Experimental and Toxicologic Pathology 66 (2014) 361–365

Contents lists available at ScienceDirect

Experimental and Toxicologic Pathology

journal homepage: www.elsevier.de/etp

The effects of grape seed and colchicine on carbon tetrachloride induced hepatic damage in rats

Ayhan Atasever, Duygu Yaman*

Erciyes University Faculty of Veterinary Medicine, Department of Pathology, Kayseri, Turkey

ARTICLE INFO

Article history:
Received 14 February 2014
Received in revised form 26 April 2014
Accepted 29 April 2014

Keywords:
Carbon tetrachloride
Colchicine
Grape seed
Liver
Rat

ABSTRACT

This study aims to determine the effects of grape seed and colchicine on carbon tetrachloride (CCl_4) induced hepatic damage and on some serum biochemical parameters. Sixty male Wistar albino rats (200–250 g) were randomly divided into six groups (ten rats/group) and included the control group the group were given isotonic sodium chloride (1 mL/kg b.w) intraperitonealy (i.p.), group 2 the group treated i.p. injection of CCl_4 (1.0 mL/kg b.w) in corn oil twice in the first week, Groups 3 and 4 injected with CCl_4 as described for group 2 and the rats were orally given (100 mg/kg b.w) GSE and i.p. injected (10 μg/rat) with colchicine for four weeks, respectively and groups 5 and 6 were the grape seed and colchicine control groups in which rats were orally given grape seed (100 mg/kg b.w) and i.p. injected with colchicine (10 μg/rat), respectively. Anorexia, weight loss, motionlessness and hepatic colour variation at necropsy were observed in groups 2, 3, and 4. Hyperemia, focal bleeding, fat degeneration, changes ranging from degenerative to necrotic, increase in connective tissue elements, pronounced in portal sites in particular, and infiltration of lymphoid series cell observed in the livers of the rats in group 2, treated with CCl_4. Histological hepatic changes in the rats in group 3 and 4 were similar to those in group 2. The levels of serum total protein, albumin and globulin decreased in groups 2, 3, and 4, compared with groups 1, 5 and 6; aspartate transaminase (ALT) activities increased. The lowest alkaline phosphatase (ALP) activities were in groups 4 and 5. We concluded that GSE and colchicine have not sufficient ameliorative effects to CCl_4 induced acute hepatic damage.

1. Introduction

Carbon tetrachloride (CCl_4) is one of the most commonly used for inducing liver injury in experimental animal studies (Nielsen et al., 1991; Johnston and Kroening, 1998). The toxicity of CCl_4 probably depends on formation of the trichloromethyl radical (CCl_3), which can rapidly react with oxygen to trichloromethyl peroxyl radical (CCl_3O_2) (Packer et al., 1978; Basu, 2003). These lipid radicals quickly add molecular oxygen to form lipid peroxyl radicals, by that initiating the process of lipid peroxidation (Freeman and Crapo, 1982; McCay et al., 1984; Reddy and Mannaerts, 1994). Reactive oxygen free radicals have been known to produce tissue injury through lipid peroxidation (Singh and Gupta, 2011). Scavenging of free radicals by antioxidants could reduce the fibrosis process in the tissues (Haggag, 2011).

Plant origin polyphenolic compounds are intensely studied in the recent years (Nichols and Katiyar, 2010). Grape (*Vitis vinifera*) is particularly rich source of proanthocyanidins (Silva et al., 1991). Proanthocyanidins (GSPs) have been shown to be potent antioxidants and free radical scavengers, being more effective than either ascorbic acid or vitamin E (Uchida, 1980; Shi et al., 2003). Grape seed extract (GSE) has a preventive role in production of not only extra-cellular matrix elements which cause hepatic fibrosis (Bouhamidi et al., 1998; Bagchi et al., 2000; Feng et al., 2005) but peroxyl radicals which have the role in starting and/or maintaining lipid peroxidation (Kara and Kocaoglu Guclu, 2012).

