ORIGINAL RESEARCH

Medicine Science 2019; ():

Effects of L-Carnitine and N-Acetylcysteine on nonalcoholic hepatic steatosis in rats

Cemal Nas ORCID: 0000-0002-5616-8625
Mehmet Tahir Gokdemir ORCID: 0000-0002-5546-9653
Naime Canoruc ORCID: 0000-0001-5987-9812
Mehmet Yaldiz ORCID: 0000-0003-0640-4551

1Health Sciences University, Gazi Yasargil research and training Hospital, Department of Biochemistry, Diyarbakir, Turkey
2Health Sciences University, Gazi Yasargil research and training Hospital, Department of Emergency, Diyarbakir, Turkey
3Ankara University, Elderly Care School, Ankara, Turkey
4Mersin University, Faculty of Medicine, Department of Pathology, Mersin, Turkey

Received 16 October 2018; Accepted 28 November 2018
Copyright © 2019 by authors and Medicine Science Publishing Inc.

Abstract
This study was performed to investigate whether the antioxidants L-carnitine and N-acetylcysteine (NAC) have therapeutic effects on Nonalcoholic hepatic steatosis (NHS) in rats with carbon tetrachloride (CCl4)-induced fatty liver disease. Twenty-four healthy male and female Wistar Albino rats, weighing 220–250 g and obtained from our university health research institute, were used in this study. The animals were divided into four groups of six rats each: Group 1 [Diet + normal saline solution (NSS), control group], Group 2 [Diet + CCl4], Group 3 [Diet + CCl4 + L-carnitine] and Group 4 [Diet + CCl4 + NAC]. For biochemical examinations blood samples were obtained from the right ventricle of the heart and liver samples for histopathological were also obtained. The mean Aspartate aminotransferase (AST) (P = 0.043) and lactate dehydrogenase (LDH) levels (P=0.021) were significantly lower in rats with l-carnitine treatment. The mean ALT level were significantly lower in rats with NAC and (P = 0.014). Microscopic steatosis severity was decreased in the rats with NAC treatment than the controls. However administration of l-carnitine did not sufficiently prevent hepatic steatosis or inflammation. Our study showed that L-carnitine and NAC treatment resulted in significant regression of steatosis in rats with NHS. However, these findings must be confirmed by further studies including larger populations.

Keywords: L-Carnitine, N-Acetylcysteine, nonalcoholic hepatic steatosis, rats

Introduction
Nonalcoholic hepatic steatosis (NHS) is an important health problem, the prevalence of which has been gradually increasing with the general increase in the rate of obesity. There have been a number of recent improvements in the diagnosis and treatment of NHS, which is a type of hepatitis observed in people who do not consume alcohol that shares histopathological features with alcoholic fatty liver disease [1]. Although this pathological entity was previously referred to as pseudo-alcoholic liver disease, fatty liver hepatitis, diabetic nonalcoholic Laennec’s disease, and steatonecrosis, the term “nonalcoholic steatohepatitis” was adopted in the 1980s. Finally, the term “NHS” was applied [2]. Pharmacological therapies for NHS include lipid-lowering agents, such as statins and fibrates; metformin; insulin sensitizers, such as thiazolidinedione; cytoprotective and antioxidant agents, such as bile acid and vitamin E; and anti-obesity drugs, such as orlistat. However, these drugs are not specific to the liver, and side effects associated with prolonged use may occur. Triglyceride (TG) accumulation in hepatocytes, and inflammation of the liver parenchyma, play roles in the pathogenesis of hepatic steatosis [3]. Future treatment approaches will focus on agents capable of preventing the release of proinflammatory cytokines and TG accumulation. Previous studies indicated that N-acetylcysteine (NAC) and carnitine have anti-inflammatory effects [4]. Therefore, as L-carnitine deficiency is the primary defect in hepatic steatosis, the necessity of L-carnitine treatment has been emphasized in these patients. NAC and L-carnitine may be options for treatment of NHS. This study was performed to investigate whether the antioxidants L-carnitine and NAC have therapeutic effects on NHS in rats with carbon tetrachloride (CCl4)-induced fatty liver disease.

