



CHAPTER IV

RESULTS

Effect of 100% fat diet for 6 weeks on serum biochemical parameters, oxidative stress marker, and liver histopathology in rats

1. Body Weight

The 100% fat diet-fed rats lost weight significantly compared with normal controls after 6 weeks (197.00 ± 8.07 g. vs 438.38 ± 9.70 g., $P < 0.05$) (Table 1 and Figure 15). Despite weight loss, the general condition of 100% fat diet-fed rats remained good throughout the experiment.

2. Total Glutathione Level in Whole Blood

The total glutathione level in whole blood in 100% fat diet-fed rats was increased significantly as compared with normal control group (2066.67 ± 93.81 μM vs 1337.54 ± 31.48 μM , $P < 0.05$) (Table 1 and Figure 16).

3. Serum Biochemical Parameters

Serum AST and ALT in the normal control and experimental groups were shown in Table 1 and Figure 17 and 18. Serum AST and ALT were decreased significantly in 100% fat diet-fed rats as compared with normal controls (AST; 53.63 ± 9.31 U/L vs 86.75 ± 4.28 U/L, ALT; 23.00 ± 1.92 U/L vs 40.13 ± 2.35 U/L, $P < 0.05$). Additionally, serum cholesterol level was significantly higher in the 100% fat diet-fed rats than that in the normal controls (94.75 ± 3.11 mg/dL vs 71.75 ± 1.84 mg/dL, $P < 0.05$) (Table 1 and Figure 19), whereas there was no significant difference in serum triglycerides between the two groups (Figure 20).

4. Hepatic Malondialdehyde (MDA) Content

MDA was elevated significantly in the 100% fat diet-fed rats compared with that in the normal controls (209.88 ± 43.83 nmol/mg protein vs 3.80 ± 1.68 nmol/mg protein, $P < 0.05$) (Table 1 and Figure 21).

5. Histopathological Examination

The pathological grading for steatosis and necroinflammation was summarized in Table 2. Liver sections from rats fed with the regular dry rat chow had shown normal morphological appearance (Figure 29). In rats fed with 100% fat diet for 6 weeks, all livers developed moderate to severe macrovesicular steatosis, hepatocyte ballooning, mild to moderate inflammation, and regeneration of hepatocytes (Figure 30).

Effect of N-acetylcysteine on serum biochemical parameters, oxidative stress markers, and liver histopathology in rats with nonalcoholic steatohepatitis

1. Body Weight

The body weight at 6 weeks of rats fed with 100% fat diet was decreased when compared to the beginning. After the first 6 weeks, rats were fed with regular dry rat chow for additional 4 weeks. As a result, the body weight was significantly increased in all groups (Table 3 and Figure 22).

2. Total Glutathione Level in Whole Blood

Total glutathione levels in NASH+diet, NASH+diet+NAC₂₀, and NASH+diet+NAC₅₀₀ were not different as compared with the normal controls

(1287.15 ± 23.27 μM , 1332.76 ± 23.31 μM , and 1455.94 ± 46.17 μM vs 1337.54 ± 31.48 μM , respectively, $P > 0.05$) (Table 3 and Figure 23).

3. Serum Biochemical Parameters

The analysis of serum AST level in the normal controls (86.75 ± 4.28 U/L), NASH+diet (93.00 ± 4.08 U/L), NASH+diet+NAC₂₀ (81.42 ± 2.61 U/L), and NASH+diet+NAC₅₀₀ (95.22 ± 8.18 U/L) showed no significant difference. There was no significant difference in serum ALT level among the normal controls (40.13 ± 2.35 U/L), NASH+diet (44.18 ± 1.30 U/L), NASH+diet+NAC₂₀ (39.83 ± 1.40 U/L), and NASH+diet+NAC₅₀₀ (41.89 ± 2.02 U/L). For cholesterol levels in the normal controls (71.75 ± 1.84 mg/dL), NASH+diet (70.82 ± 2.26 mg/dL), NASH+diet+NAC₂₀ (71.50 ± 1.46 mg/dL), and NASH+diet+NAC₅₀₀ (75.78 ± 2.01 mg/dL) were not statistically significant. Also, the result of triglyceride levels in the normal controls (90.25 ± 19.09 mg/dL), NASH+diet (121.36 ± 15.36 mg/dL), NASH+diet+NAC₂₀ (114.00 ± 5.24 mg/dL), and NASH+diet+NAC₅₀₀ (97.11 ± 10.66 mg/dL) showed no significant difference (Table 3, Figure 24, 25, 26 and 27).

4. Hepatic Malondialdehyde (MDA) Content

By week 10, hepatic MDA contents of the rats in NASH+diet, NASH+diet+NAC₂₀, and NASH+diet+NAC₅₀₀ were not different from that of normal controls (3.10 ± 0.90 , 2.85 ± 0.87 , and 2.23 ± 0.14 vs 3.80 ± 1.68 nmol/mg protein, respectively, $P > 0.05$) (Table 3 and Figure 28).

5. Histopathological Examination

The histopathological grading for steatosis and necroinflammation were summarized in Table 4. Histopathological study demonstrated that the liver sections in

the control group had normal morphological appearance (Figure 29). The pathological changes of liver were obviously improved in NASH+diet, NASH+diet+NAC₂₀, and NASH+diet+NAC₅₀₀ groups compared with the NASH group (Figure 31, 32 and 33).

Table 1. Effects of 100% fat diet for 6 weeks on body weight, serum biochemical parameters, and oxidative stress markers in rats

Parameters	Normal control (n=8)	100% fat diet for 6 weeks (NASH, n=8)
Body weight at the beginning (g)	239.00±2.27	245.13±0.99*
Body weight at 6 weeks (g)	438.38±9.70	197.00±8.07*
Total GSH (μM)	1337.54±31.48	2066.67±93.81*
AST (U/L)	86.75±4.28	53.63±9.31*
ALT (U/L)	40.13±2.35	23.00±1.92*
Cholesterol (mg/dL)	71.75±1.84	94.75±3.11*
Triglycerides (mg/dL)	90.25±19.09	147.75±32.57
Hepatic MDA (nmol/mg protein)	3.80±1.68	209.88±43.83*

Values expressed as mean ± SEM

*Significant difference (P<0.05) compared with normal control group

Table 2. Effects of 100% fat diet for 6 weeks on liver histopathology in rats : summary of the scores of steatosis and necroinflammation.

Group	Number	Level of steatosis				Level of necroinflammation			
		0	1	2	3	0	1	2	3
Normal control	8	8	-	-	-	8	-	-	-
100% fat diet for 6 weeks (NASH)	8	-	-	5	3	-	5	2	1

The severity of steatosis was graded by

0 = No steatosis

1 = Fewer than 33% of the hepatocytes were affected.

2 = 33%-66% of the hepatocytes were affected.

3 = More than 66% of the hepatocytes were affected.

