pathologist



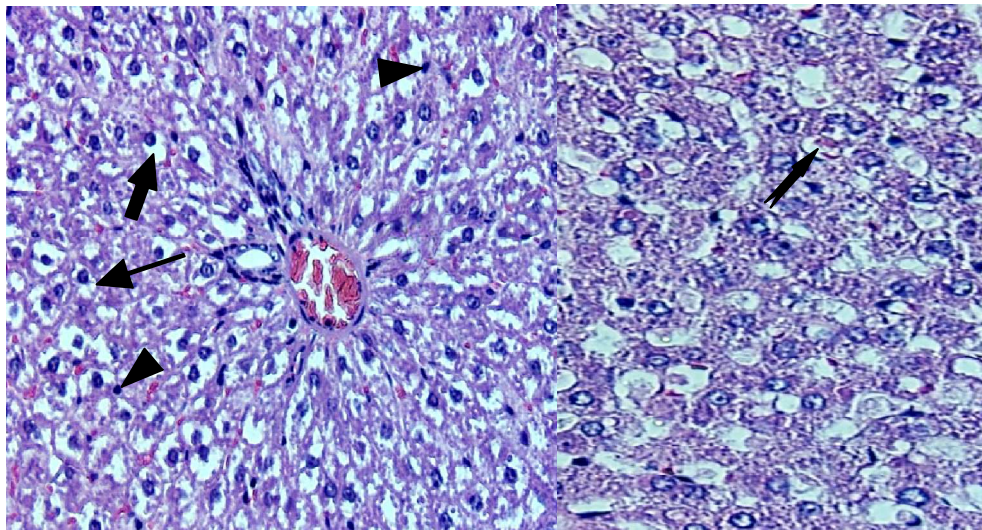
Examination of liver tissue slides with first pathologist in Pathology Anatomy
Department of Diponegoro University Semarang



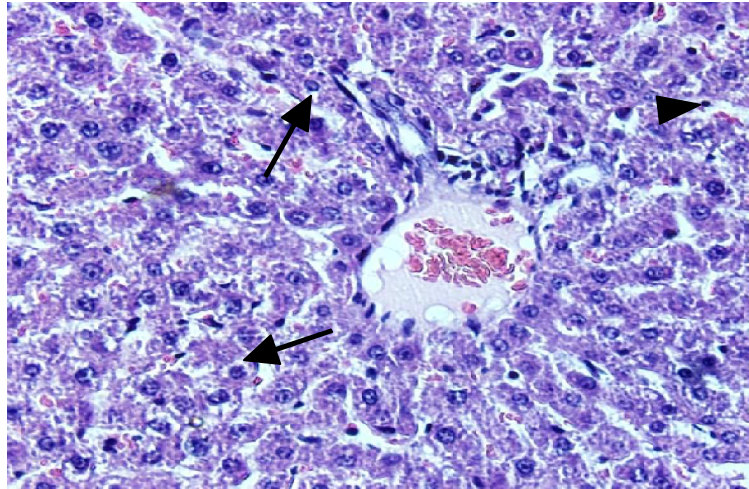
A, B, C ; Representative liver sections from Wistar rats with ethanol induced hepatic steatosis, inflammation, Mallory bodies present on control group with Haematoxylin Eosin (H&E) staining examination by using (A, 20× B, 20× and C, 40×). **D, E, F** ; Representative liver sections from Wistar rats pretreated with *Nigella sativa* seeds extract on treatment group3 with Haematoxylin Eosin (H&E) staining examination by using (D, 20× E, 20× and F, 40×).



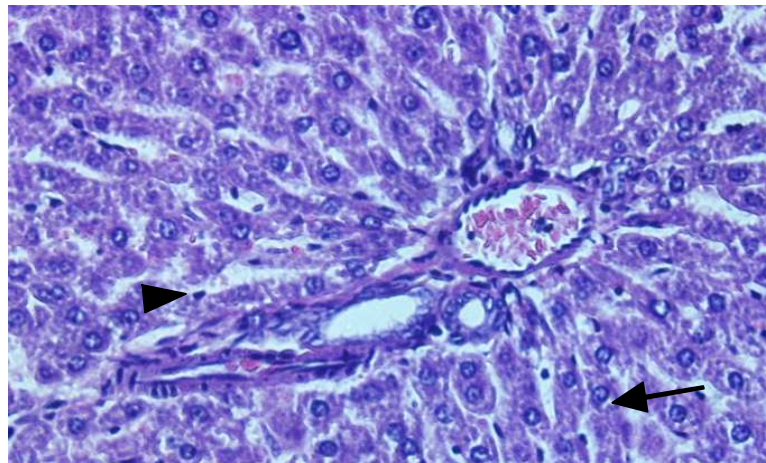
Haematoxylin Eosin (H&E) staining examination by using 400X magnification. Liver section in the control group, shows hepatocytes with severe hepatic steatosis as form small fat droplets (thin arrow), large fat droplets (thick arrow), marked inflammatory cells infiltrated as form lymphocytic(arrowhead), Mallory bodies is present (tailed arrow).



Haematoxylin Eosin (H&E) staining examination by using 400X magnification. Liver section in group 1, shows most of field with severe hepatic steatosis (long thin arrow, thick arrow) and moderate hepatic inflammation (arrowhead), some of hepatocytes contain Mallory bodies (tailed arrow).



Haematoxylin Eosin (H&E) staining examination by using 400X magnification. Liver section in group2, shows a hepatocyte with moderate most hepatic steatosis as form small fat droplets (long thin arrow), mild inflammatory cells infiltrated as form lymphocytic (arrowhead). Inflammation is usually mixed but it can predominantly be either neutrophilic or lymphocytic. Mallory bodies none present.



Haematoxylin Eosin (H&E) staining examination by using 400X magnification. Liver section in group 3, shows hepatocyte with mild hepatic steatosis (long thin arrow). Most of the fields showed mild inflammatory cells infiltrated (arrowhead). Mallory bodies none present in hepatocytes.