Material and Methods
Twenty-four healthy male and female Wistar Albino rats, weighing 220–250 g and obtained from our university health research institute, were used in this study. The animals were divided into four groups of six rats each: Group 1 [Diet + normal saline solution (NSS), control group], Group 2 [Diet + CCl4], Group 3 [Diet + CCl4 + L-carnitine] and Group 4 [Diet + CCl4 + NAC]. For biochemical examinations blood samples were obtained from the right ventricle of the heart and liver samples for histopathological were also obtained. The mean Aspartate aminotransferase (AST) (P = 0.043) and lactate dehydrogenase (LDH) levels (P=0.021) were significantly lower in rats with l-carnitine treatment. The mean ALT level were significantly lower in rats with NAC and (P = 0.014). Microscopic steatosis severity was decreased in the rats with NAC treatment than the controls. However administration of l-carnitine did not sufficiently prevent hepatic steatosis or inflammation. Our study showed that L-carnitine and NAC treatment resulted in significant regression of steatosis in rats with NHS. However, these findings must be confirmed by further studies including larger populations.

Keywords: L-Carnitine, N-Acetylcysteine, nonalcoholic hepatic steatosis, rats

*Corresponding Author: Mehmet Tahir Gokdemir, Health Sciences University, Gazi Yasargil research and training Hospital, Department of Emergency, Diyarbakir, Turkey, E-mail: drtahircokdemir@gmail.com
institute, were used in this study. The rats were kept in cages and fed at the Health Sciences Research Center of the Faculty of Medicine of our university. The animals were divided into four groups of six rats each:

Group 1 [Diet + normal saline solution (NSS)]: rats received 1 ml of NSS for 4 weeks intraperitoneally (i.p.).

Group 2 [Diet + CCl4]: rats received a single i.p. injection of CCl4 (0.5 ml/rat) in week 1. Throughout the following 4 weeks, 40% CCl4 solution in olive oil was injected (0.3 ml/rat) i.p., twice weekly.

Group 3 [Diet + CCl4 + L-carnitine]: rats received i.p. CCl4 injections in the first 4 weeks, and a single dose of 100 mg/kg L-carnitine (CARNITENE®; Santa Farma İlaç AS, Istanbul, Turkey) was administered i.p. in the last week (week 5). These animals underwent the same fattening procedure as Group 2 and the same dose of CCl4 was administered.

Group 4 [Diet + CCl4 + NAC]: rats received intraperitoneal CCl4 injections in the first 4 weeks and a single dose of 150 mg/kg NAC (ASIST®; Hüsnü Arsan İlaç AS, Istanbul, Turkey) was administered i.p. in the final week (week 5). These animals underwent the same fattening procedure as Group 2 and the same dose of CCl4 was administered.

The ethical rules were applied in the study. Ethical date 16 April 2002, Ethics committee approval number: 1496

**Diet**

The rats received a diet consisting of a mixture of 79.5% corn, 20% animal fat, and 0.5% CCl4 for 2 weeks. In the last week, the feed was changed to corn only. Animals received medications according to their group allocation.

At 24–48 hours following the end of medication period, the rats in the four groups were anesthetized with xylazine (Rompun, 1 ml/rat, intramuscularly [i.m.]) + ketamine hydrochloride (Ketalar, 1 ml/rat, i.m.) on the day of the experiment for biochemical and histopathological examinations, and blood samples were obtained from the right ventricle of the heart by opening the thoracic cavity. Liver samples were also obtained. Following these procedures, the animals were sacrificed by exsanguination from the carotid artery.

Blood samples obtained from the rats were transferred into plain tubes for biochemical analysis, and the serum was separated by centrifugation at 3,500 rpm for 5 minutes. A portion of the liver tissue was placed in 10% formaldehyde solution for histopathological examination, and the remaining tissue was kept at −80°C until the day of analysis of malondialdehyde (MDA) levels. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), albumin, bilirubin (total and direct), total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), very low-density lipoprotein (VLDL), TG, total protein, and lactate dehydrogenase (LDH) levels in the serum samples were analyzed using an Auto Analyzer (Toshiba, Tokyo, Japan) operating based on the principles of Abbott enzymatic-colorimetric chemical measurement.