The severity of necroinflammation was graded by

0 = No hepatocyte injury/inflammation

1 = Sparse or mild focal zone 3 hepatocyte injury/inflammation

2 = Noticeable zone 3 hepatocyte injury/inflammation

3 = Severe zone 3 hepatocyte injury/inflammation

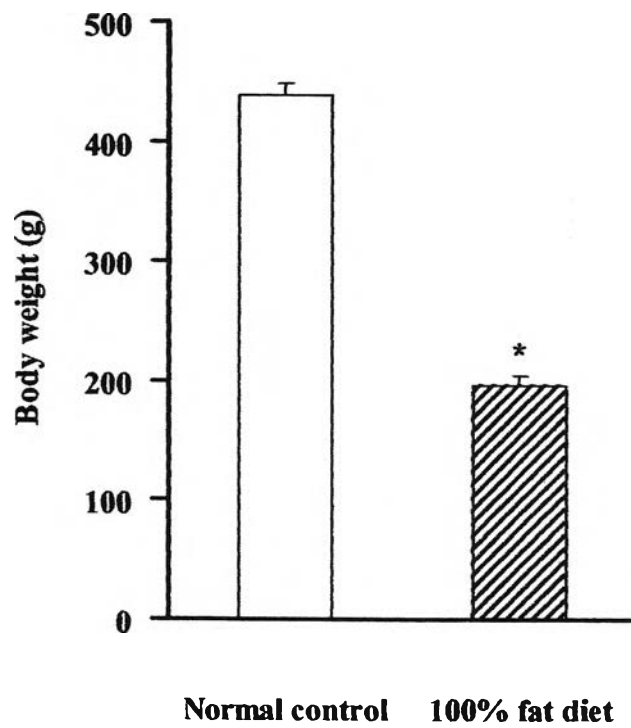


Figure 15. Effect of 100% fat diet for 6 weeks on body weight (mean±SEM)

*Significant difference ($P < 0.05$) compared with normal control group

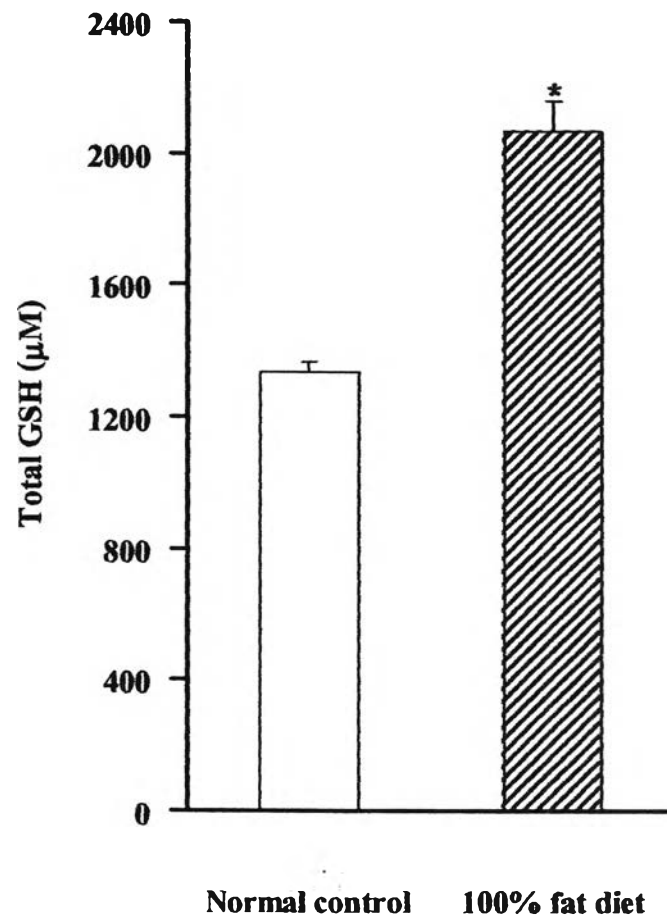


Figure 16. Effect of 100% fat diet for 6 weeks on total GSH (mean±SEM)

*Significant difference ($P < 0.05$) compared with normal control group

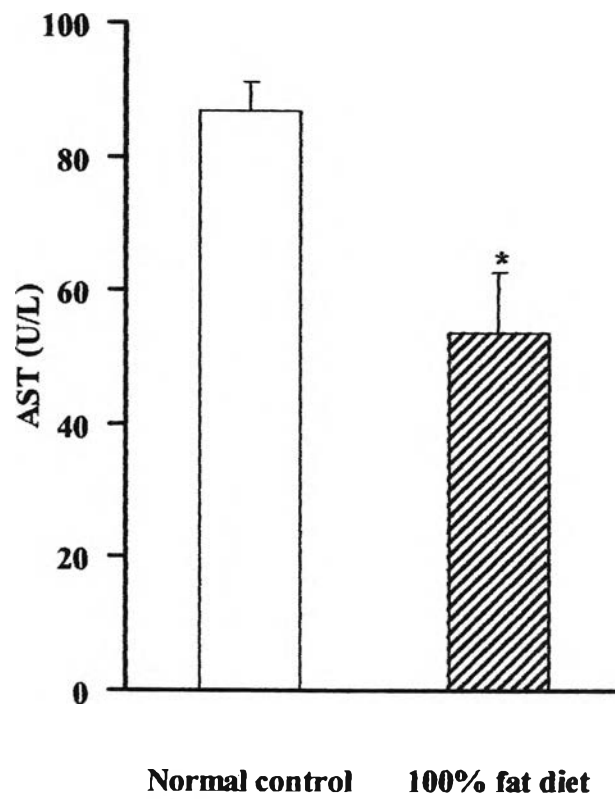


Figure 17. Effect of 100% fat diet for 6 weeks on serum AST (mean±SEM)

*Significant difference ($P < 0.05$) compared with normal control group

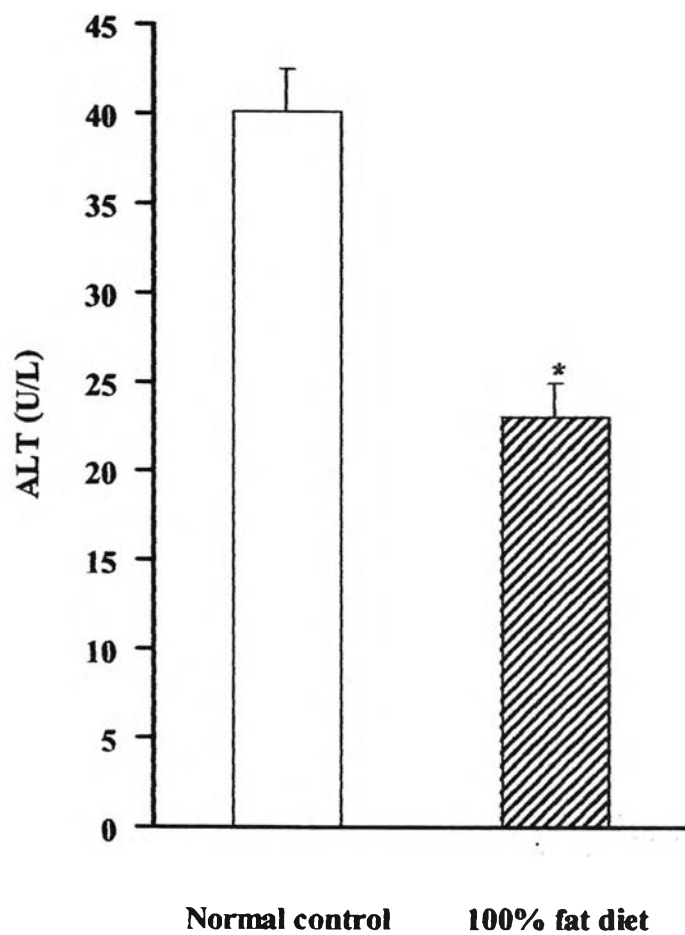


Figure 18. Effect of 100% fat diet for 6 weeks on serum ALT (mean±SEM)

