CHAPTER IV

RESULT

5.1 RESULT OF LIVER TISSUE DAMAGE FROM H&E STAINING.

Tissue damage were observed from H&E staining examination by Olympus PX51 light microscope with ocular lens 10 and objective lens 20 examination done by 5 randomized choosing filed , the examiner use the central vein as anatomic landmark and divide the distant far from the central vein by rows and describe the hepatic cell damage and inflammation according to this rows, the result where expressed according to the system that using the grading of normal, mild ,moderate and severe damage , from total 24 rats, 24 livers of rats were made into tissue slides.

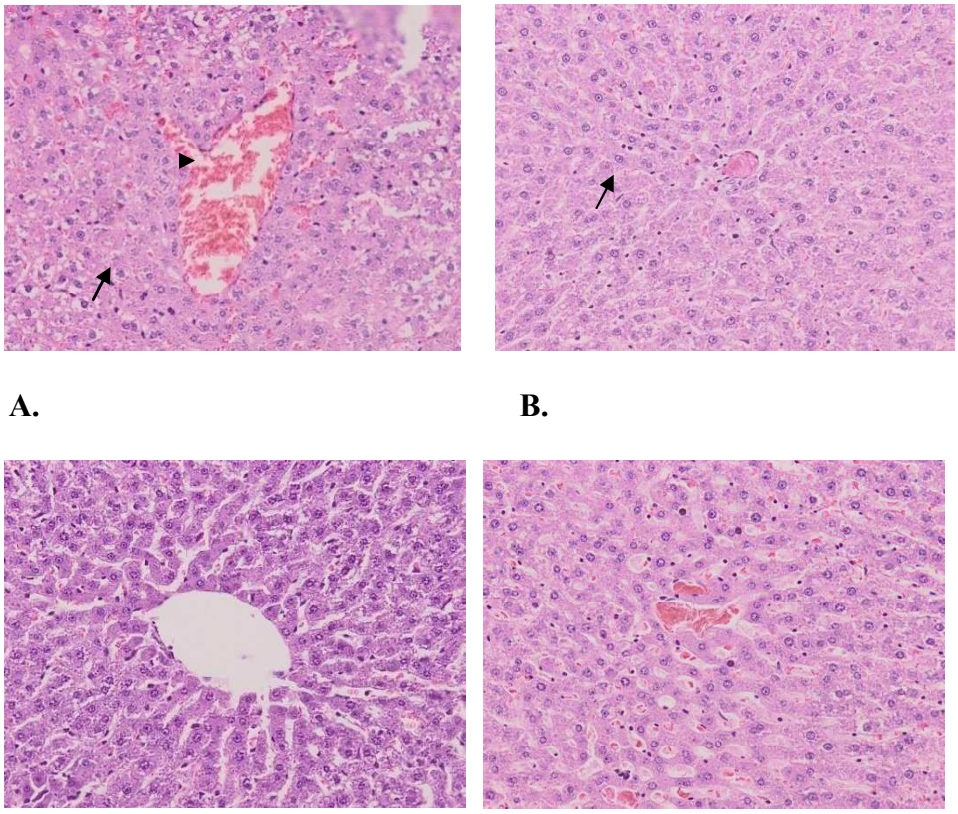

Figure 9. H&E staining examination using 200X magnification from A. Control group shows severe liver damage, B. Group 1, shows moderate damage of liver tissue, C. Group 2 shows mild damage, D. group 3 shows Mild liver tissue damage, arrow head: endothelial Injury, arrow: cell necrosis**.**

Table 4. Group's descriptive statistic for liver tissue damage from H&E staining examination.

Groups	Median	SD
Control	3	0.408
Group 1	2	0.516
Group2	1	0.167
Group3	0.50	0.548

As shown in Table 4, control group has the more liver tissue damage (Median 3) and group 3 has the smallest tissue damage among all groups (Median 0.50), Figure 10 shows that box plot median liver tissue damage in the smallest in group 3. From the box plot its notice that reading number 3 in control group is outlier reading because all the reading was severe and this reading was moderate, it's also notice that reading number 14 in group 2 was outlier reading because all the reading in this group was mild and this reading was moderate.