**Histopathological examination**

Liver tissues that had been kept in 10% formaldehyde solution were stained with hematoxylin and eosin, and histopathological examination was performed under a light microscope in the Department of Pathology at our university. On histopathological examination, the samples were graded according to Angulo’s fatty liver grading system [5].

The study protocol was approved by the Ethics Committee of our university (Approval No: 2002/1496).

**Statistical analysis**

SPSS software (ver. 11.5; SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The results of biochemical tests are expressed as means and standard deviation. Due to the small number of rats in each group, the median, minimum, and maximum values were also calculated. The Mann–Whitney U test was used for comparison of test results among groups. In all analyses, P < 0.05 was taken to indicate statistical significance.

**Results**

The mean, standard deviation, median, and minimum–maximum values of the biochemical tests in Groups 1 and 2, and the significance of differences in values between the two groups, are presented in Table 1.

As shown in Table 2, the mean AST (187 ± 52.74 U/L vs. 392 ± 232.31 U/L, P = 0.043) and LDH (582.75 ± 320.88 U/L vs. 1263.25 ± 131.5 U/L, P = 0.021) levels were significantly lower in rats with L-carnitine treatment (Group 3) than in rats without L-carnitine treatment (Group 2).

The results of biochemical analyses of Groups 4 and 2 are presented in Table 3. The mean ALT level was 64.25 ± 28.89 U/L in Group 4 and 232.4 ± 76.19 U/L in Group 2, and this difference was significant (P = 0.014).

**Results of histopathological examinations**

The liver parenchyma, portal area, and sinusoidal structure of rats in Group 1, which were treated with saline only, were determined to be normal (Figure 1). On histopathological examination, CCl4 was found to cause grade 2 macrovesicular steatosis and grade 4 inflammation according to the fatty liver grading system described by Angulo et al. (5) (Figure 2a–b). Figures 2a (×10 magnification) and 2b (×20 magnification) show tissue sections described by Angulo et al. (5) (Figure 2a–b). Figures 2a (×10 magnification) and 2b (×20 magnification) show tissue sections of the liver from rats in Group 2 that had been treated with CCl4. Microscopic examination showed moderate macrovesicular steatosis around both periportal and central veins. Swelling was seen in the hepatocytes, the sinusoidal structure was partially disrupted, and patchy mononuclear cell infiltration was detected in the portal area.

Administration of L-carnitine did not sufficiently prevent hepatic steatosis or inflammation in Group 3 (CCl4 + L-carnitine) (Fig. 3a–b). Moderate macrovesicular steatosis was observed, which was more prominent around the central vein. The rats in Group 3 showed no decrease in steatosis grade compared to those in Group 2.
The results of microscopic examination of liver tissue sections from rats in Group 4 (CCl4 + NAC) are presented in Figs. 4a–b. NAC was shown to improve the histopathological features, with reduction in histological grade by 1. Moderate diffuse macrovesicular steatosis, disruption of the sinusoidal structure of the liver, swelling in the hepatocytes, and mild congestion in the portal area were observed. The rats in Group 4 showed a decrease in steatosis severity compared to those in Group 2.
Table 2. Distribution of biochemical values for Groups 2 and 3

