CHAPTER V

RESULTS

V.1. RESULT OF HEPATIC TISSUE CHANGES FROM HE STAINING EXAMINATION

All results of hepatic tissue changes were examined from total 24 rats ,24 livers of the rats which were prepared into tissue slides after end experiment.

In control group showed hepatic steatosis of both macrovesicular and microvesicular type (Fig. 8A), and inflammatory cells of portal tract were densely packed in all fields (Fig. 11A), Mallory bodies were present, but not to the greatly extent (Fig. 14A). In group 1 showed hepatic steatosis. Moreover, the hepatic steatosis appeared were not to greatly reduce, as well as inflammatory cells infiltrates of portal tract were packed and Mallory bodies were presented, but not to the extent seen in the liver of those rats treated with alcohol only. (Fig. 8B,11B,14B). In group 2 showed reduced of accumulation of small or large fat droplets in hepatocytes (Fig. 9C). In addition, inflammatory cells infiltrates were reduced (Fig. 12C), Mallory bodies were not present in the most of fields (Fig. 15C). In group 3 showed greatly reduced of accumulation of small or large fat droplet in hepatocytes (Fig. 9D).Also inflammatory cells infiltrates were greatly reduced (Fig. 12D), whereas Mallory bodies were not present in the most of fields (Fig. 15D).

V.2. RESULT OF HEPATIC STEATOSIS FROM HE STAINING EXAMINATION

In control group showed severe hepatic steatosis both of macro- and microvesicular type in the hepatocytes (Fig. 9A). In group1 showed hepatic steatosis. Moreover, the hepatic steatosis appeared were not to greatly reduced in the hepatocytes (Fig. 9B). In group 2 showed moderate hepatic steatosis in the hepatocytes (Fig. 10C). In group 3 showed greatly reduced in accumulation of small or large fat droplets in the hepatocytes (Fig. 10D).

Table 4. Groups descriptive statistic for hepatic steatosis from H&E staining examination.

Groups	Mean	Std.Diviation	Median (Min – Max)
Control	2.83	0.408	3.00 (2 – 3)
Group 1	2.50	0.548	2.50 (2 – 3)
Group2	1.50	0.548	1.50 (1 – 2)
Group3	0.83	0.408	1.00 (0 – 1)

The normality statistical tests were revealed by a non parametric statistical test called Shapiro-Wilk test (Test full result was attached at appendix 4), it's found that the hepatic steatosis data has not normal distribution (p= 0.002). The significance of differences in terms of hepatic steatosis between experimental groups were revealed by performing Kruskal-Wallis test. The hepatic steatosis data appear to be statistically significance (P= 0.000), subsequently carried output

Mann-Whitney test as post Hoc test to know the statistic differences between each two groups.

Box plot of the hepatic steatosis data

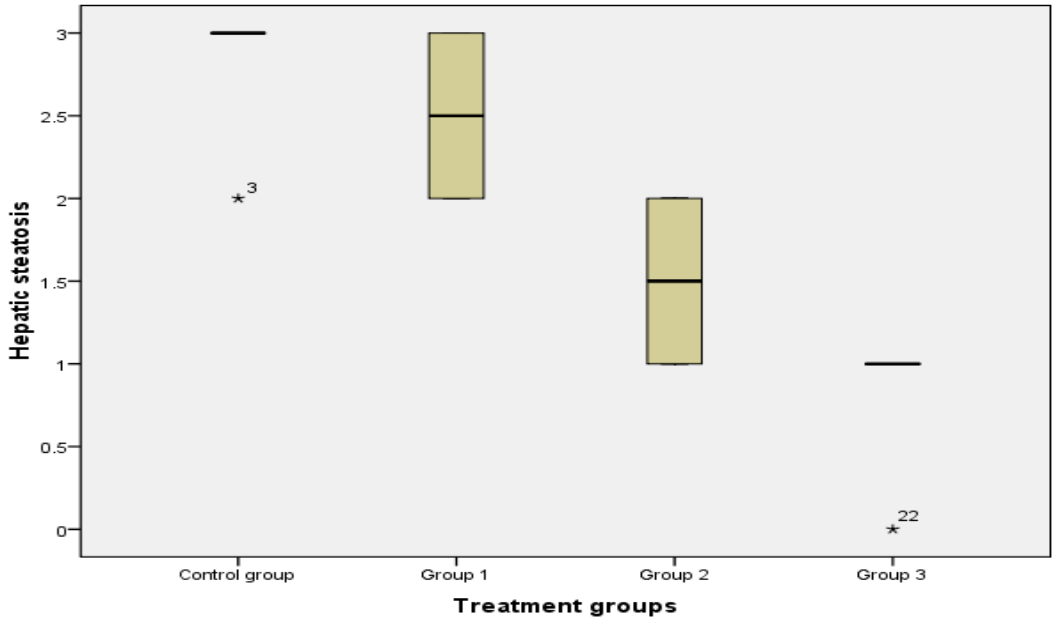

Figure 8. Box plot median hepatic steatosis of Wistar rats in control group, group1, group 2, and group 3.