Colchicine, which is an alkaloid obtained from *Colchium autumnale*, has a suppressive effect on collagen synthesis and enhances collagenase activity (Leighton et al., 1990; Mourelle and Villalon, 1988). Colchicine represses mitosis of cells in liver fibrosis and decreases functions of inflammation cells by its anti-inflammatory effect. Therefore it prevents fibrosis and lipid peroxidation (O'Conner, 1988; Leighton et al., 1991; Menino et al., 1993).

* Corresponding author at: Erciyes University Faculty of Veterinary Medicine, Department of Pathology, Melikgazi, Kayseri, 38039, Turkey.
Tel.: +90 352 207 66 66/29928; fax: +90 352 337 27 40.
E-mail addresses: dyguyaman@hotmail.com, dyaman@erciyes.edu.tr (D. Yaman).

http://dx.doi.org/10.1016/j.etp.2014.04.008
0940-2993/

This study aims to determine the effects of grape seed and colchicine on carbon tetrachloride (CCl_4) induced hepatic damage with histopathology and some biochemical parameters.

2. Materials and methods

All animal experimental procedures were approved by ethical committee of Erciyes University, Faculty of Veterinary Medicine, Kayseri (#2007-04/05), Turkey and the experimental procedures were performed in Erciyes University Experimental Research and Application Center, Kayseri, Turkey. Carbon tetrachloride (CCl_4) was purchased from Merck (1.94504) limited, France. Colchicine was obtained from Biological Industries Israel Beit Haemek Ltd, Israel and GSE was obtained from GNC Corporation (19801), Turkey. Five-month old male Wistar albino rats (200–250 g) purchased from Erciyes University Experimental Research and Application Center, Kayseri, Turkey. All rats were fed with pellet diet and kept at 21–25 °C under a 12 h dark/light cycle. Rats were randomly divided into six groups (10 rats/group) and included the control group the group were given isotonic sodium chloride (1 mL/kg b.w) intraperitonealy (i.p.), group 2 the group treated i.p. injection of CCl_4 (1.0 mL/kg b.w) in corn oil twice in the first week, groups 3 and 4 injected with CCl_4 as described for group 2 and the rats were orally given (100 mg/kg b.w) (Ragab et al., 2013) GSE and i.p. injected (10 μg/rat) (Muriel et al., 2005) with colchicine for four weeks, respectively and groups 5 and 6 were the grape seed and colchicine control groups in which rats were orally given grape seed (100 mg/kg b.w) and i.p. injected with colchicine (10 μg/rat), respectively. Rats were anesthetized with intramuscular of ketamine (50 mg/kg) and xylazine (10 mg/kg) injection and blood samples were collected by heart puncture 24 h after the last CCl_4 administration. Blood samples were centrifuged at 3000 r.p.m. for 10 min and serum was taken in eppendorf tube. All serum samples were kept at −20 °C until analysis. All serum parameters were assayed spectrophotometrically using an autoanalyzer (AbbottArchitect, C802713).

All animals were killed by decapitation at the end of the experiment. For each rat, the liver was removed and fixed in neutral formalin solution (10%). Tissue specimens were embedded in paraffin wax and sectioned (thickness, 5 μm), for histopathological evaluation. After staining with haematoxylin and eosin (H&E), they were examined with light microscope.

Statistical analyses were carried out using SPSS 15.0 for windows software and performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. All values were expressed as mean values ± standard error of means (SEM).

3. Results

In the experiment; weakness, hunched posture, staggered gait, excessive salivation, ptosis, ataxia and corneal opacity were clinically observed in rats. There is no colour change observed in livers in the groups 1, 5 and 6 but in the groups 2, 3 and 4, dark red and grey-white colour changes were noted. There was also recorded hepatomegaly.

