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Protective effects of vitamin D_3 against D-galactosamine-induced liver injury in rats

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ABSTRACT

In this study, we examined liver damage induced by D-galactosamine (D-GaIN) and the protective effects of vitamin D_3 in relation to D-GaIN toxicity.

Twenty Wistar albino rats were used in this study. The rats were divided into four groups. Group I rats were used as the control group. Group II rats were given a single intraperitoneal injection of D-GaIN. Group III rats were given a single intraperitoneal injection of D-GaIN, intramuscular vitamin D_3 for five days. Group IV rats were given intramuscular vitamin D3 for five days. All of rats were euthanized by cervical decapitation on the fifth day of experiment. Upon completion of the experiment, a midsaggital incision was performed, and the livers of all rats were removed and fixed. The livers were processed to perform TUNEL technique and histochemical staining.

During the microscope examination, we observed inflamatory cell infiltration, sinusoidal dilatation, and apoptotic bodies due to D-GaIN exposure. In addition, glycogen content of the group II hepatocytes was significantly decreased. Vitamin D_3 treatment provided better structural apperance of the livers in group III. TUNEL positive cells were extremly pervasive in the group II livers. The study found group III TUNEL positive cells at a reduced rate in relation to group II due to vitamin D_3 treatment.

This findings indicate that D-GaIN causes inflamation in the liver. This inflamation triggers the apoptotic process gradually. Vitamin D_3 has potency to decrease the severity of D-GaIN-caused structural liver damage.

1. Introduction

Acute liver failure is a clinical syndrome characterized with high mortality (Gu et al., 2014) by the sudden loss of hepatic function in the absence of preexistent liver disease (Xie et al., 2014). Hepatic encephalopathy, coagulopathy, jaundice and hydroperitoneum are life-threatening symptoms (Lee, 1994) of acute liver failure.

D-GaIN/lipopolysaccharide (D-GaIN/LPS) combination is often used as a hepatotoxin to perform massive hepatic inflammation, necrosis, and apoptosis. The combination has the capability to induce acute hepatic damage within a few hours after injection (Yan et al., 2013). The toxic effects of D-GaIN is a result of deficiency of UDP-glucose, UDP-galactose, and lack of intracellular calcium homeostasis. These alterations affect cellular membranes and organelles and the synthesis of proteins and nucleic acids (Devaki et al., 2009). D-GaIN also blocks the energy metabolism of hepatocytes (Mangeney-Andreani et al., 1982) and changes the phospholipid composition of membranes (Devaki et al., 2009). Hepatic injury induced D-GaIN closely resembles human viral hepatitis (Catal and Bolkent, 2008). Devaki et al. (2009) demonstrated that administration of D-GaIN decreased activities of the urea cycle enzymes, ornithine transcarbomylase, and arginase in rats.

Vitamin D_3, is an active metabolite of vitamin D, and has different biological functions. One of these biological properties is antioxidative action. Systemic-administred vitamin D_3 inhibited the elevated lipid peroxidation which was detected in vitamin D_3-deficient rats. Moreover, vitamin D_3 reduced oxidative stress by elevating the antioxidative defense system, including glutathione content, glutathione peroxidase and superoxide dismutase in astrocytes and the liver (Lin et al., 2005). The liver plays a key role in metabolism of vitamin D. Epidermal and diet sourced vitamin D is hydroxylated in the liver into 25-hydroxyvitamin D [25(OH)D]. 25(OH)D, is the major circulating form of vitamin D, and is used to determine a patient's vitamin D level. 25(OH)D is transported to the kidney via circulated blood and, subsequently undergoes a