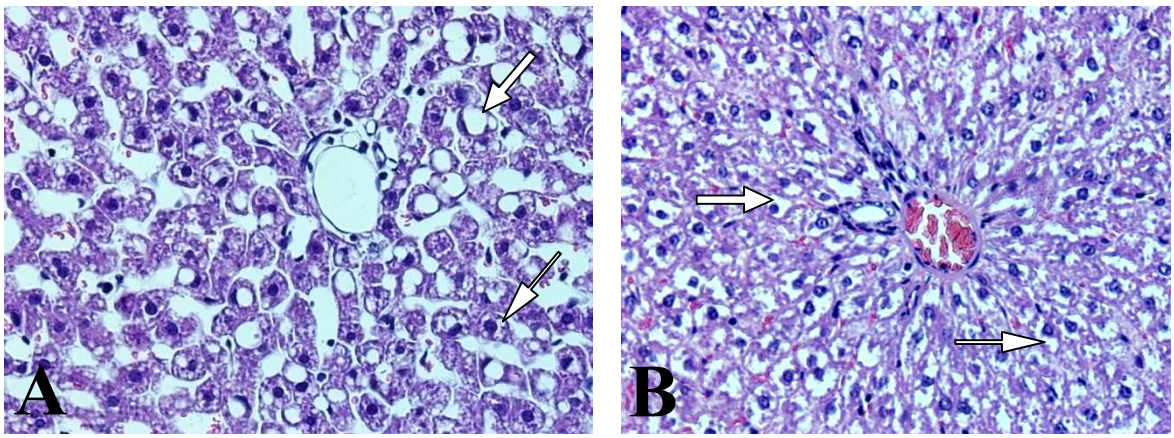

Figure 9. Haematoxylin Eosin (H&E) staining examination by using 400X magnification. (A) Control group, shows hepatocytes with severe hepatic steatosis as form small fat droplets (thin arrow), large fat droplets (thick arrow). (B) Group 1, shows most hepatocytes with severe hepatic steatosis of both small fat droblets (thin arrow), large fat droplets (thick arrow).

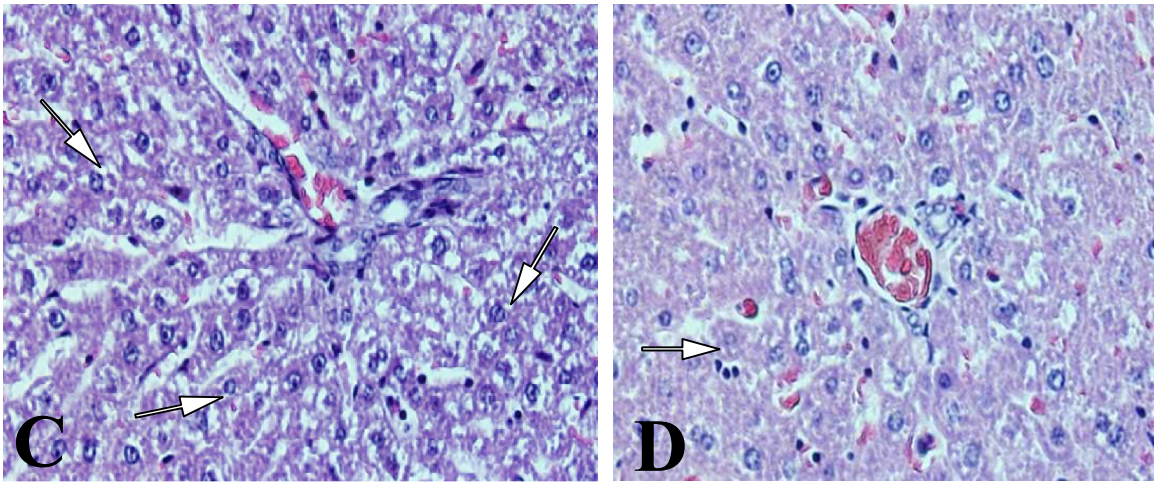

Figure 10. Haematoxylin Eosin (H&E) staining examination by using 400X magnification. (C) Group2, shows hepatocytes with moderate, most hepatic steatosis as form small fat droplets (thin arrow). (D) Group 3, shows hepatocytes with mild hepatic steatosis (thin arrow).

