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The protective effect of vitamin D against carbon tetrachloride damage to the rat liver

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Abstract

We investigated the protective effect of vitamin D against liver damage caused by carbon tetrachloride (CCl₄). Twenty-four male rats were divided into four equal groups: G1, untreated controls; G2, administered CCl₄; G3, administered both CCl₄ and vitamin D for 10 weeks; G4, administered CCl₄ for 10 weeks and vitamin D for 12 weeks. At the end of experiment, intracardiac blood samples were taken and liver samples were removed. Hepatic damage due to CCl₄ was assessed using biochemistry and histopathology. Glutathione (GSH) levels decreased, while malondialdehyde (MDA) levels increased in liver tissues of G2. Alanine transaminase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl-transaminase (GGT) levels increased, while albumin (ALB) levels decreased. Hepatocyte degeneration, lobular disorder, sinusoid dilation, focal necrotic areas, hyperemia, and glycogen loss was noted. Hepatic fibrosis was observed around portal areas and central veins. Bridging fibrous septa were formed between portal veins. By immunohistochemistry, both matrix metalloproteinase-9 (MMP-9) and desmin reactivity were increased. All aspects of liver damage were at least partially prevented in rats treated with vitamin D. Vitamin D appears to act as an antioxidant and anti-fibrotic to protect the rat liver against damage.

Key words: carbon tetrachloride, desmin, hepatic fibrosis, matrix metalloproteinase-9, oxidative stress, vitamin D

Hepatic fibrosis is an accumulation of the extracellular matrix (ECM) in the liver (Xia et al. 2006, Prosser et al. 2006, Török 2008). Accumulation of ECM proteins deforms the normal structure and disturbs normal liver function. The rates of ECM production and degradation are balanced in normal liver; consequently fibrosis does not occur. Fibrosis occurs when the destruction and formation of ECM becomes unbalanced (Hernandez-Gea and Friedman 2011). Disturbances in connective tissue metabolism are characterized by increased expression of matrix metalloproteinases (MMPs) (Matrisian 1994). MMP-9

(gelatinase B) is produced by Kupffer cells, hepatocytes, and hepatic stellate cells (HSC) (Hernandez-Gea and Friedman 2011). MMP-9 activates latent TGF- β by proteolysis (Yu and Stamenkovic 2000), which in turn stimulates collagen synthesis and transforms hepatic stellate cells (HSHs) into myofibroblasts (Gressner et al. 2002).

Oxidative stress also plays an important role in the etiology of hepatic fibrosis (Castilla-Cortasar et al. 1997, Parola and Robino 2001, Ogeturk et al. 2008, Hong et al. 2009). Free radicals that are released in cells when toxic substances are not fully metabolized can cause liver damage. Treatment with carbon tetrachloride (CCl₄) is widely used to create a hepatotoxicity model for experimental studies (Lee et al. 2003, Ding et al. 2005, Zhang et al. 2011, Huang et al. 2014). When metabolized by hepatic microsomal enzymes, CCl₄ forms free radicals that cause liver damage by triggering multiple harmful reactions

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including lipid oxidation (Debnath et al. 2013, Ingawale et al. 2014).

Vitamin D exhibits both direct and indirect antioxidant effects. Directly, vitamin D is a membrane antioxidant (Wiseman 1993, Preedy 2014). Indirectly, it affects the antioxidant defense system, by up-regulating endogenous antioxidant effectors like superoxide dismutase, catalase, glutathione, and glutathione peroxidase (Preedy 2014, Mokhtari et al. 2017). Major sources of vitamin D are exposure to ultraviolet radiation and diet. The bioavailability of vitamin D after oral consumption depends on the absorption capacity during digestion. Specific enzymes including 25(OH)D-1 α -hydroxylase, vitamin D-25-hydroxylase, CYP24, and CYP3A4 that are involved in the metabolism of vitamin D regulate its bioavailability (Tsiaras and Weinstock 2011).