*Significant difference ($P < 0.05$) compared with normal control group

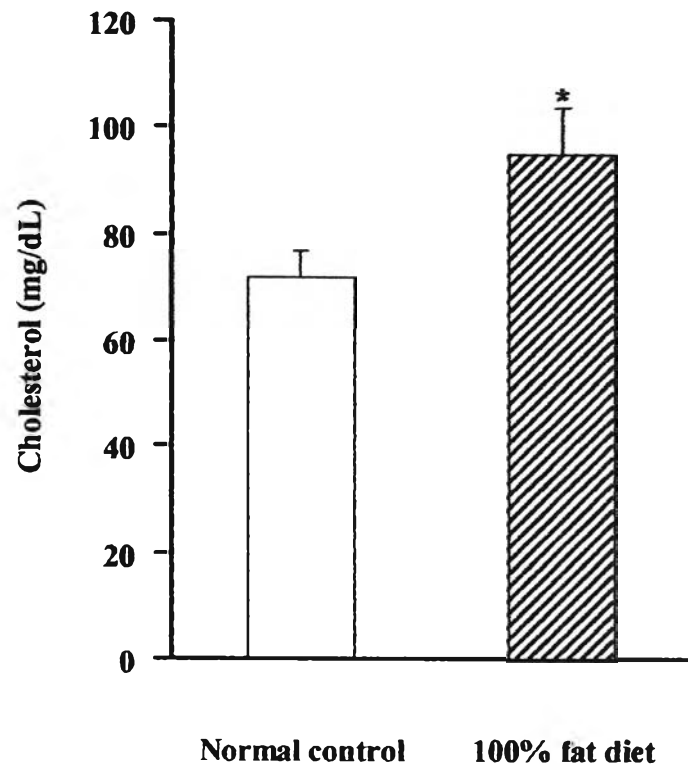


Figure 19. Effect of 100% fat diet for 6 weeks on serum cholesterol (mean \pm SEM)

*Significant difference ($P < 0.05$) compared with normal control group

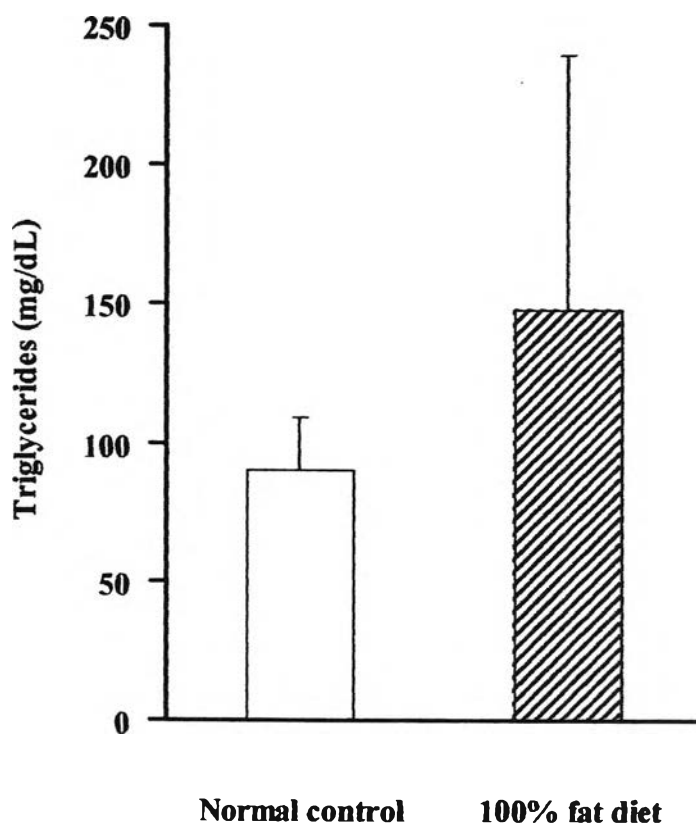


Figure 20. Effect of 100% fat diet for 6 weeks on serum triglycerides (mean \pm SEM)

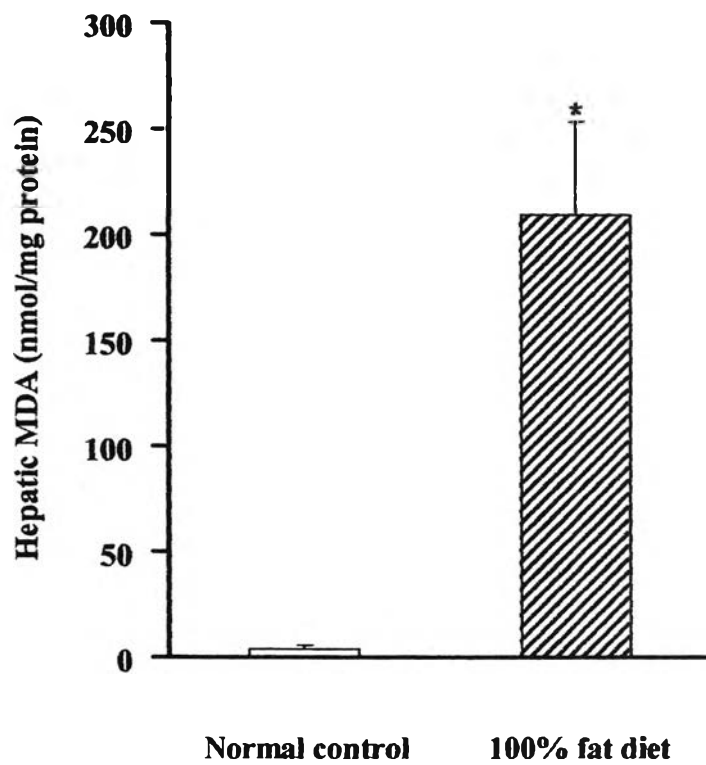


Figure 21. Effect of 100% fat diet for 6 weeks on hepatic MDA contents
(mean±SEM)

*Significant difference ($P < 0.05$) compared with normal control group

Table 3. Effect of NAC on body weight, serum biochemical parameters, and oxidative stress markers in rats with NASH

Parameters	Normal control (n=8)	NASH 6 weeks (n=8)	NASH+diet (n=11)	NASH+diet+NAC ₂₀ (n=12)	NASH+diet+NAC ₅₀₀ (n=9)
Body weight at the beginning (g)	239.00±2.27	245.13±0.99*	246.82±1.45	241.75±1.65	245.89±1.78
Body weight at 6 weeks (g)	438.38±9.70	197.00±8.07*	183.82±4.24*	181.42±4.72*	182.00±6.55*
Body weight at 10 weeks (g)	-	-	411.73±4.91	403.50±4.97*	383.67±4.28*#
Total GSH (µM)	1337.54±31.48	2066.67±93.81*	1287.15±23.27	1332.76±23.31	1455.94±46.17
AST (U/L)	86.75±4.28	53.63±9.31*	93.00±4.08	81.42±2.61	95.22±8.18
ALT (U/L)	40.13±2.35	23.00±1.92*	44.18±1.30	39.83±1.40	41.89±2.02
Cholesterol (mg/dL)	71.75±1.84	94.75±3.11*	70.82±2.26	71.50±1.46	75.78±2.01
Triglycerides (mg/dL)	90.25±19.09	147.75±32.57	121.36±15.36	114.00±5.24	97.11±10.66
Hepatic MDA(nmol/mg protein)	3.80±1.68	209.88±43.83*	3.10±0.90	2.86±0.87	2.23±0.14

*Significant difference (P<0.05) compared with normal control 6 weeks

#Significant difference (P<0.05) compared with NASH+Diet

Table 4. Effects of NAC on liver histopathology in rats with NASH : summary of the scores of steatosis and necroinflammation.

Group	Number	Level of steatosis				Level of necroinflammation			
		0	1	2	3	0	1	2	3
Normal control	8	8	-	-	-	8	-	-	-
NASH	8	-	-	5	3	-	5	2	1
NASH+diet	11	11	1	-	-	10	1	-	-
NASH+diet+NAC ₂₀	12	9	3	-	-	12	-	-	-
NASH+diet+NAC ₅₀₀	9	9	-	-	-	9	-	-	-

The severity of steatosis was graded by

0 = No steatosis,

1 = Fewer than 33% of the hepatocytes were affected.

2 = 33%-66% of the hepatocytes were affected.

3 = More than 66% of the hepatocytes were affected.