Table 5. Comparison between the groups (post Hoc test) as Mann-Whitney test, it is considered as statistically significant if P<0.05

	Control group	Group1	Group2
Group1	p= 0.241		
Group2	p= 0.005	p=0.019	
Group3	p= 0.002	p= 0.002	p= 0.043

From the table 5 its notice that the difference in terms of hepatic steatosis was statistically significant between the groups, except between control group and group1. From the analysis above its noted that hepatic steatosis induced by ethanol is relatively correlated with administration of *Nigella sativa* seeds extract, this applicable by the fact that the least hepatic steatosis occur in the group2, 3

comparing to the most hepatic steatosis that occur in control group (Full result was attached at appendix 4), however it should be noted that there was no statistically significant changes between control group and group 1 and this is mean that the almost of hepatocytes in two groups have shown hepatic steatosis (P= 0.241).

V.3. RESULT OF HEPATIC INFLAMMATION FROM HE STAINING EXAMINATION

In the control group, inflammatory cells of portal tract were densely packed in all fields (Fig. 12A). In group 1 showed inflammatory cells infiltrates of portal tract were packed, but not to the extent seen in the liver of those rats treated with alcohol only (Fig. 12B). In group 2 showed reduced of inflammatory cells infiltrates less than in group 1 (Fig. 13C). In group 3 showed greatly reduced of inflammatory cells infiltrates (Fig. 13D).

Table 6. Groups descriptive statistic for hepatic inflammation from H&E staining examination.

Groups	Mean	Std.Diviation	Median (Min - Max)
Control	3.83	0.408	4.00 (3 - 4)
Group 1	2.67	0.816	3.00 (1 - 3)
Group2	1.33	0.816	1.00 (1 - 3)
Group3	0.50	0.548	0.50 (0 - 1)

The significance of these differences was revealed by using non parametric statistical test was called Shapiro-Wilk test (Full result of test was attached at appendix 4), it's found that the hepatic inflammation data has not normal distribution as the Shapiro-Wilk test reveals significant result (P= 0.001). The significance of differences in terms of hepatic inflammation between experimental groups were revealed by performing Kruskal-Wallis test. The hepatic inflammation data appear to be statistically significance (P= 0.000), subsequently carried output Mann-Whitney test as post Hoc test to know the statistic differences between each two groups.