The liver participates in the metabolism of vitamin D (Arteh et al. 2010). Vitamin D is converted to 25-hydroxyvitamin D [25(OH)D] in the liver and into its active form, 1,25 dihydroxyvitamin D [1,25(OH)₂D], in the kidney (Holick 2007, Abramovitch et al. 2011). Vitamin D functions through the vitamin D receptor (VDR), which is a ligand-bound transcription factor (Abramovitch et al. 2011). Vitamin D activation by binding to VDR regulates directly or indirectly the expression of more than 200 genes (Holick 2007, Kitson and Robert 2012). These genes control proliferation and differentiation of both normal and cancer cells (Nagpal et al. 2005, Holick 2006 and 2007, Kitson and Roberts 2012). Low levels of vitamin D have been reported in a wide range of disorders due to inflammation (Autier et al. 2014). Vitamin D deficiency results in progressive fibrosis in the liver (Arteh et al. 2010, Petta et al. 2010, Lange et al. 2011, Terrier et al. 2011, Baur et al. 2012). Therefore, we investigated the biochemical, histochemical, and immunohistochemical effects of 1,25 (OH)₂D₃ treatment of rats with hepatic damage caused by CCl₄.

Material and methods

Animals and experimental design

We used 24 2.5–3-month-old 200–220 g male Wistar albino rats after securing approval from the Ethics Committee of Gazi University Experimental Animal Center. Animals were kept in rooms with a 12 h light:12 h dark cycle at constant temperature (22 $\pm$ 2° C) and provided standard pellet feed and tap water *ad libitum*. The rats were divided into four groups of six: control (G1), CCl₄ (G2), CCl₄ + vitamin D for 10 weeks (G3), CCl₄ for 10 weeks + vitamin D for 12 weeks (G4) (Table 1). Corn oil was used as the vehicle for both CCl₄ and vitamin D (1,25(OH)₂D₃) delivery. G1 rats were injected with 1.5 ml/kg corn oil subcutaneously (s.c.) twice each week for 10 weeks as the control. G2 rats were injected s.c. with sterile 1:1 CCl₄ dissolved 1.5 ml/kg in corn oil twice each week for 10 weeks to promote hepatic fibrosis. Every day a 0.5 μ g/kg dose of intraperitoneal (i.p.) 1,25(OH)₂D₃ was administered to G3 and G4 animals (Abramovitch et al. 2011). At the end of the experiment, rats were anesthetized using 5–10 mg/kg ketamine/xylazine i.p. and samples of liver tissues were excised, frozen in liquid nitrogen and stored at –80° C for biochemical analysis. Other tissue samples were fixed with 10% formalin for 12 h for histochemical and immunohistochemical studies. Intracardiac blood was collected in tubes containing EDTA and separated by centrifugation for 5 min at 3,000 $\times$ g. Blood serum was stored at –80° C until testing.

Malondialdehyde (MDA)

Tissue samples were homogenized at 4° C in cold trichloroacetic acid (TCA) (Sigma Aldrich, St. Louis, MO), 1 g tissue + 9 ml 10% TCA, then centrifuged at 1792 $\times$ g for 15 min. The supernatant was centrifuged again at 1792 $\times$ g for 8 min. To 750 μ l of the second supernatant, 750 μ l 0.67% thiobarbituric acid (TBA)

Table 1. MDA, GSH, ALT, GGT, ALP and ALB levels in all groups

Groups	Name	MDA	GSH	ALT	GGT	ALP	ALB
Control	(G1)	14.12 $\pm$ 7.53	4.65 $\pm$ 0.89	62.67 $\pm$ 5.6	5.00 $\pm$ 0.01	201.33 $\pm$ 51.3	4.18 $\pm$ 0.1
CCl ₄	(G2)	27.63 $\pm$ 3.06	3.84 $\pm$ 0.48	92.67 $\pm$ 19.5	10.67 $\pm$ 6.01	294.17 $\pm$ 70.5	3.13 $\pm$ 0.4
CCl ₄ + vitamin D for 10 weeks	(G3)	10.17 $\pm$ 0.14*	4.46 $\pm$ 0.89	46.67 $\pm$ 2.6*	3.00 $\pm$ 0.00*	98.33 $\pm$ 12.5	3.68 $\pm$ 0.1
CCl ₄ for 10 weeks + vitamin D for 12 weeks	(G4)	13.46 $\pm$ 0.72*	4.47 $\pm$ 0.87	49.83 $\pm$ 6.6	3.00 $\pm$ 0.00*	114.17 $\pm$ 25.3	3.45 $\pm$ 0.1

Data are means $\pm$ SEM. **p* < 0.05 vs. CCl₄.

(Sigma Aldrich) was added and samples were incubated in a boiling water bath at 100° C for 15 min, followed by centrifugation at $1792 \times g$ at 4° C. The absorbance at 532 nm of each supernatant was determined. The lipid oxidation level for liver tissues was expressed as an MDA equivalent using an extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1}/\text{cm}^{-1}$ (Casini et al. 1986).