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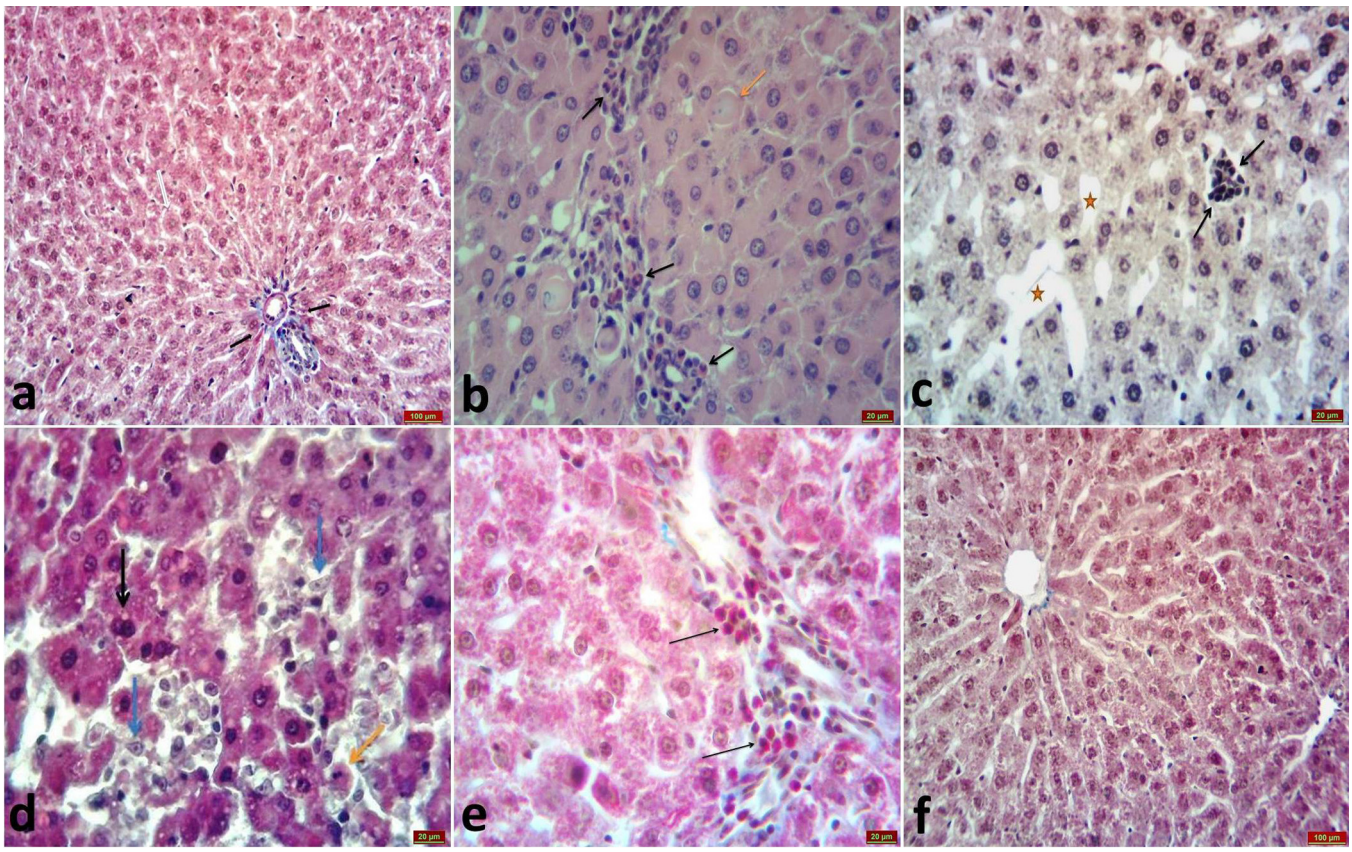

Fig. 1. Histopathological appearance of all groups. Periportal area (black arrow), hepatocytes (white arrow), sinusoids are normal in group I (a). Polymorphnuclear cell infiltration (black arrow) and apoptotic body (orange arrow) in the periportal area (b), sinusoidal dilatation (*) and inflammatory cell infiltration (arrow) (c) apoptotic body (orange arrow), active fibroblasts (blue arrow), vacuolization in the hepatocytes cytoplasm (black arrow) were evident in the group II (d), Periportal inflammatory cells in group III (e) and normal appearanced liver structure in of group IV. Techniques; MT, H&E, H&E, MT, MT, MT staining respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

second hydroxilation which then converts it into 1,25(OH)D, the active form of vitamin D (Arteh et al., 2010).

In light of these observations, we planned this experimental study to induce hepatic injury and in order to improve alternative therapy and protection choices.

2. Materials and methods

2.1. Animals

Ethical approval for this study has been obtained from Firat University Faculty of Medicine Ethics Board, and all procedures conformed to the "Guide for the Care and Use of Laboratory Animals". Twenty adult male Wistar albino rats (weighing 220–230 g) were used in this study. The rats were randomly divided into four groups. The rats were kept in Plexiglas cages (five animals per cage) and received standard food and water ad libitum in an air-conditioned room with automatically-regulated temperature (22 ± 1 °C) and light cycle (light: 07.00–19.00).

2.2. Experimental protocol

D-Galactosamine (Fluka, Italy) (D-GalN) dissolved in % 0.9 isotonic NaCl.

Group I (control) rats (n:5), received the normal diet and used as control.

Group II (D-GalN) rats (n:5), were given single intraperitoneal injection of D-GalN (500 mg/kg) and euthanized by cervical decapitation five days later.

Group III (D-GalN + vitamin D_3) rats (n:5), were given single intraperitoneal injection of D-Galactosamine (D-GalN, 500 mg/kg) and intramuscular vitamin D_3 for five days and euthanized by cervical decapitation on the fifth day.

Group IV (vitamin D_3) rats (n:5), were given intramuscular vitamin D_3 for five days and euthanized by cervical decapitation on the fifth day.

A midsaggital incision was performed at the end of the experiment, and the livers of all rats were removed and fixed in 10% formaldehyde for 24 h. After adequate fixation, the tissues were dehydrated through a graded ethanol series, cleared in xylol, and then embedded in paraffin wax. The tissue blocks were sectioned at a thickness of 5 μm to perform TUNEL technique and histochemical staining.

2.3. Histochemical staining

Tissue samples of all groups were stained by using standard Hematoxylin & Eosin (H&E), Masson's Trichrome (MT) and Periodic acid Schiff (PAS) tecniques.

2.4. TUNEL technique

Apoptotic cell deaths in liver were detected by TUNEL assay using ApopTag plus Peroxidase in situ Apoptosis Detection Kit (Chemicon, Catno: S7101,USA) based on the instructions of the producer. TUNEL method makes DNA breaks in the cells visible. Percentages of TUNEL-positive cells were quantified by counting 100 cells from ten random microscopic fields. Apoptotic index (the per-