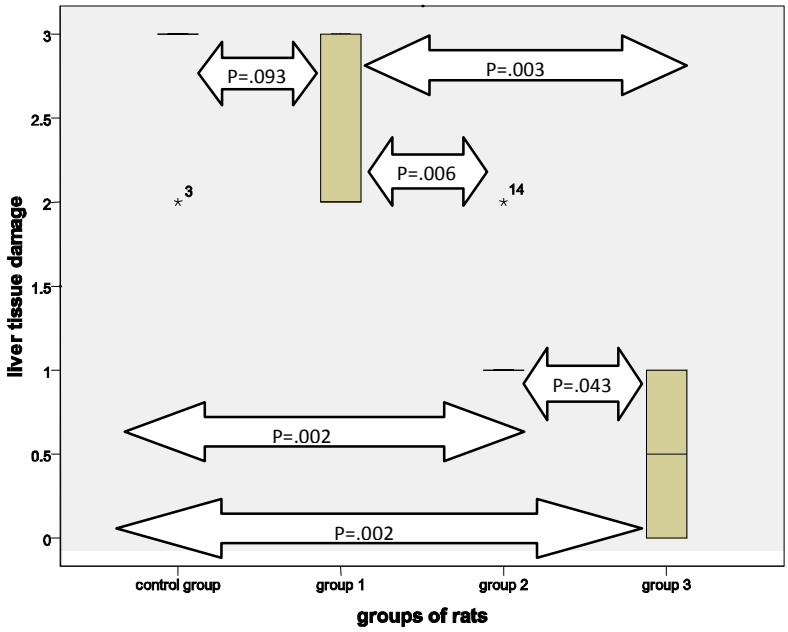

Figure10. Box plot median liver tissue damage of Wistar rats in control group, group1, group 2, and group 3.

The significance of these differences was revealed using statistic test.

Using Shapiro-Wilk test (test full result was attached on the appendix 3) , it's found that the data has not normal distribution as the Shapiro-Wilk test reveal

significant result (P =0.004) **,** by performing Kruskal-Wallis test the data appear to be statistically significance (P=0.000), subsequently carried out Mann-Whitney test as post Hoc test to know the statistic comparison between the groups.

From the box plot above its notice that the difference was statistically significance between the groups, except between control group and group 1 (P=0.093).

From the analysis above its noted that the liver tissue damage that induced by alcohol has statistical difference with giving Nigella sativa extract , this applicable by the fact that as the dose increase between group the damage become less and less and the least damage occur in the group 3 comparing to the most damage that occur in control group (full result at appendix 3),however its should be noted that there was no statistcally significant changes between control group and group 1 and this is mean that the two group almost have the same or near degree of damage .

5.2 RESULT OF TNFα EXPRESSION BY IMMUNOHISTOCHEMISTRY EXAMINATION.

Expression of TNFα observed by immunohistochemistry examination using TNFα antibody ,examination of slide done by Olympus PX51 light microscope using 10X ocular lens and 40X objective lens, examination and reading of the slides were done by counting the percentage of TNFα cells stained brown color in the cytoplasm as proportion score adding to it the intensity of the staining rated as none, mild, intermediate and strong, the result of the these two

score is a number which is called Allred score which then categorized into: : 0 – Allred (0*); 1 – Allred (2, 3, 4); 2 – Allred (5, 6); 3 – Allred (7, 8).