Glutathione (GSH)

Liver tissues were homogenized and centrifuged as above, then two volumes of supernatant were mixed with eight volumes 0.3 M $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ (Sigma Aldrich) and one volume 5,5'-dithiobis-(2-nitrobenzoic) acid (DTNB) (Sigma Aldrich) dissolved in 0.4 mg/ml 1% sodium citrate buffer solution. Samples were incubated at room temperature for 5–10 min, then absorbance at 412 nm was measured. GSH levels were calculated using an extinction coefficient of $13,600 \text{ M}^{-1}/\text{cm}^{-1}$ (Aykaç et al. 1985).

Determination of liver enzymes

Rats were anesthetized using ketamine/xylazine and blood samples were collected into non-heparinized tubes by cardiac puncture. Serum was obtained after centrifugation at $1792 \times g$ for 10 min. Serum samples were used for biochemical assays using a VetScan analyzer (Abaxis, Union City, CA). Serum biochemical parameters including alkaline phosphatase (ALP), albumin (ALB), alanine aminotransferase (ALT) and gamma glutamyltransferase (GGT) were evaluated.

Histochemistry

Tissue samples were fixed in 10% formalin, then placed under running tap water overnight and transferred to 70% alcohol. Samples were passed through an ascending alcohol series, cleared with xylene and embedded in paraffin. Sections were cut at 4–5 μm for histochemical examination using a microtome (Thermo Scientific, Waltham, MA). Sections were stained with hematoxylin and eosin (H & E) (Sigma Aldrich). Fibrosis was assessed using Masson's trichrome (Sigma Aldrich) staining and glycogen was assessed using periodic acid-Schiff (PAS) (Merck, Darmstadt Germany) staining. All staining was performed according to Bancroft and Gamble (2008).

Immunohistochemistry

Immunohistochemistry was performed using the avidin-biotin-HRP method. Desmin and MMP-9 (Millipore, Darmstadt, Germany) staining protocols followed the manufacturer's instructions. The antibody complexes were visualized using DAB500 kit (Millipore).

Statistical analysis

The SPSS 20.0 software was used for statistical analysis. In cases where more than two groups of independent variables were obtained, Kruskal-Wallis analysis of variance was used. With categorical variables, a *post-hoc* multiple comparisons test was applied to determine differences or correlations between groups. Qualitative variables were expressed as ratios, while quantitative variables were averaged. Values for $p \leq 0.05$ were considered statistically significant.

Results

Lipid oxidation and antioxidant activity

To confirm that hepatotoxicity is associated with oxidative stress in a rat model, we measured MDA and GSH levels in CCl_4 treated animals and also evaluated the protective effect of vitamin D. MDA levels, which indicate lipid oxidation, were higher in liver tissues of G2 than in G1 animals, which is consistent with increased oxidative stress in these animals. By contrast, the MDA levels in G3 and G4 animals were reduced compared to G2. The differences between G3 and G2, and between G4 and G2 were statistically significant ($p < 0.05$) (Table 1). MDA levels following vitamin D treatment were similar to the control, particularly for G4 rats (Fig. 1a). The GSH content of G4 liver was significantly higher than for G2 ($p < 0.05$) (Table 1, Fig. 1b). Both MDA and GSH experiments indicated that CCl_4 causes lipid oxidation and decreases glutathione levels in rat liver, but that vitamin D can reverse this effect.

Biochemistry

The levels of serum ALT, ALP, and GGT were higher in G2 than in G1 rats, while the level of ALB was lower. In vitamin D treated animals (G3 and G4), ALT, ALP, and GGT serum levels decreased compared to G2, while the ALB level increased (Fig. 2). The mean ALT level of G3 was significantly lower than the mean ALT level of

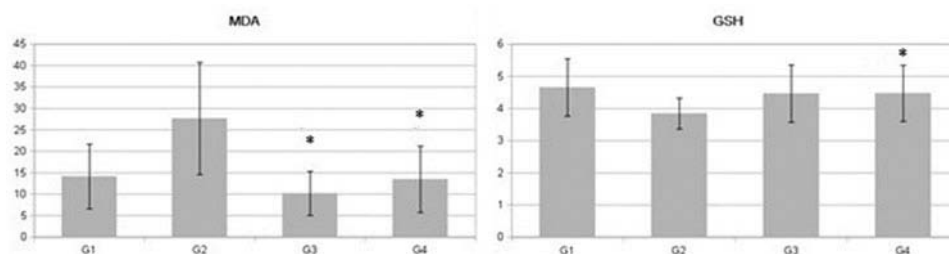


Fig. 1. Comparison of MDA and GSH levels in experimental groups. a) MDA. b) GSH. *Significantly different from CCl₄ group (G2).