The severity of necroinflammation was graded by

0 = No hepatocyte injury/inflammation

1 = Sparse or mild focal zone 3 hepatocyte injury/inflammation

2 = Noticeable zone 3 hepatocyte injury/inflammation

3 = Severe zone 3 hepatocyte injury/inflammation

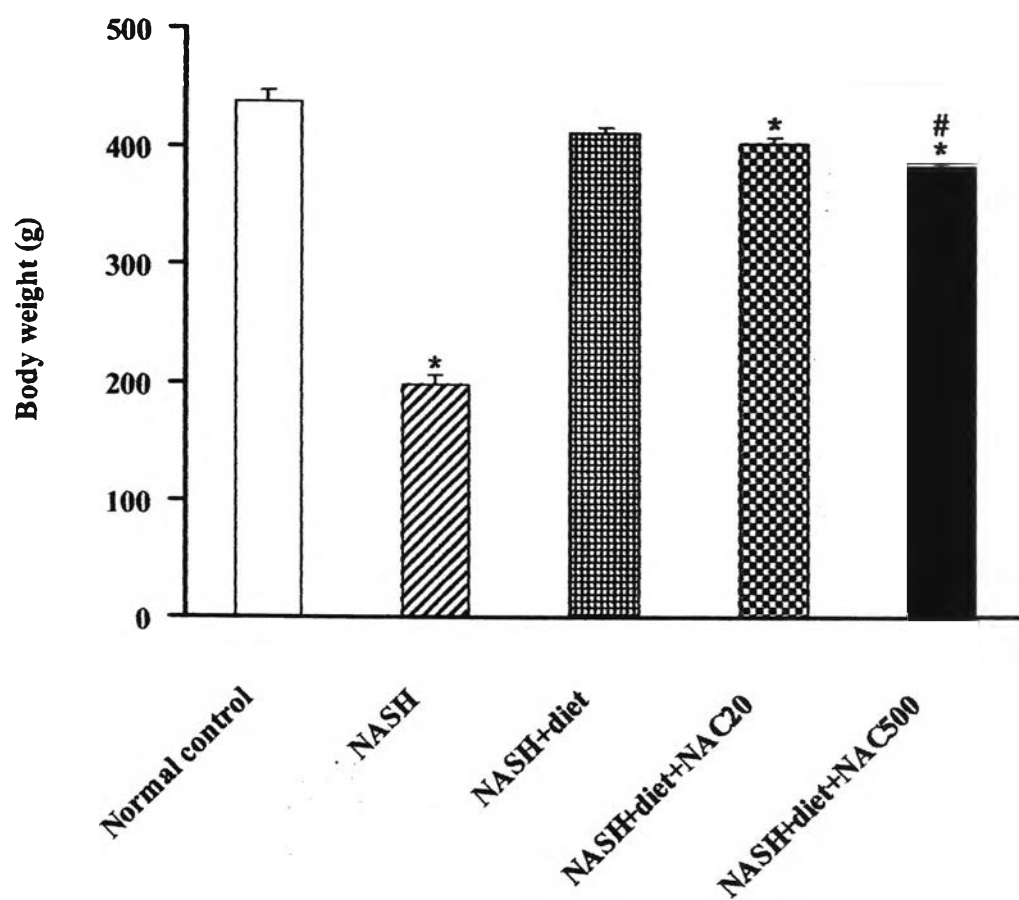


Figure 22 . Effect of NAC on body weight in rats with NASH (mean±SEM)

*Significant difference ($P < 0.05$) compared with normal control group

#Significant difference ($P < 0.05$) compared with NASH+diet group

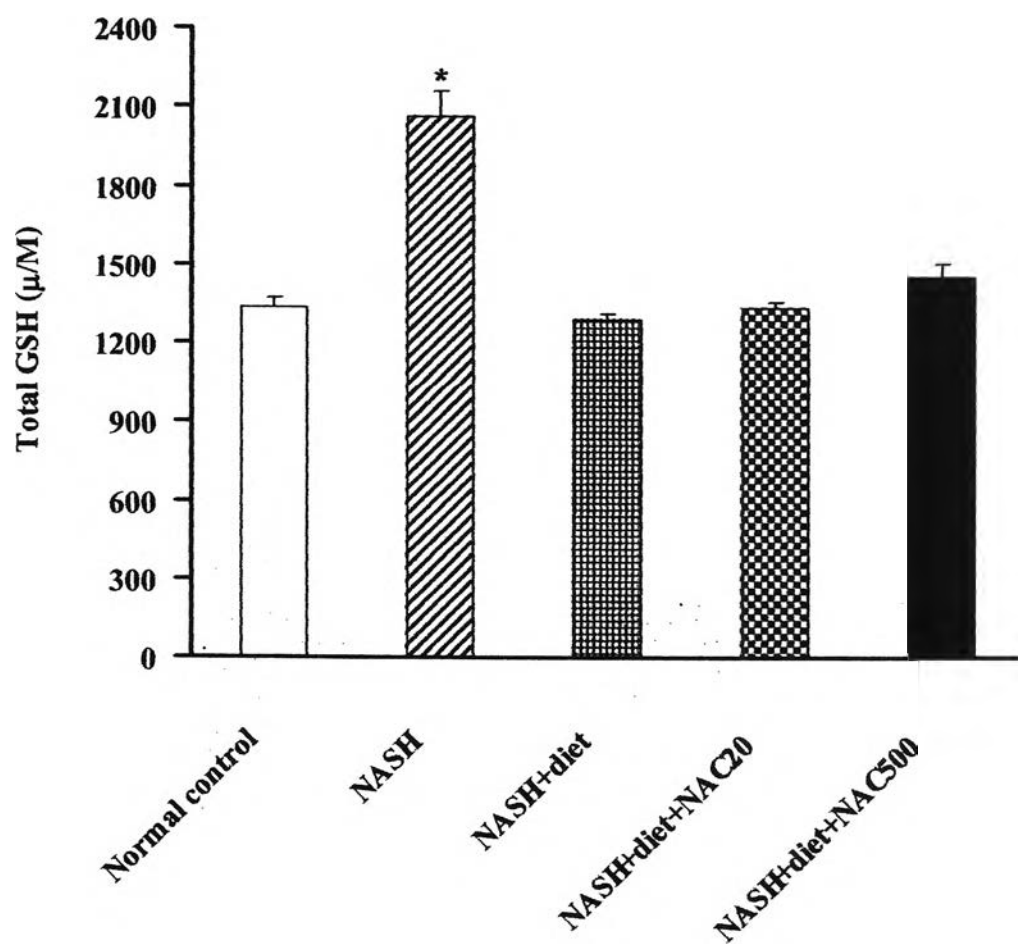


Figure 23 Effect of NAC on total GSH level in rats with NASH (mean±SEM)