Box plot of the hepatic inflammation data

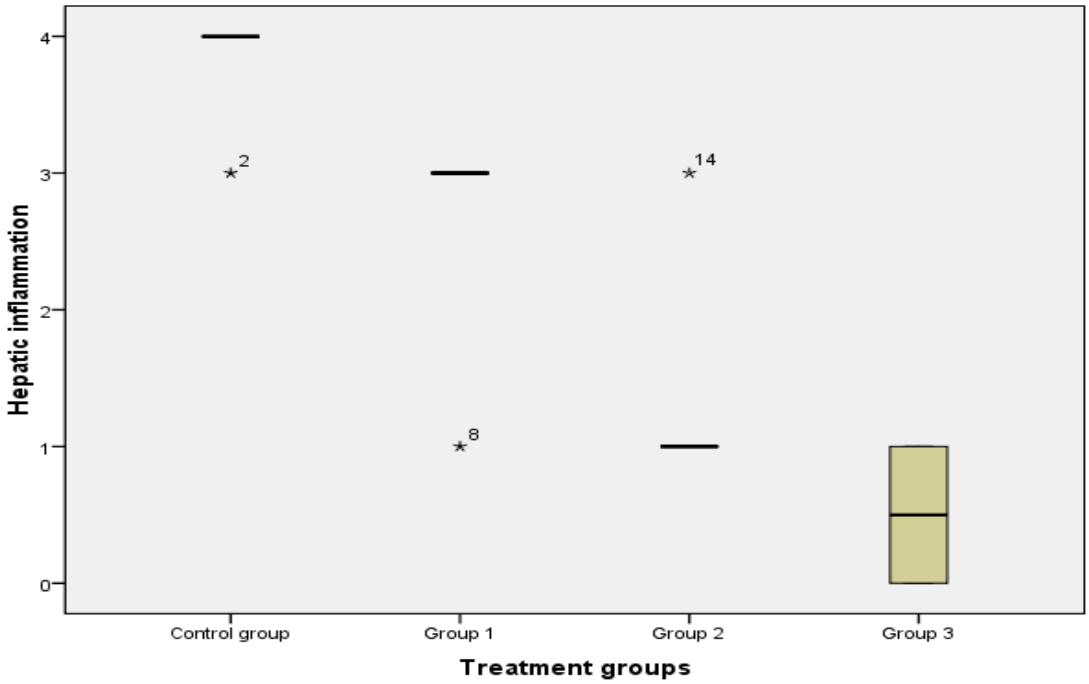

Figure11. Box plot median of hepatic inflammation of Wistar rats in control group, group1, group 2, and group 3.

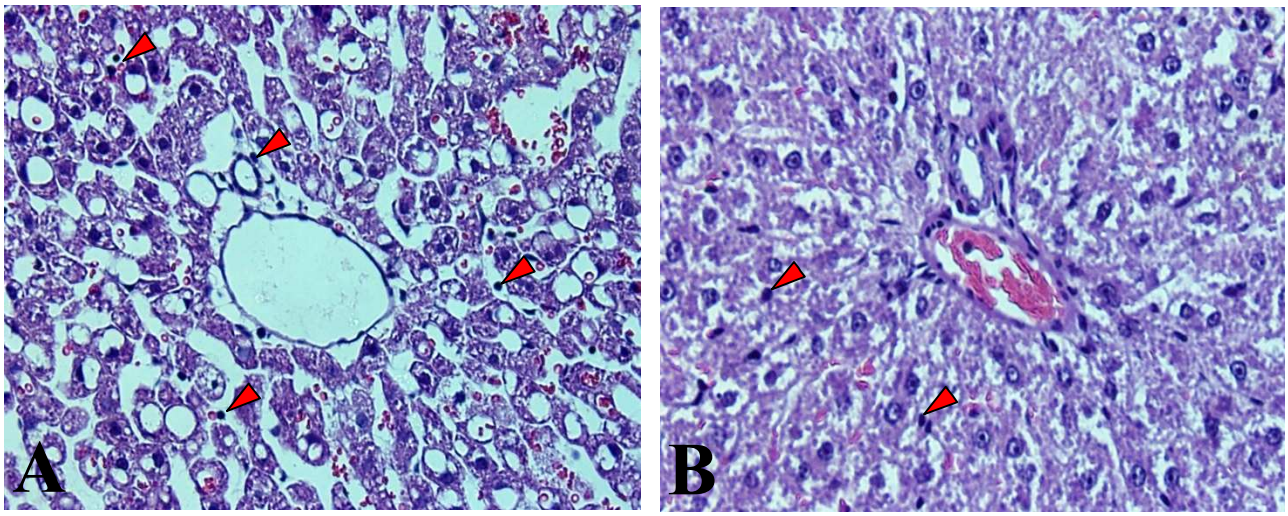

Figure 12. Haematoxylin Eosin (H&E) staining examination by using 400X magnification. (A) Control group, shows marked inflammatory cells infiltrated as form lymphocytic(arrowhead). (B) Group 1, shows moderate inflammatory cells infiltrated (arrowhead). Inflammation is usually mixed but it can predominantly be either neutrophilic or lymphocytic.