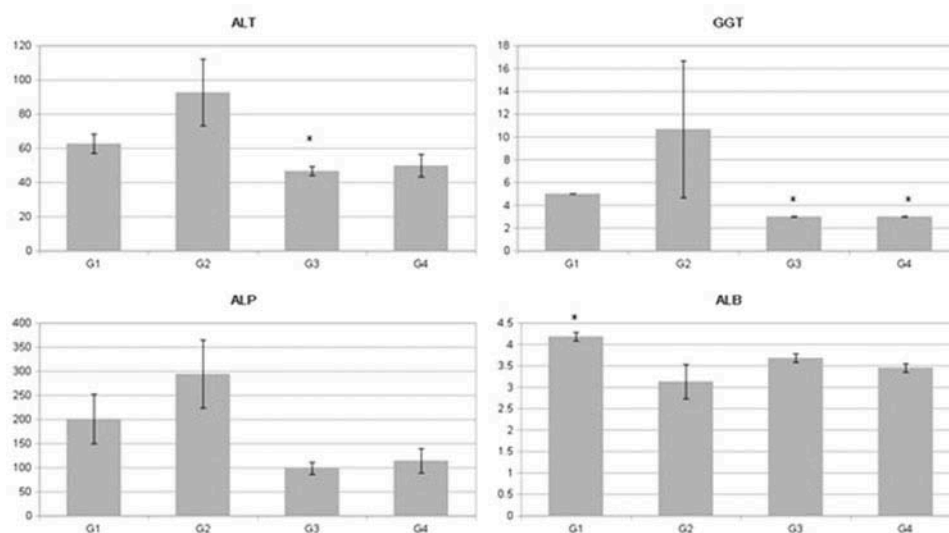


Fig. 2. Comparison of liver enzyme activities in experimental groups. a) ALT. b) GGT. c) ALP. d) ALB. *Significant difference from CCl₄ group (G2).

G2 ($p < 0.05$). Similarly, the mean GGT levels of G3 and G4 were significantly lower than the mean GGT level of G2 ($p < 0.05$) (Table 1). These findings demonstrate further the protective effect of vitamin D in CCl₄ treated rats.

Histochemistry

In sections of G1 liver stained with H & E, hepatocytes were arranged radially around the central vein and were flanked by the sinusoids (Fig. 3a).

Liver tissues of G2 rats exhibited damage to hepatocytes, sinusoids, central vein and the hepatic lobular structure. The cytoplasmic contents were lost and necrotic foci of “ghost-like” hepatocytes were apparent (Fig. 3b). The sinusoids were dilated and the central vein, portal vein and sinusoids exhibited hyperemia (Fig. 3c). Mononuclear cell infiltration was less frequent in the portal area (Fig. 3d).

In G3 rats, we observed that liver tissue integrity was partially protected. In G4 rats, the overall appearance of the liver was closer to that of the G1 animals (Fig. 3e, f).

Collagen fibers were found only rarely (Fig. 4a, 4b) in G1 sections stained with Masson’s trichrome. In G2 sections, however, collagen fibers were increased around the portal tracts and central vein (Fig. 4c). Fibrous bridges between two portal veins also were observed (Fig. 4d). Fibrosis was not observed in vitamin D treated animals (G3 and G4) (Fig. 4e, f). Therefore, CCl₄ promoted fibrosis, but simultaneous treatment with vitamin D prevented this.

Hepatocytes of G1 rats were stained by PAS, which indicated the presence of glycogen (Fig. 5a). G2 rat livers exposed to CCl₄ contained PAS stained hepatocytes around the central vein, but also PAS unstained foci that corresponded to necrotic areas that had lost their glycogen (Fig. 5b). In the vitamin D treated groups, the distribution of PAS stained cells was closer to that of the control (Fig. 5c, d),