*Significant difference ($P < 0.05$) compared with normal control group

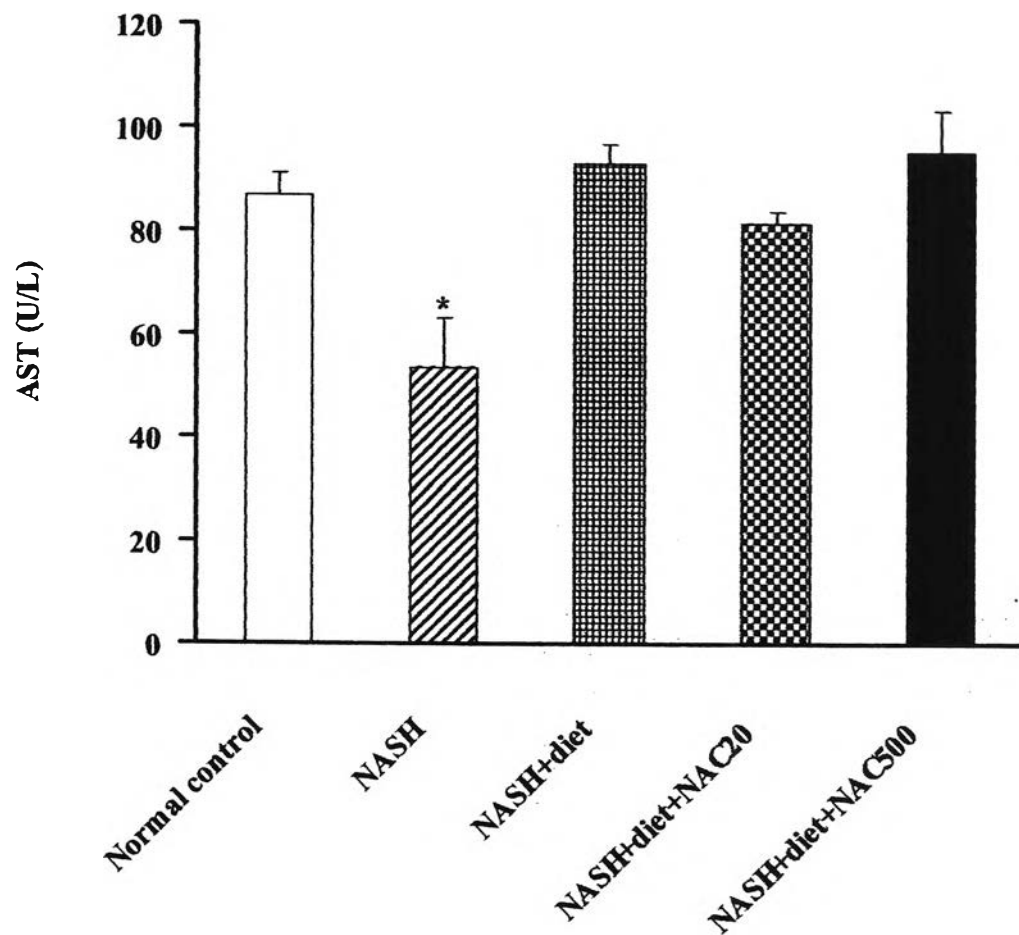


Figure 24. Effect of NAC on serum AST level in rats with NASH (mean±SEM)

*Significant difference (P<0.05) compared with normal control group

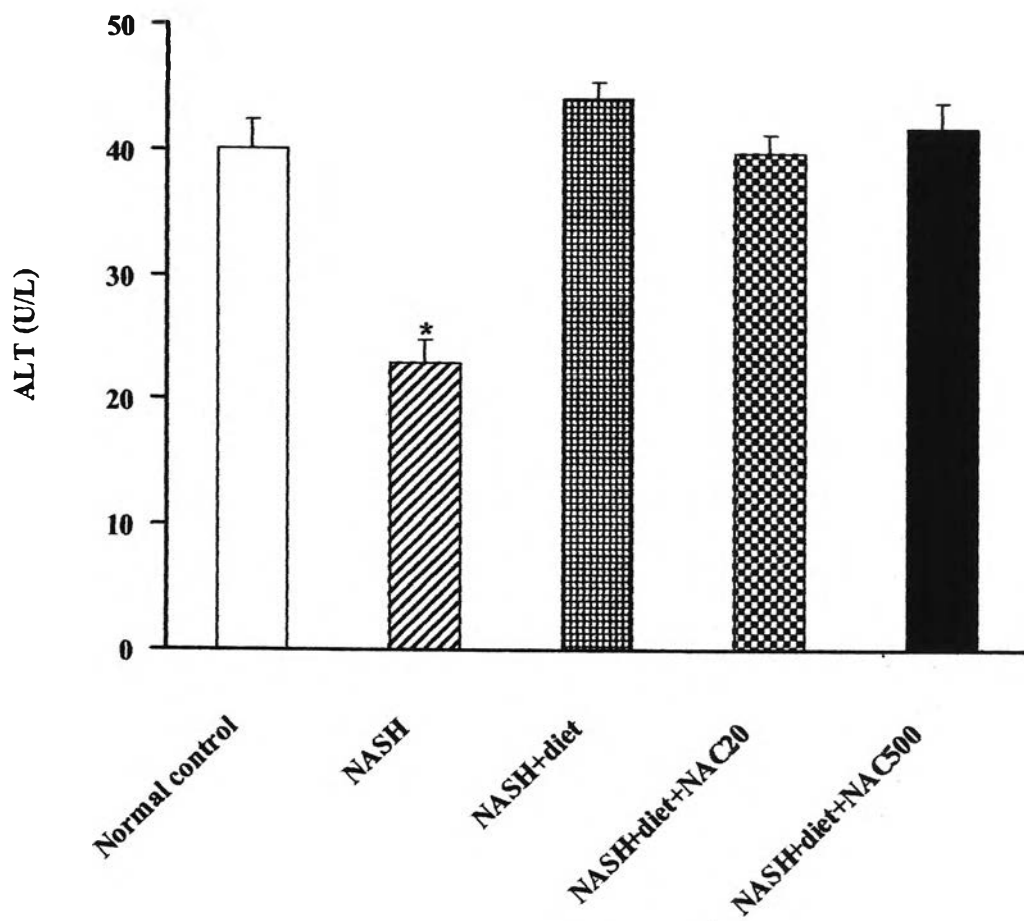


Figure 25. Effect of NAC on serum ALT level in rats with NASH (mean±SEM)

*Significant difference (P<0.05) compared with normal control group

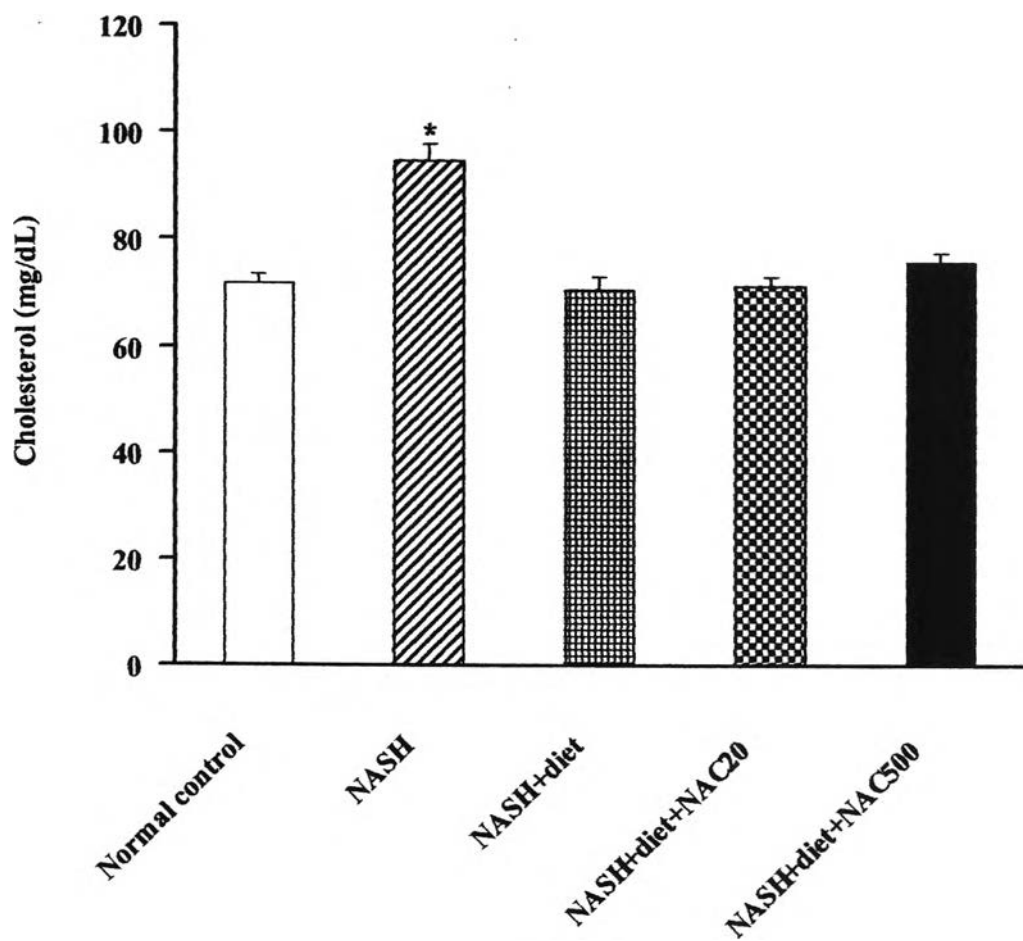


Figure 26. Effect of NAC on serum cholesterol in rats with NASH (mean \pm SEM)