<table>
<thead>
<tr>
<th>TESTS</th>
<th>GROUP 2 (Mean ± SD) Median (Min-Max)</th>
<th>GROUP 3 (Mean ± SD) Median (Min-Max)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBL, mg/dl</td>
<td>0.14 ± 0.054</td>
<td>0.1 ± 0.0</td>
<td>0.317</td>
</tr>
<tr>
<td>DBIL, mg/dl</td>
<td>0.14 ± 0.054</td>
<td>0.05 ± 0.057</td>
<td>0.096</td>
</tr>
<tr>
<td>AST, U/L</td>
<td>422.4 ± 211.0</td>
<td>187 ± 52.74</td>
<td>0.043</td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>128 ± 78.69</td>
<td>64.25 ± 28.89</td>
<td>0.149</td>
</tr>
<tr>
<td>ALP, U/L</td>
<td>282.25 ± 80.26</td>
<td>367.75 ± 81.13</td>
<td>0.149</td>
</tr>
<tr>
<td>LDH, U/L</td>
<td>126.35 ± 131.5</td>
<td>582.75 ± 320.88</td>
<td>0.021</td>
</tr>
<tr>
<td>TP, g/dl</td>
<td>6.3 ± 0.42</td>
<td>5.75 ± 0.77</td>
<td>0.186</td>
</tr>
<tr>
<td>ALB, g/dl</td>
<td>1.1 ± 0.14</td>
<td>1.12 ± 0.22</td>
<td>0.999</td>
</tr>
<tr>
<td>CHOL, mg/dl</td>
<td>58.25 ± 22.36</td>
<td>80 ± 27.86</td>
<td>0.248</td>
</tr>
<tr>
<td>TG, mg/dl</td>
<td>36.5 ± 10.78</td>
<td>41 ± 13.6</td>
<td>0.561</td>
</tr>
<tr>
<td>HDL, mg/dl</td>
<td>27.5 ± 9.67</td>
<td>39.25 ± 12.68</td>
<td>0.191</td>
</tr>
<tr>
<td>LDL, mg/dl</td>
<td>23.3 ± 10.5</td>
<td>32.65 ± 15.26</td>
<td>0.248</td>
</tr>
<tr>
<td>VLDL, mg/dl</td>
<td>7.3 ± 2.18</td>
<td>8.17 ± 2.73</td>
<td>0.564</td>
</tr>
<tr>
<td>MDA, nmol/g</td>
<td>53.2 ± 16.7</td>
<td>75.99 ± 6.03</td>
<td>0.149</td>
</tr>
</tbody>
</table>

Table 3. Distribution of biochemical values for Group 2 and Group 4

<table>
<thead>
<tr>
<th>TESTS</th>
<th>GROUP 2 (Mean ± SD) Median (Min-Max)</th>
<th>GROUP 4 (Mean ± SD) Median (Min-Max)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBL, mg/dl</td>
<td>0.14 ± 0.054</td>
<td>0.1 ± 0.0</td>
<td>0.176</td>
</tr>
<tr>
<td>DBIL, mg/dl</td>
<td>0.14 ± 0.054</td>
<td>0.05 ± 0.057</td>
<td>0.190</td>
</tr>
<tr>
<td>AST, U/L</td>
<td>422.4 ± 211.0</td>
<td>187 ± 52.74</td>
<td>0.110</td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>128 ± 78.69</td>
<td>64.25 ± 28.89</td>
<td>0.014</td>
</tr>
<tr>
<td>ALP, U/L</td>
<td>282.25 ± 80.26</td>
<td>367.75 ± 81.13</td>
<td>0.806</td>
</tr>
<tr>
<td>LDH, U/L</td>
<td>126.35 ± 131.5</td>
<td>582.75 ± 320.88</td>
<td>0.221</td>
</tr>
<tr>
<td>TP, g/dl</td>
<td>6.3 ± 0.42</td>
<td>5.75 ± 0.77</td>
<td>0.138</td>
</tr>
<tr>
<td>ALB, g/dl</td>
<td>1.24 ± 0.089</td>
<td>1.12 ± 0.22</td>
<td>0.381</td>
</tr>
<tr>
<td>CHOL, mg/dl</td>
<td>57.2 ± 26.36</td>
<td>80 ± 27.86</td>
<td>0.221</td>
</tr>
<tr>
<td>TG, mg/dl</td>
<td>36.5 ± 10.78</td>
<td>41 ± 13.6</td>
<td>0.561</td>
</tr>
<tr>
<td>HDL, mg/dl</td>
<td>27.5 ± 9.67</td>
<td>39.25 ± 12.68</td>
<td>0.191</td>
</tr>
<tr>
<td>LDL, mg/dl</td>
<td>23.3 ± 10.5</td>
<td>32.65 ± 15.26</td>
<td>0.248</td>
</tr>
<tr>
<td>VLDL, mg/dl</td>
<td>7.3 ± 2.18</td>
<td>8.17 ± 2.73</td>
<td>0.564</td>
</tr>
<tr>
<td>MDA, nmol/g</td>
<td>53.2 ± 16.7</td>
<td>75.99 ± 6.03</td>
<td>0.149</td>
</tr>
</tbody>
</table>