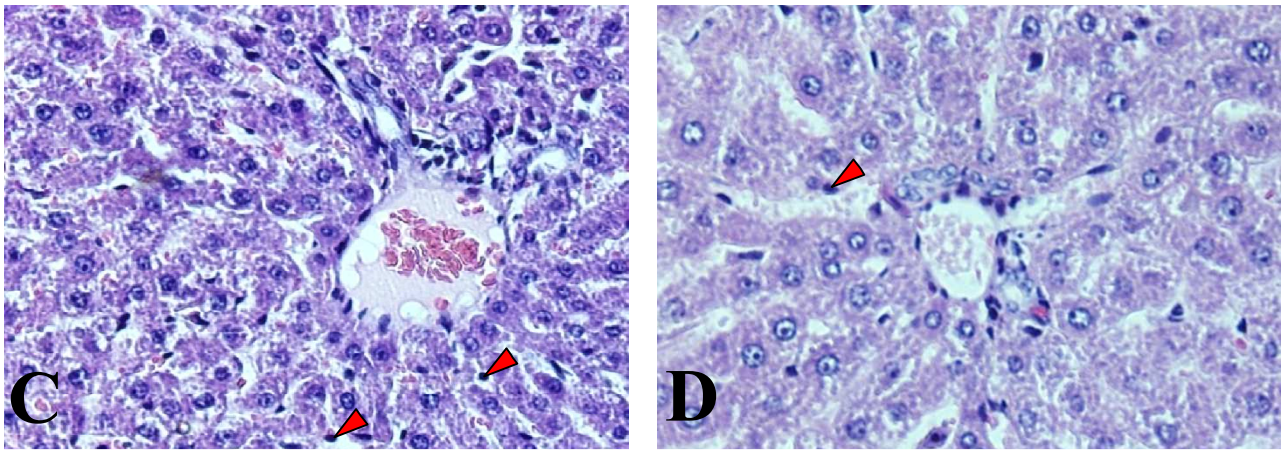

Figure 13. Haematoxylin Eosin (H&E) staining examination by using 400X magnification. (C) Group 2, shows mild inflammatory cells infiltrated as form lymphocytic (arrowhead). (D) Group 3, most of fields showed the least inflammatory cells infiltrated (arrowhead).

Table 7. Comparison between the groups (post Hoc test) as Mann-Whitney test, it is considered as statistically significant if P<0.05

	Control group	Group1	Group2
Group1	p= 0.006		
Group2	p= 0.002	p= 0.027	
Group3	p= 0.002	p= 0.005	p= 0.043

From the table 7 its notice that the difference in terms of hepatic inflammation was statistically significant between the groups. From the analysis above its noted that the hepatic inflammation that induced by ethanol is relatively correlated with administration of *Nigella sativa* seeds extract, this applicable by the fact that as an increase in the dose of *Nigella sativa* seeds extract between groups the hepatic inflammation become less and less and the least hepatic inflammation occur in the group 3 comparing to the most hepatic inflammation that occur in the control group. (Full results of the test were attached at appendix 4).

V.4. RESULT OF MALLORY BODIES FROM HE STAINING EXAMINATION

In control group showed Mallory bodies in the hepatocytes (Fig. 14A). In group1 showed Mallory bodies in some of hepatocytes, but not to the extent seen in the hepatocytes in the control group (Fig. 14B). In group 2, 3 greatly reduced in

Mallory bodies in the hepatocytes (Fig. 14C), whereas in group 3 was not shown Mallory bodies in most of the fields (Fig. 14D).

Table 8. Groups descriptive statistic for Mallory bodies from H&E staining examination.

Groups	Mean	Std.Diviation	Median (Min - Max)
Control	0.33	0.516	0.00 (0 - 1)
Group 1	0.67	0.516	1.00 (0 - 1)
Group2	0.83	0.408	1.00 (0 - 1)
Group3	0.83	0.408	1.00 (0 - 1)

Mallory bodies were decreased after treating with various doses of *Nigella sativa* extracts. The association between the presence of Mallory bodies and administrate with various doses of *Nigella sativa* extracts were statistically revealed by using an ordinal statistical test called A Kendall's tau-b test instead chi square test. Chi square test cannot be used for data analysis of examination results of Mallory bodies because there were empty cells and 8 cells (100%) have expected count less than 5 ; 50 the chi square isn't testing validity to this data analysis. Kendall's tau-b test has used to measure the association between treatment with *Nigella sativa* seeds extract and present or none present Mallory bodies in treatment groups. The Kendall's tau-b test was showed that there was a significant association ($p=0.001$) between the administration of *Nigella sativa* seeds extract and Mallory bodies in the treatment groups. (Full results of the test were attached at appendix 5).