*Significant difference ($P < 0.05$) compared with normal control group



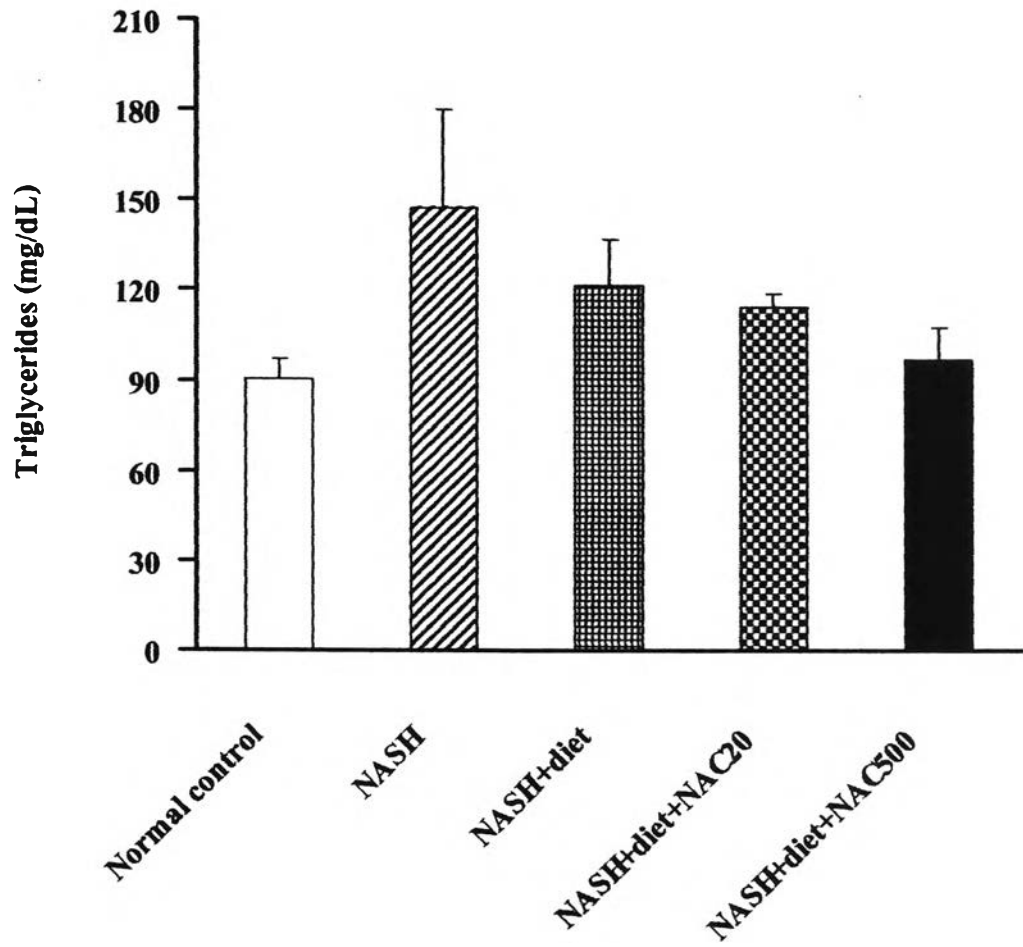


Figure 27. Effect of NAC on serum triglycerides in rats with NASH (mean \pm SEM)

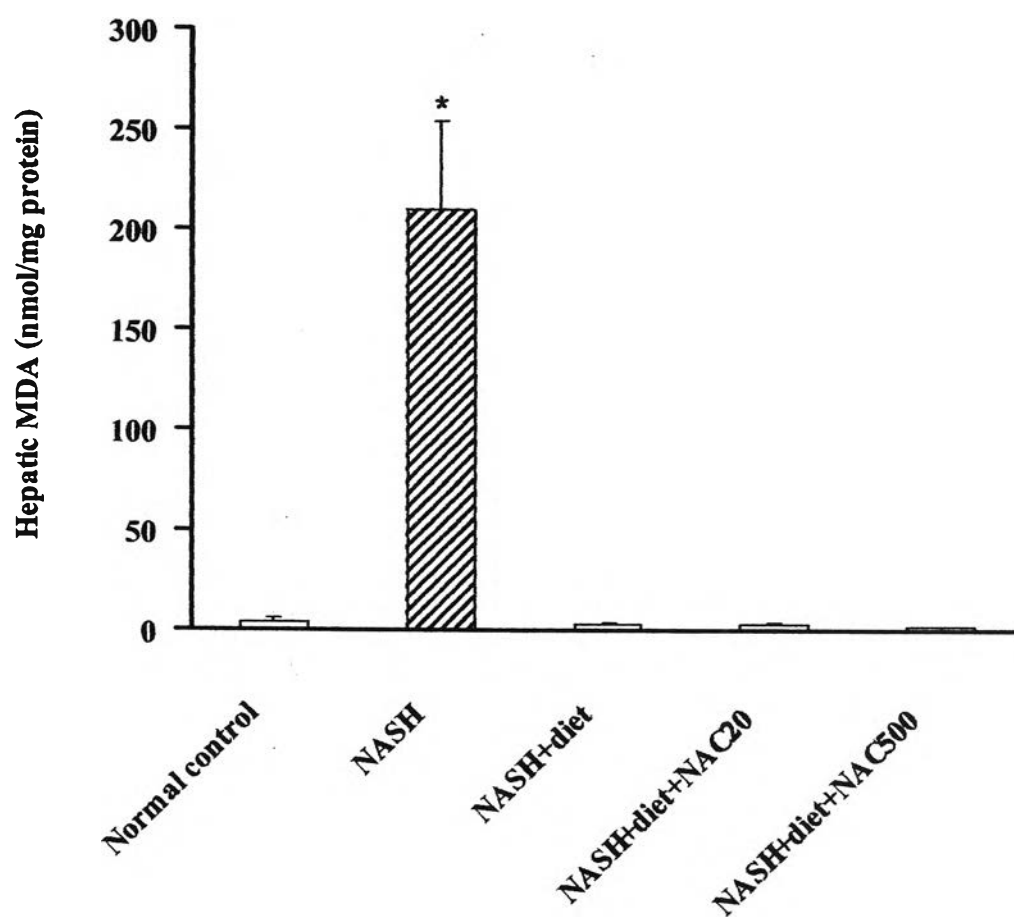


Figure 28. Effect of NAC on hepatic MDA contents in rats with NASH (mean±SEM)