Discussion

This study was performed to evaluate the effects of L-carnitine and NAC on hepatic steatosis in rats given various treatments. Significant decreases in blood LDH and AST levels were observed in NHS-induced rats that received L-carnitine (Group 3) compared to those in Group 2 treated with CCl4, whereas no significant improvement was observed on histopathological examination of the liver. Histopathological examination indicated that the serum ALT level was decreased, and hepatic steatosis improved, in NHS-induced rats administered NAC (Group 4).

Nonalcoholic fatty liver disease (NAFLD) is an important health problem, the prevalence of which has gradually increased with increasing rates of obesity. There have been a number of recent improvements in the diagnosis and treatment of NAFLD, which manifests with various symptoms. NAFLD is thought to be more prevalent than previously estimated, and is known to be associated with many different agents and diseases [7].

CCl4 induces fatty degeneration and necrosis of the liver. It damages the membrane structure by increasing lipid peroxidation and releasing free radicals. Collagen synthesis in the liver increases according to the period and dose of CCl4 administration, leading to fibrosis and eventually, the development of cirrhosis. Following experimental administration of CCl4 to animals, necrosis, centrilobular degeneration and steatosis develop in the third zone of the liver [8]. Grade 2 macrovesicular steatosis was seen in the third zone of the liver on liver sections from rats in the control group treated with CCl4.

Accumulation of free fatty acids in the liver may be responsible for liver dysfunction, because they are highly reactive and may damage biological membranes. Acute and chronic increases in liver fat have been reported to lead to lipid peroxidation, the severity of which is closely correlated to the amount of fat in the liver. Lipid peroxidation leads to the release of toxic substances (MDA and 4-hydroxynonenal), which may trigger inflammatory responses in the liver. These toxic substances lead directly to cell damage, or induce an inflammatory response by attracting inflammatory cells to the liver parenchyma [9]. However, isolated fatty liver in the absence of hepatitis occurs more frequently than steatohepatitis. Therefore, there is some doubt as to whether fat accumulation in the liver is responsible for inflammation, but experimental findings...
suggest that increases in fatty acid levels lead to increased fibrous tissue formation in the liver [2].

TG accumulation and severe mitochondrial β-oxidation insufficiency are the most likely reasons for liver pathology [10].

L-Carnitine plays an important role in the transportation of long-chain fatty acids into the mitochondria from the cytoplasm. This transport induces β-oxidation of fatty acids. Therefore, energy production occurs with the entry of fatty acids into the Krebs and citric acid cycles. In addition, L-carnitine plays a role in the elimination of toxic metabolites. In L-carnitine deficiency, which may occur due to various etiologies and genetic defects, long-chain fatty acids that do not enter the mitochondria from the cytoplasm and that are not metabolized, lead to fatty liver by accumulating as a single fat lobule, and then accumulating in the liver [11]. There have been very few studies of fatty liver resulting from L-carnitine deficiency, and few reports regarding its treatment, in contrast to the large numbers of studies on alcoholic fatty liver [7]. The levels of L-carnitine in human subjects with type 1 diabetes have been shown to be decreased due to increased urinary excretion of L-carnitine [11]. In a randomized controlled clinical study, elevations in AST and ALT levels compared to the control group were significantly attenuated by L-carnitine treatment. The same study demonstrated that high serum ALT levels in 89.7% (35/39 persons) of patients treated with L-carnitine orotate complex normalized after 12 weeks of treatment. Computed tomography of the liver demonstrated an increase in the liver attenuation index of subjects administered L-carnitine orotate complex compared to placebo controls, and a significant change was reported in the liver attenuation index following 12 weeks of therapy [12]. In the present study, a single i.p. dose of 200 mg/kg L-carnitine was administered to rats. Significant decreases were detected in LDH and AST levels compared to the control group. However, no significant improvement was observed on histopathological examination of the liver compared to the control group.