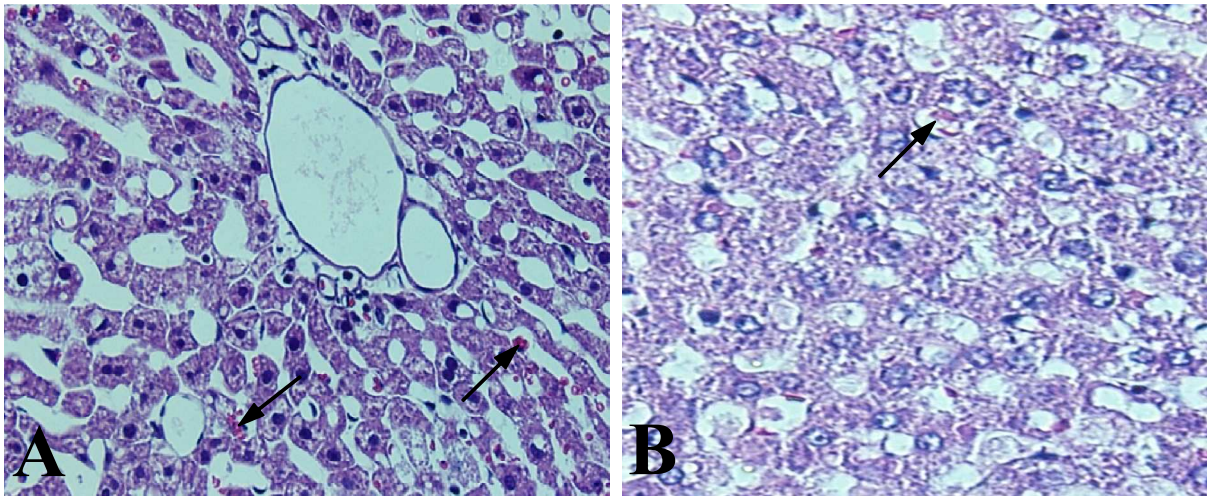

Figure 14. Haematoxylin Eosin (H&E) staining examination by using 400X magnification. (A) Control group, shows hepatocytes contain Mallory bodies. (B) Group 1, shows some of hepatocytes contain Mallory bodies (long thin arrow).

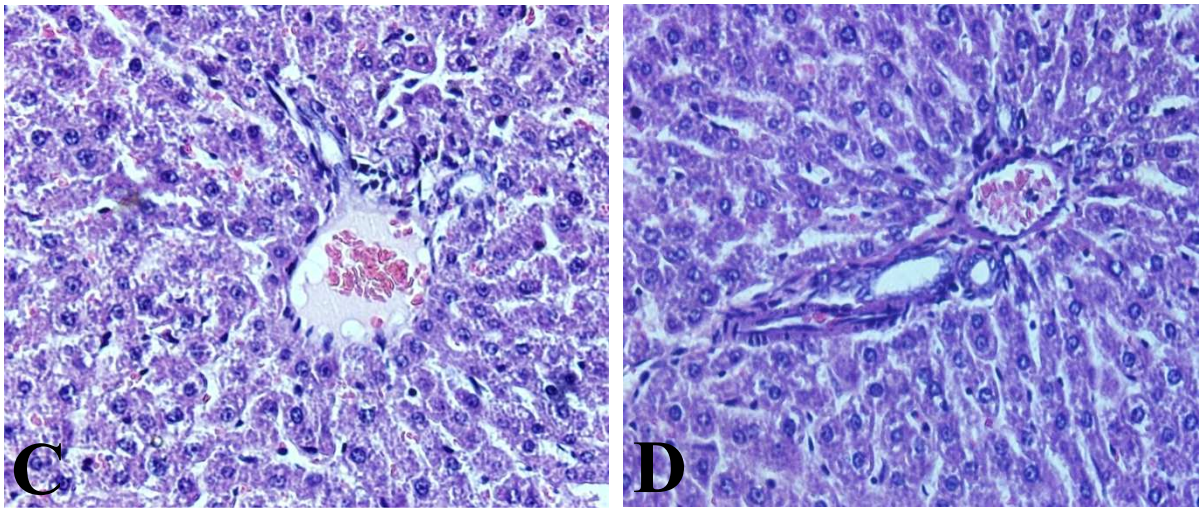

Figure 15. Haematoxylin Eosin (H&E) staining examination by using 400X magnification. (C) Group 2, shows hepatocytes without Mallory bodies. (D) roup 3, shows a hepatocytes without Mallory bodies.

V.5. Calculation of the inter-pathologists agreement, kappa score (k)

To get reliable test results (reliability), and valid result (validity) measurement of hepatic steatosis, hepatic inflammation and Mallory bodies were

diagnosed by two expert pathologists. Kappa Statistic (K) was used to assess inter-pathologists agreement for the reliability and validity to each diagnostic score. Kappa values for hepatic steatosis, hepatic inflammation and Mallory bodies was 0.764, 0.650 and 0.798 respectively (Kappa values $\geq$ 0.6). According to these values that, the inter-pathologists agreement was great between two pathologists. (Full results of the test were attached at appendix 3).