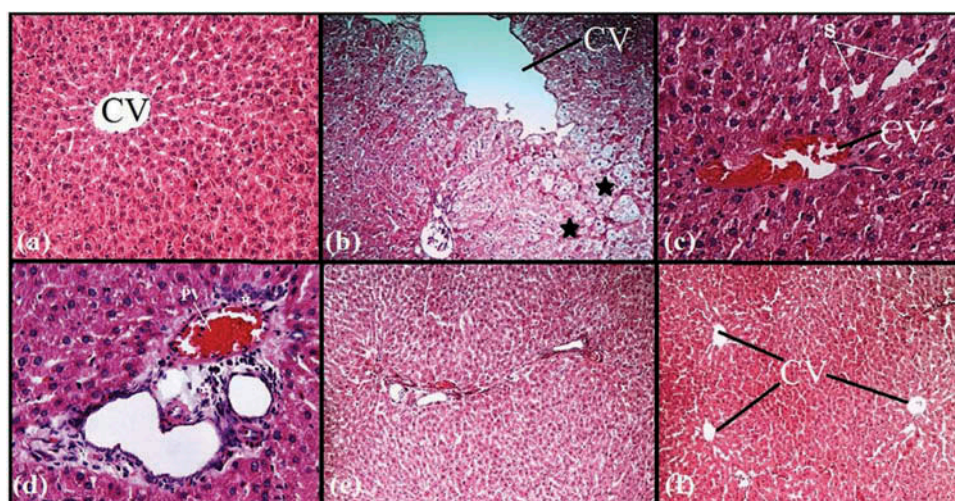


Fig. 3. Histological structure. a) Control animals (G1). CV, central vein. Scale bar = 100 µm. b) CCl₄ group (G2). Necrotic foci around degenerated central vein (asterisk). Scale bar = 200 µm. c) CCl₄ group (G2). Hyperemia in the central vein and dilatation in the sinusoids are evident. Scale bar = 50 µm. d) CCl₄ group (G2). Mononuclear cell infiltration (asterisk) was observed rarely in portal area and hyperemia in the portal vein (PV) is shown. Scale bar = 50 µm. e) CCl₄ + vitamin D for 10 weeks (G3). General appearance of liver. Scale bar = 200 µm. f) CCl₄ for 10 weeks + vitamin D for 12 weeks (G4). Histological appearance of liver. CV, central vein. Scale bar = 200 µm. All panels, H & E staining.

which is consistent with protection against CCl₄ induced damage.

Immunohistochemistry

G1 liver sections were MMP-9 negative (Fig 6a). Sections of G2, however, were MMP-9 positive. Stained regions were found in these animals, especially around the sinusoids and portal areas. MMP-9 was localized in the cytoplasm of hepatocytes (Fig. 6b). In the G3 liver, MMP-9 staining was less pronounced compared to G2 (Fig. 6c), while the G4 liver was MMP-9 negative (Fig. 6d).

Desmin positive cells were rare in G1 rats (Fig. 7a) by contrast to G2 animals, which generally reacted strongly to antibodies against desmin (Fig. 7b). Treatment with vitamin D dramatically reduced the number of desmin positive cells in G3 animals (Fig. 7c) and in G4 rats desmin expression was restored to levels comparable to G1 animals (Fig. 7d).

Discussion

Liver fibrosis is a consequence of many chronic liver diseases (Bataller and Brenner 2005) and oxidative stress has been implicated in its development (Poli 2000, Medina and Moreno-Otero 2005, Sánchez-Valle et al. 2012). CCl₄ is toxic and causes oxidative stress

(Lee et al. 2003, Ding et al. 2005, Starkel and Leclercq 2011, Huang et al. 2014).

We found by measuring MDA and GSH levels that CCl₄ administration caused lipid oxidation and decreased GSH levels in liver tissues. MDA is an indicator of lipid oxidation, while GSH is an important reducing agent in the antioxidant defense system (Yang et al. 2008, Anand et al. 2011, Sun et al. 2013, Chen et al. 2014, Ozerkan et al. 2015). Accordingly, MDA levels were increased significantly in G2 rats exposed to CCl₄, while GSH concentrations decreased significantly. Both groups of CCl₄ treated rats that also received vitamin D (G3 and G4) exhibited lower MDA levels than G2 animals, while their GSH levels were higher than for G2. Therefore, vitamin D can reduce lipid oxidation and increase GSH levels in CCl₄ treated rat liver to levels comparable to untreated control animals.