L-Carnitine increases the activities of antioxidant enzymes, such as glutathione peroxidase, catalase, and superoxide dismutase, and has metal ion-chelating activity (i.e., ferrous) that catalyzes the production of reactive oxygen species (ROS). Their antioxidant activities can be compared to those of standard antioxidant agents, such as alpha-tocopherol, and may alleviate ischemia-reperfusion injury by decreasing the inhibitory effects of ROS on aerobic metabolism [13,14]. In another study, mitochondrial dysfunction was induced in rats fed a high-fat diet (48 kcal% fat) for 6 weeks. The mitochondrial functions of these rats were shown to improve following L-carnitine (100 mg/kg/day) administration via oral gavage for 2 weeks [10]. Amin et al. reported that L-carnitine supplementation resulted in significant decreases in serum TG, VLDL, TC, and LDL-C levels, and significant increases in HDL-C levels, in obese mice [15]. There have been a few studies regarding the treatment of fatty liver resulting from L-carnitine deficiency [12,15]. Acetyl-L-carnitine produced dose-dependent improvements in the liver tissue of rats with experimentally induced fatty liver [5]. The necessity for L-carnitine treatment may be due to the principal defect in fatty liver being L-carnitine deficiency. Further controlled and experimental studies are needed to investigate the effects of L-carnitine in fatty liver disease caused by L-carnitine deficiency and other etiologies.

Oxidative damage and glutathione-sulfate consumption are known to be due to the effects of cytokines [13]. This leads to reduced antioxidant capacity and a genetic predisposition toward NAFLD. Studies on the use of substances with known antioxidative effects, such as vitamins E and C, selenium, and NAC, for the treatment of NAFLD are of great interest [8,16]. NAC is a thiol-containing agent that eliminates free oxygen radicals, binds directly to reactive metabolites, and replenishes mitochondrial and cytosolic glutathione stores by acting as a glutathione substitute, thereby preventing hepatic damage. Decreased glutathione levels and marked increases in oxidative stress parameters were observed in alcoholic liver disease, similar to what is seen in paracetamol intoxication [12]. In the present study, we examined the effects of NAC on rats in an experimental setting, as a strong antioxidant that can replenish glutathione stores in NAFLD, the pathogenesis of which is similar to that of alcoholic liver damage. In a prospective randomized case-control study, Nabi et al. demonstrated significant improvement in the survival of patients treated with NAC, and a lower mortality rate, of 53%, in the control group compared to 28% in patients treated with NAC. In the same study, the use of NAC was concluded to be safe and was shown to decrease the length of hospital stay [8]. NAC is an antioxidant, and a reducing and chelating agent, which replenishes glutathione stores except in the case of acetaminophen intoxication [12]. Zhou et al. compared NAC-treated rats to controls and demonstrated that NAC reduced ALT and AST activity, decreased TG and LDL-C levels, and improved the liver tissue to varying degrees [16].

In the present study, a single dose of 150 mg/kg NAC was administered i.p., and the serum ALT level was significantly decreased compared to the control group. On histopathological examination, NAC was shown to decrease fatty liver.

One of the major limitation of our study was that it was planned in 2002 and it was studied in 2004. Although more than a decade of work has been done, studies on L-Carnitine and N-Acetylcysteine are still up to date. However, this can be considered as an important limitation.

Conclusion

In conclusion, elucidation of the etiopathogenesis of, and new treatment approaches for, NHS are crucial because this condition can result in serious diseases, such as cirrhosis. Changes in the levels of oxidative stress parameters may be important for the diagnosis of liver damage caused by NHS, as well as for prediction of its prognosis. Our study showed that L-carnitine and NAC treatment resulted in significant regression of steatosis in rats with NHS. However, these findings must be confirmed by further studies including larger populations.

Acknowledgment
We are grateful to Santa Farma İlaç A.S. for providing CARNITINE® ampoules and Hisnâ Arsan İlaç A.S. for providing ASSIST® ampoules (NAC)

Competing interests
The authors declare that they have no competing interest.

Financial Disclosure
All authors declare no financial support.

Ethical approval
Consent of ethics was approved by the local ethics committee.
References