Histopathological structure of liver tissues in the control group was found normal (Fig. 1A). Hepatic damage was found significant in the group 2, as compared with the control group (group 1). Necrotic changes, mild fibrosis and hepatosteatosis were determined especially in the portal sites in the group 2. Hepatosteatosis was observed in parenchyma, mostly in the cells around vena centralis (Fig. 1B). Necrotic changes in the hepatocytes around vena centralis resulted in pink homogenous mass formation and there was an increase in number of connective tissue cells in portal area (Fig. 1C). Although infiltrating lymphocyte-rich mononuclear cells were clustered in especially close to portal sites, they were less frequently spread through necrotic areas around vena centralis and all parenchyma (Fig. 1D). Histopathological findings for group 3 were similar to group 2. It was observed that hepatocytes included different sizes of lipid vacuoles where hepatosteatosis was dense. Some of the cells contained multivacuolar oil vacuoles and their nucleuses were centrally localized, while some of them were large and univacuolar oil vacuoles with sharp edges contained flattened nucleuses pushed to cell periphery (Fig. 1E). Findings of the rats for group 4 were similar to the group 2 and group 3. Hepatosteatosis were observed in all parenchyma, especially in the cells around vena centralis (Fig. 1F). Analysis of the livers of the rats in the group 5 and group 6 presented that they had normal structures that were close to the control group.

As shown in Table 1, serum total protein, albumin and globulin levels for the rats in the CCl_4-intoxicated group (groups 2, 3 and 4) were found significantly higher than GSE (group 5) and colchicine (group 6) applied groups ($p < 0.001$). In the rats of the groups 2, 3 and 4, serum ALT activities were increased as compared with the control group ($p < 0.001$); however, there was no significant difference for serum ALT activity between the groups 5 and 6. ALT activity was not affected from grape seed application in group 3, while decreased with colchicine application in group 4 ($p < 0.001$). Compared to the control group (group 1), serum ALP activity was elevated but not statistically significant in CCl_4 group (group 2). There was a significant decrease in serum ALP activities in the GSE and colchicine applied groups as compared to the control group (group 1) and CCL_4 group (group 2) ($p < 0.01$). Total cholesterol, triglyceride and serum AST enzyme levels were not affected from CCl_4 treatment ($p > 0.05$) (Table 1).

4. Discussion

It is known that oxidative stress may cause cancer, atherosclerotic cardiovascular diseases, liver diseases and toxic cell damages (Freeman and Crapo, 1982; Jiang et al., 1992). It has determined that CCL_4 causes, oxidative stress and inactivation of liver Kupffer cells (Reddy and Mannaerts, 1994; Pinzani and Rombouts, 2004). Proctor and Chatamra (1982) notified that there was a high death ratio when using CCl_4 on rats. Nielsen et al. (1991) and Jiang et al. (1992) found the ratios 19% and 20%, respectively. In the present study, there was not a significant death ratio in CCl_4 (1 mL/kg) applied groups. These results may be relevant to administration dose and routes. Wang et al. (1996) reported that a single intraperitoneal injection of CCl_4 caused degenerative changes in hepatocytes, especially around vena centralis on mice. Srica and Williams (1992) determined that single administration of 0.6 mL of $CC1_4$ by gavage after ductus ligation, necrosis occurred around the portal site not because of ligation but the CCl_4 injection on rats; Noa et al. (2003) stated that 1 mg/kg CCl_4 i.p. injection caused central necrosis on Sprague-Dawley rats. In this study, hepatosteatosis that was formed around vena centralis and parenchyma in the group two doses of CCl_4 applied was found similar to the findings of the studies mentioned above. Our findings such as hepatosteatosis that formed around vena centralis in the group given two doses of CCl_4 were applied on in the first week and the slight fibrosis in parenchyma and mononuclear cell infiltration like studies of Sokal et al. (1990) and Montfort and Tamayo (1978). They also determined hepatosteatosis, centrilobular necrosis, increase in fibrosis tissue and cell infiltration.

There are limited numbers of experimental studies on the histological effects of colchicine on liver. In these researches, it has stated that colchicine increases perivascular fibrosis, prevents lipid peroxidation and regresses hepatosteatosis (Mourelle and Villalon, 1988; Sabouraud et al., 1992). Muriel et al. (1993) researched the