Lin et al. (2012) treated male rats with 2 ml/kg CCl₄ for 12 weeks and reported that serum ALT, ALP and GGT levels increased significantly compared to controls. Similarly, Motawi et al. (2011) reported elevated serum ALT, ALP and GGT levels in male rats administered 0.5 mg/kg CCl₄ for six weeks. Yin et al. (2012) reported that male rabbits given 0.2 ml/kg CCl₄ for 4 weeks caused liver fibrosis and high GGT levels in serum, while ALP and ALB levels were low. We also found that CCl₄ caused hepatic fibrosis and that serum ALP, ALT, and GGT activities increased

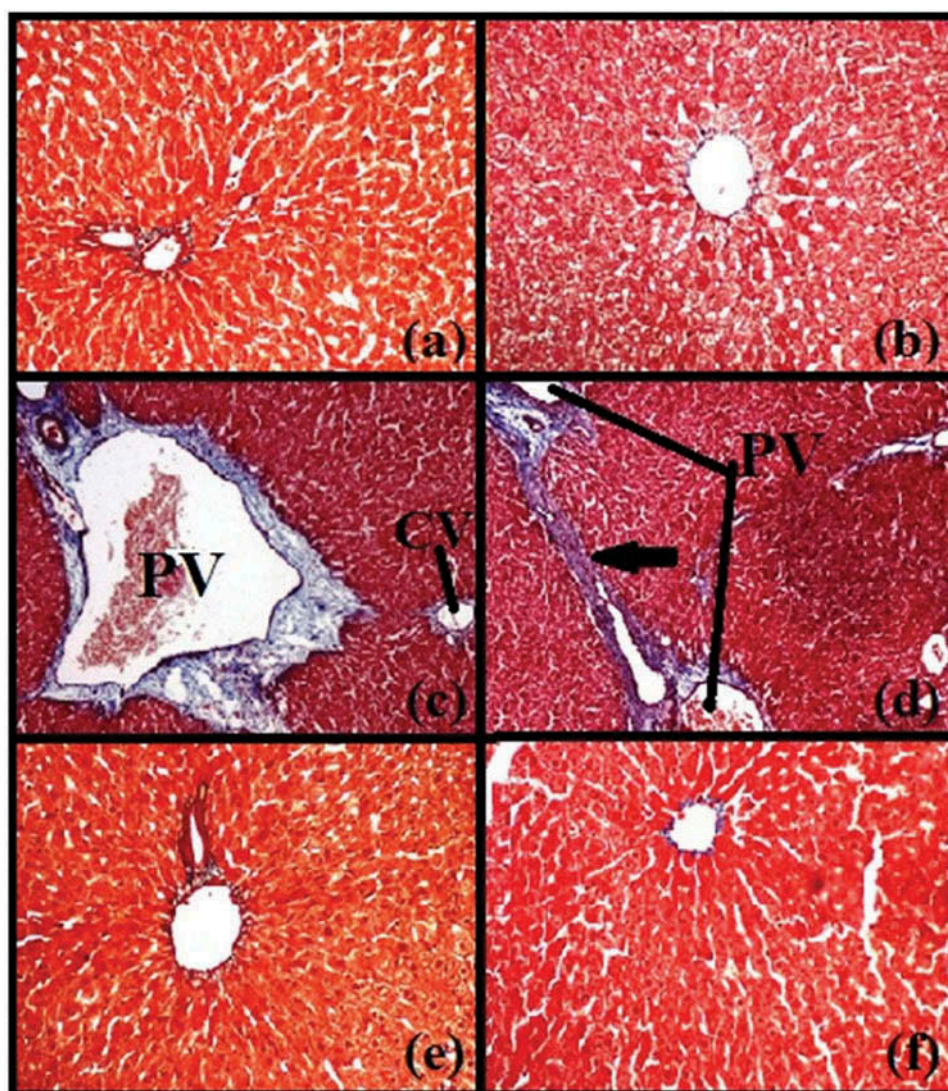


Fig. 4. Collagen fibers. a) Control group (G1) portal area. Scale bar = 200 μm . b) Control group (G2), area around central vein. Scale bar = 100 μm . c) CCl_4 (G2) group. Increased connective tissue around portal vein (PV) and central vein (CV). Scale bar = 200 μm . d) G2 group. Fibrosis (arrow) between two portal veins. Scale bar = 200 μm . e) CCl_4 + vitamin D (G3) for 10 weeks. Appearance contrasts markedly to Fig 4c and is somewhat similar to Fig 4a. Scale bar = 100 μm . f) CCl_4 for 10 weeks + vitamin D for 12 weeks (G4). Normal distribution of collagen in central vein wall. Scale bar = 100 μm . Masson's trichrome staining.

significantly, while ALB levels were decreased. We showed also that treatment of CCl_4 exposed rats with vitamin D for 10 or 12 weeks restored serum ALP, ALT, GGT, and ALB to levels that approached controls.