*Significant difference ($P < 0.05$) compared with normal control group

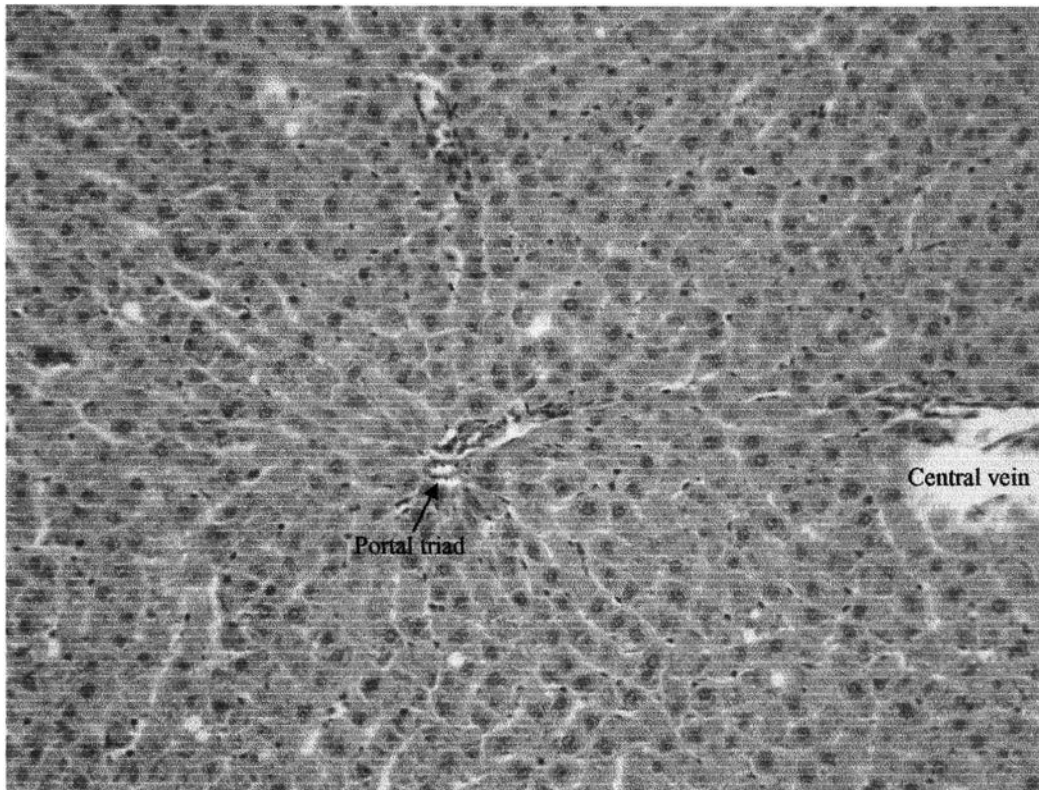
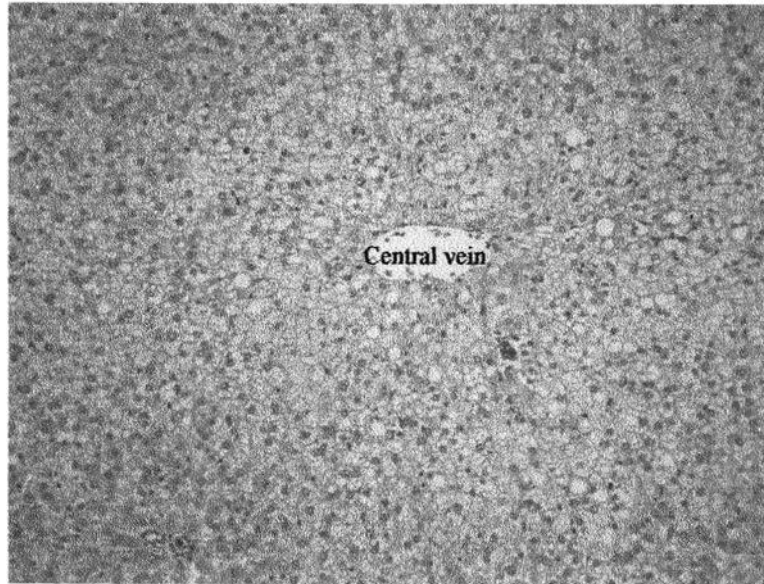
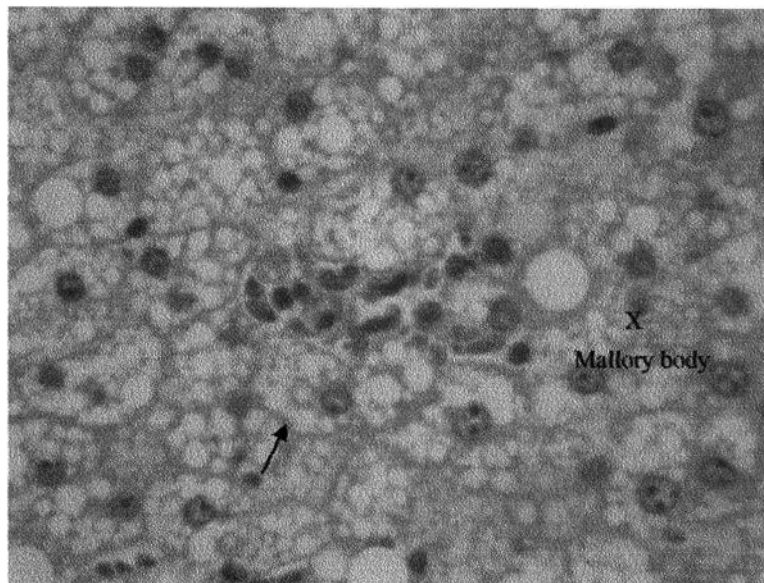


Figure 29. Hematoxylin and eosin stain (H&E, 100X) of liver sections in normal control group showed normal morphology of liver histopathology.

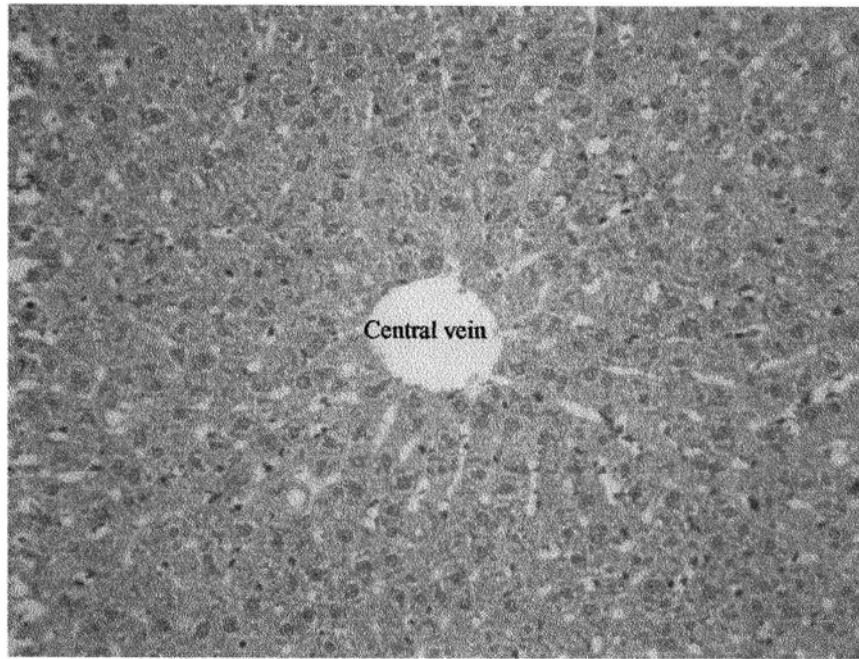


A

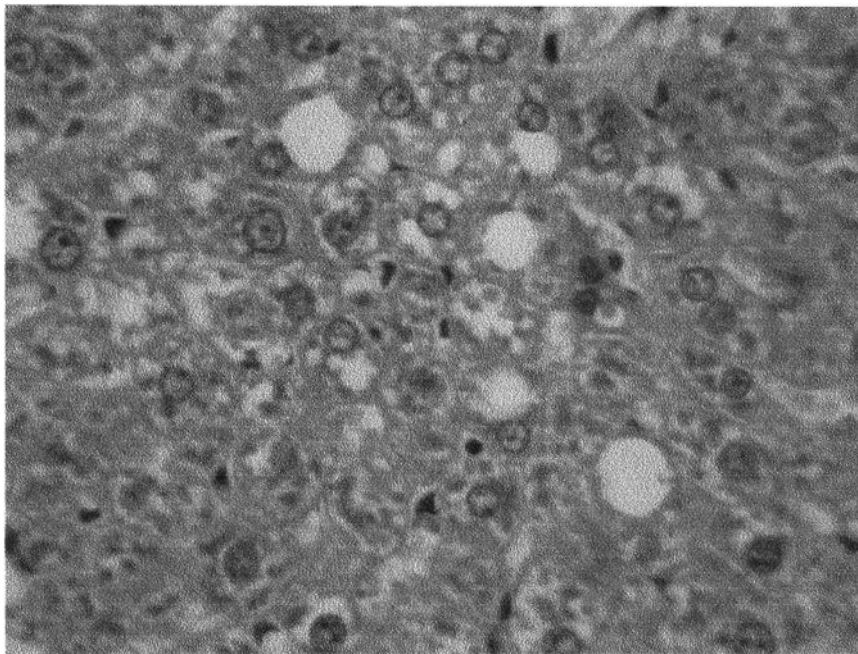


B

Figure 30. Hematoxylin and eosin stain of liver sections in rats fed with 100% fat diet group (H&E, A = 100X, B = 400X), the liver showed macrovesicular steatosis, ballooning changes (arrows), Mallory body (cross), hepatocyte necrosis, and infiltration of inflammatory cells.

