Morphological investigation of alcohol-induced hepatocyte apoptosis and liver injury in rats

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The aim of this study was to morphologically investigate alcohol-induced hepatocyte apoptosis and liver injury in rats. A total of 20 male Sprague-Dawley rats were divided into two groups: control and alcohol treated; each group contain 10 animals. The rats in alcohol treated group were given a daily dose of 6 g/kg ethanol by using intra-gastric intubation. Control group was given the same volume of saline. This application was continued daily for a total of 6 weeks. The end of the experiment all animals were anesthetized. The anesthetized rats were sacrificed and liver tissues were removed for histopathological investigation. Liver damage was examined by using hematoxylin-eosin and apoptosis was determined by terminal-deoxynucleotidyl-transferase mediated dUTP nick end labeling (TUNEL). There existed hepatocyte diffuse steatosis and hemorrhage in alcohol treated group. Our data indicate an enhancement in the activity of TUNEL in hepatocyte apoptosis of the alcohol treated group. The effects of alcohol on liver can be clearly detected as a hepatocyte cell death and liver injury.


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1. Introduction
Alcoholic liver disease is a major health and economic problem in the western world (Kerr et al., 2000). Fatty infiltration, the first manifestation of alcohol-induced liver injury, is usually followed by inflammation, focal necrosis and terminal venular sclerosis, which ultimately can develop into cirrhosis (Tsukamoto et al., 2001). Steatosis was formerly considered a benign and fully reversible condition. However, new evidence suggests that hepatic fatty infiltration may in fact be an important pathogenic factor in the development of alcoholic liver disease (Stewart et al., 2001). It is currently understood that the pathogenesis of these diseases is related to apoptosis. It has been shown that an increase in the number of apoptotic cells in the liver is correlated with the development of ethanol-induced pathological liver injury (Nanji et al., 1996; Natori et al., 2001). Apoptosis is thought to occur through two main pathways: an extrinsic route which utilises death receptors, and an intrinsic pathway that involves intracellular mitochondrial stress signals, and leads to cytochrome c release and caspase activation (Hockenbery 1995; Thornberry and Lazebnik 1998; Lemasters 2005).

The aim of the present study was to morphologically investigate alcohol-induced hepatocyte apoptosis and liver injury in rats.

2. Material and methods
Animals
Twenty healthy male Wistar albino rats, weighing 200-250 g and averaging 16 weeks old were utilized in this study. Rats were fed on a standard rat chow and tap water ad libitum. In the windowless animal quarter automatic temperature (22±2°C) and lighting controls (light on at 07 AM and off at 09 PM: 14 h light/10 h dark cycle) was performed. Humidity ranged from 50% to 55%. All animals received human care according to the criteria outlined in the “Guide for the Care and Use of Laboratory Animals” prepared by the National Academy of Sciences and published by the National Institutes of Health. The study was approved by the Institutional Animal Ethical Committee of Namik Kemal University, Tekirdag, Turkey.

Experimental design
The rats were randomly allotted into one of two experimental
groups: control, and alcohol treated; each group contain 10 animals.

Experimental procedures and sample collection
The rats in alcohol treated group was given a daily dose of 6 g/kg ethanol every day for 6 weeks by using intra-gastric intubation. Control group was given the same volume of saline. This application was continued daily for a total of 6 weeks. The end of the experiment all animals were anesthetized by i.p. administration of 90 mg/kg ketamine and 10 mg/kg xylazine. The anesthetized rats were sacrificed and liver tissues were removed for histopathological investigation.

Histopathologic evaluation
The liver specimens were embedded in the paraffin blocks after they had been fixed in Bouin’s solution. For histopathology, sections of 5 μm were obtained, deparaffinized and stained with hematoxylin–eosin (H&E) and scored blindly for the degree of steatosis. Steatosis was graded from 0–5, following a protocol previously described (Nanji et al., 1994), with 0 depicting absence of cells with fat vacuoles and 5 that >75% of cells contained fat vacuoles. The liver tissue were evaluated with a bright field microscope (Nikon Optiphot 2, Tokyo) and photographed.

Evaluation of hepatocyte apoptosis
The TUNEL method, which detects fragmentation of DNA in the nucleus during apoptotic cell death in situ, was employed using an apoptosis detection kit (TdT-FragelTM DNA Fragmentation Detection Kit, Cat. No. QIA33, Calbiochem, USA). All reagents listed below are from the kit and were prepared following the manufacturer’s instructions. Five-μm-thick liver sections were deparaffinized in xylene and rehydrated through a graded ethanol series as described previously. They were then incubated with 20 mg/ml proteinase K for 20 minutes and rinsed in TBS. Endogenous peroxidase activity was inhibited by incubation with 3% hydrogen peroxide. Sections were then incubated with equilibration buffer for 10–30 minute and then TdT-enzyme, in a humidified atmosphere at 37 °C, for 90 minutes. They were subsequently put into pre-warmed working strength stop/wash buffer at room temperature for 10 minutes and incubated with blocking buffer for 30 minutes. Each step was separated by thorough washes in TBS. Labelling was revealed using DAB, counter staining was performed using Methyl green, and sections were dehydrated, cleared and mounted.

The number of TUNEL positive hepatocytes in each specimen was also scored according to Kanter (2010). Ten randomly selected septa were scored for each specimen in every experiment as follows: 0 = no positive response; 1 = less than 10% of hepatic cells; 2 = 11–20% of hepatic cells; 3 = 21–40% of hepatic cells; 4 =more than 40% of hepatic cells.

Statistical analysis
All statistical analyses were carried out using SPSS statistical software (SPSS for windows, version 11.0). All data were presented in mean (±) standard deviations (S.D.). Differences in measured parameters among the three groups were analyzed with a nonparametric test (Kruskal-Wallis). Dual comparisons between groups exhibiting significant values were evaluated with a Mann–Whitney U-test. These differences were considered significant when probability was less than 0.05.

3. Results
Histopathological changes
Histopathological examination of control liver showed normal morphology (Fig. 1a, b). The 6 week ethanol treatment resulted in marked steatosis and hemorrhage in liver tissue of rats (H&E, scale bar, 50 μm).

4. Discussion
Alcohol increases the risk of liver diseases. The pathogen-
Quantitative analysis of apoptosis in the liver of control and alcohol treated groups. Kruskal-Wallis test was used for statistical analysis. Values are expressed as means ± SD, n = 10 for each group. (ap < 0.01) compared to control group.

REFERENCES