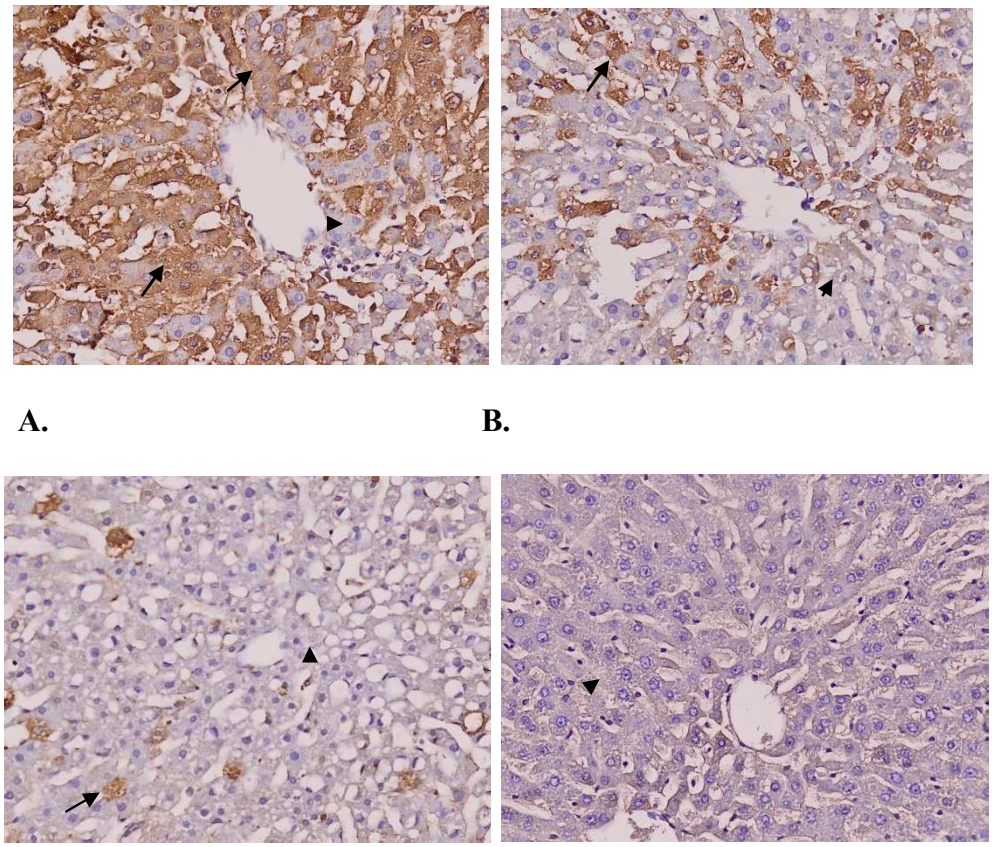

Figure 11. morphology of TNFα expression of Wister rats using 400X magnification ,the cells was strongly positively stained brown in the cytoplasm(arrow) , and cell that is negatively stained (arrow head)in control group figure A, Allred score 8(5+3) ,group 1 figure B ,Allred score 5 (3+2),figure C,group 2, Allred Score 2(1+1) ,figure D, group 3 ,Allred Score 0 (0+0).

Table 5. TNFα expression statistical analysis for all groups.

Groups	Median	SD
Control	3	0.408
Group 1	2	0.408
Group2	1	0.516
Group3	0.00	0.408

As shown in Table 5, control group has the most TNFα expression value (Median 3) and group 3 has the smallest TNFα expression value among all groups (Median 0.00) ,Figure 12 shows that box plot median TNFα expression is the smallest in group 3 and the most in control group, from the box plot its notice that reading number 5 in control group is outlier reading because all the reading was 3 Allred and this reading was 2 Allred, reading number 11 in group 2 was outlier also cause all the reading in this group was 2 Allred and this reading was 3 Allred ,its also notice that reading number 21 in group 3 was outlier reading because all the reading in this group was 0 Allred and this reading was 1 Allred .

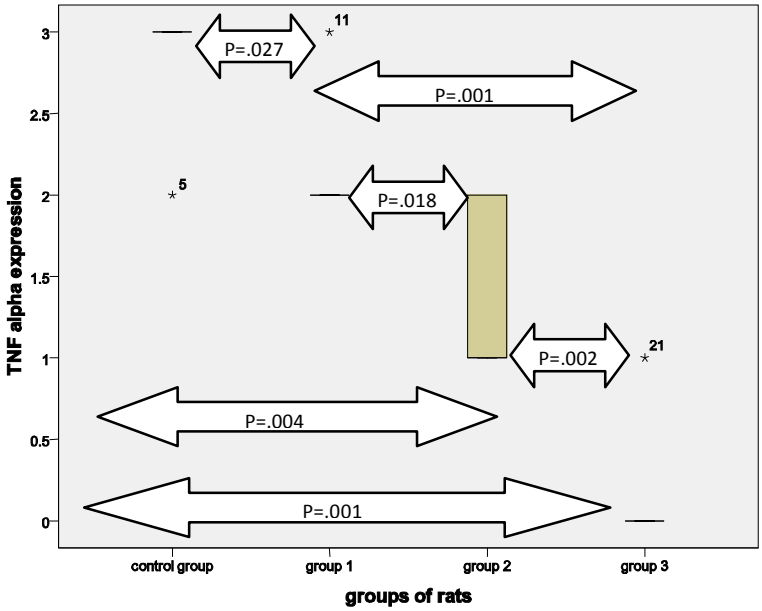

Figure 12. Box plot median liver TNFα of Wistar rats in control group, group1, group 2, and group 3.

The significance of these differences was revealed using statistic test. Using Shapiro-Wilk test (test full result was attached on the appendix 3) , it's found that the data has not normal distribution as the Shapiro-Wilk test reveal significant result (P 0.002) **,** by performing Kruskal-Wallis test the data appear to be statistically significance (P=0.000), subsequently carried out Mann-Whitney test as post Hoc test to know the statistic comparison between the groups.

From the box plot above its notice that the difference was statistically significance between all the groups, From the analysis above its noted that the TNFα expression that induced by alcohol is statistically difference with giving Nigella sativa extract , this applicable by the fact that as the dose increase between groups the TNFα expression become less and less and the least expression occur

in the group 3 comparing to the most expression that occur in control group (full result at appendix 3).

5:3 Calculation the Kappa (k).

To get reliable test results (reliability) ,and valid result (validity) measurement of liver tissue damge and TNFα expression measured by two expert pathologist. To assess the reliability and validity of the second The data was the determination of the value of Kappa. Kappa values for liver tissue damge was 0.965 and the Kappa values for TNFα expression was 0.992 (full result at appendix 3).