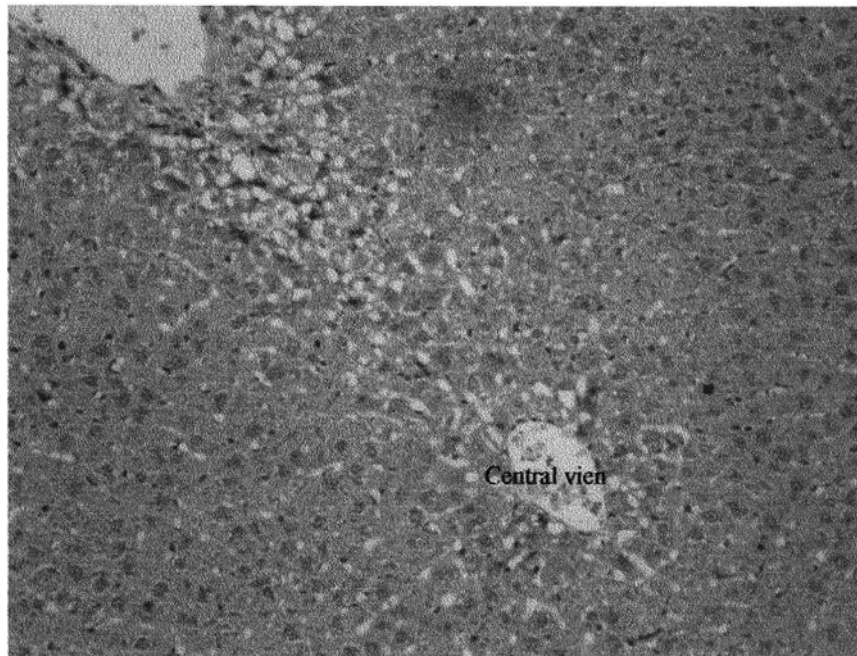


A

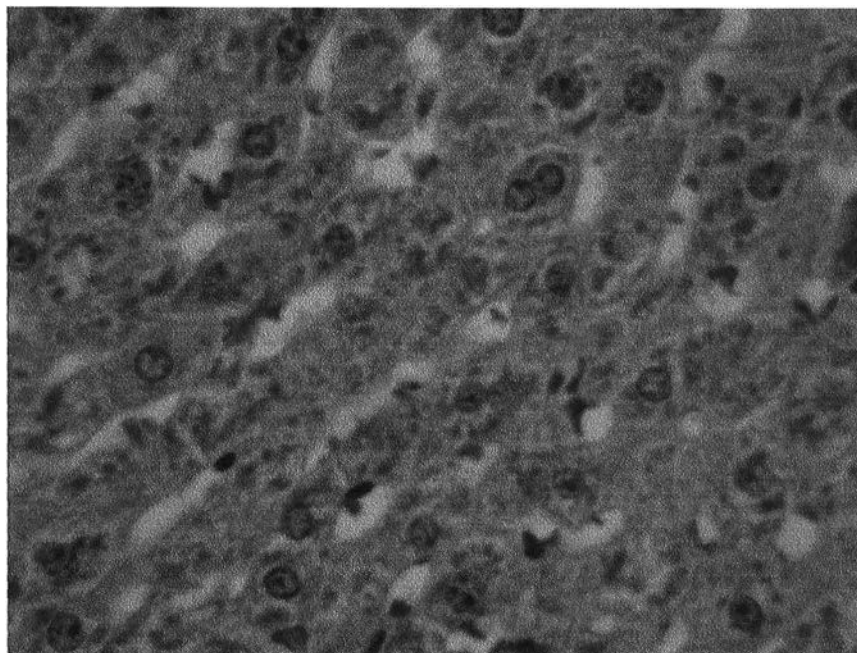


B

Figure 31. Hematoxylin and eosin stain of liver sections in NASH+diet group (H&E, A = 100X, B = 400X), example of hepatic histopathology showed improvement in steatosis and necroinflammation ; only mild steatosis and mild necroinflammation found in one rat.



A



B

Figure 32. Hematoxylin and eosin stain of liver sections in NASH+diet+NAC₂₀ group (H&E, A = 100X, B = 400X), example of hepatic histopathology showed improvement in steatosis and necroinflammation ; only mild steatosis found in three rats.

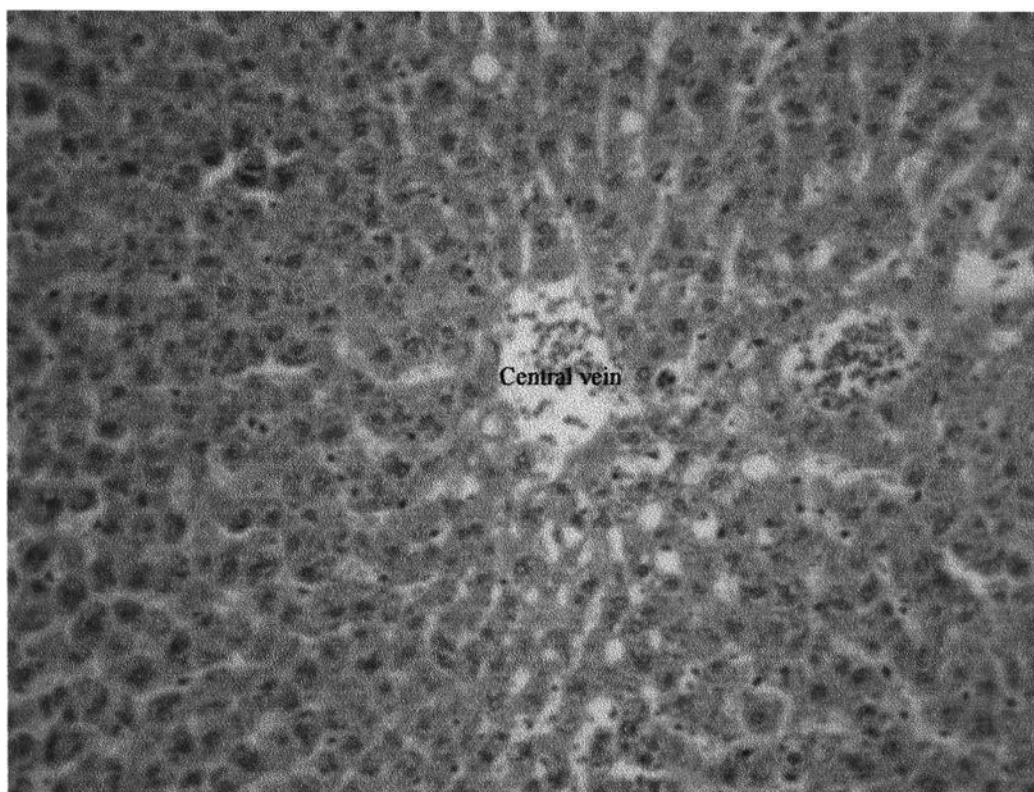


Figure 33. Hematoxylin and eosin stain of liver sections in NASH+diet+NAC₅₀₀ group (H&E, 100X) showed no steatosis, no hepatocyte ballooning, and no inflammation.