That CCl_4 causes hepatic fibrosis is well documented (Ding et al. 2005, Gui et al. 2006, Liu et al. 2006). CCl_4 exposure for ≥ 7 weeks leads to bridging fibrosis (Lee et al. 2003, Huang et al. 2014). Consistent with earlier studies, we found that administration of CCl_4 for 10 weeks caused increased collagen

deposition around the portal areas and central veins, which we identified as bridging fibrosis in some sections.

Abramovitch et al. (2011) demonstrated both in vitro and in vivo that $1,25(\text{OH})_2\text{D}_3$ prevents liver fibrosis in thioacetamide treated rats. Ding et al. (2013) used molecular and histological techniques to show that calcipotriol, a calcemic vitamin D analogue, reduced CCl_4 induced liver fibrosis in rats. We also found that $1,25(\text{OH})_2\text{D}_3$ exhibited an anti-fibrotic effect on liver fibrosis caused by CCl_4 .

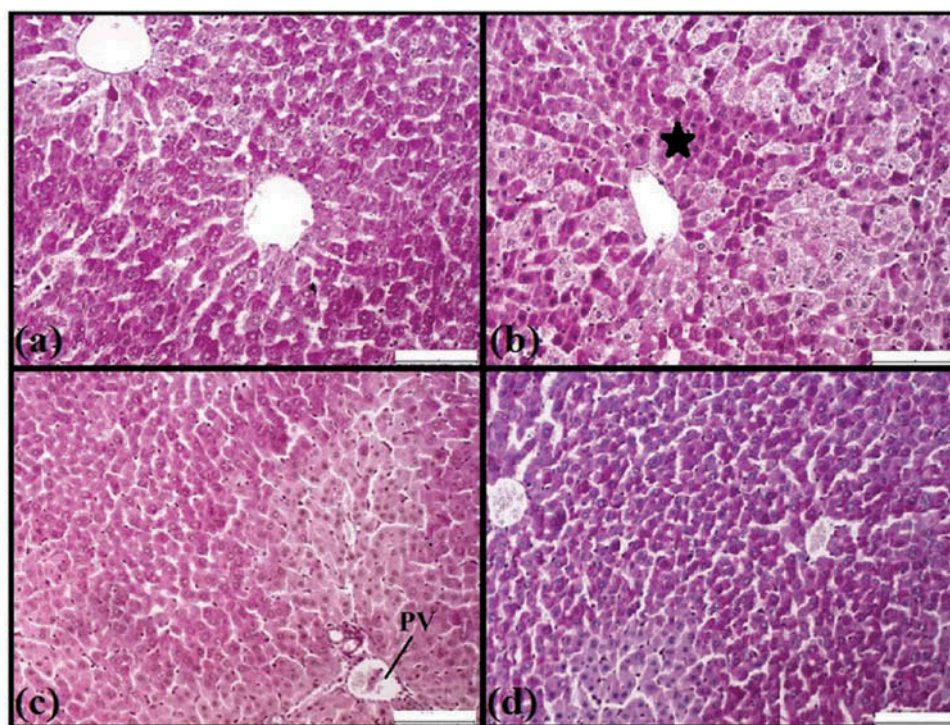


Fig. 5. Distribution of glycogen. a) Control rats (G1). General appearance of liver. PAS stained hepatocytes are shown. b) G2 rats (administered of CCl_4 for 10 weeks). PAS stained (asterisk) cells and PAS unstained necrotic foci around the central vein. c) G3 rats (CCl_4 plus vitamin D for 10 weeks). PAS stained hepatocytes. PV, portal vein. d) G4 rats (given CCl_4 for 10 weeks and vitamin D for 12 weeks). Liver showing PAS stained hepatocytes similar to the control group. Scale bars = 100 μm .

In a histological study of male Sprague-Dawley rats that were exposed to CCl_4 , Yang et al. (2008) reported hepatocellular degeneration, necrosis, inflammatory cell infiltration, sinusoidal dilation and congestion. We also found focal necrotic areas with degenerated hepatocytes, sinusoidal dilation and hyperemia in rats treated with CCl_4 . These pathological findings were nearly eliminated by vitamin D, particularly in the 12 week treatment group.

Matté et al. (2009), using PAS staining, reported decreased liver glycogen in liver sections from Wistar albino rats with chronic hyperhomocysteinemia, whereas Savransky et al. (2007), using light and electron microscopy, demonstrated accumulation of glycogen in hepatocytes of rats with chronic intermittent hypoxia (CIH). We found that glycogen content was decreased in liver tissues of CCl_4 treated animals. Necrotic foci in which the cells were devoid of glycogen were particularly evident. By contrast, the glycogen content in the liver of vitamin D treated rats was similar to that of control animals.

Increased levels of MMP-9 appear to be associated with liver damage. Knittel et al. (2000) reported that although there were few MMP-9-positive cells in

normal liver, both acute and chronic liver damage increased MMP-9 reactivity. Zhang et al. (2010) evaluated MMP-9 reactivity in a rat model of hepatic fibrosis, and reported a lack of reactivity in the liver of control animals, but intense MMP-9 reactivity in rats treated with CCl_4 for eight weeks. These investigators localized MMP-9 to the cytoplasm of hepatocytes, vascular endothelial cells and sinusoidal endothelial cells. Similarly, we found that MMP-9 reactivity was absent in control liver, but there was strong reactivity in CCl_4 treated animals, especially in hepatocyte cytoplasm and around the sinusoids and portal areas.

Vitamin D regulates the activity of MMPs (Dean et al. 1996), which is why we investigated MMP-9 levels. Timms et al. (2002) showed that levels of circulating MMP-9 was increased in individuals with vitamin D insufficiency in chronic disorders such as coronary heart disease and diabetes. We found that compared to G2 animals, rats also given vitamin D (G3 and G4) exhibited decreased MMP-9 immunoreactivity to near control levels. Our immunohistochemical findings suggest that vitamin D regulates the expression of MMP-9 in rat liver.

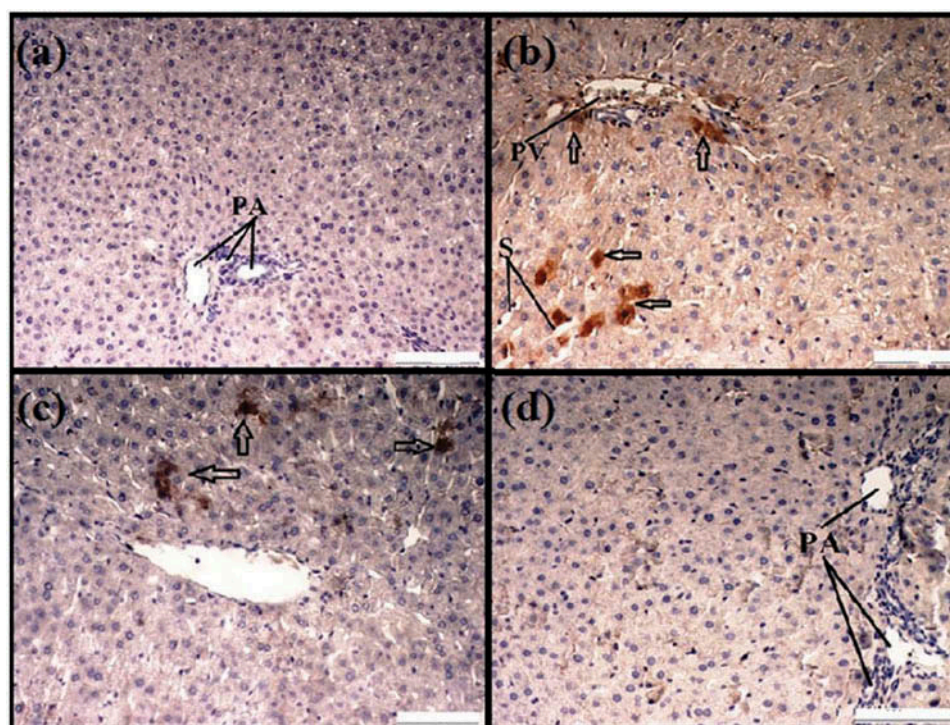


Fig. 6. MMP-9 immunoreactivity. a) Control rats (G1). Histological appearance of MMP-9-negative liver. PA, portal area. b) G2 rats (CCl₄-treated, 10 weeks). MMP-9-positive cells distributed in portal area and around sinusoids (S). PV, portal vein. c) G3 rats (CCl₄ plus vitamin D for 10 weeks). Sparse labeling for MMP-9-positive cells (arrows). d) G4 rats (given CCl₄ for 10 weeks and vitamin D for 12 weeks). MMP-9-negative tissue. DAB staining. Scale bars = 100 μm.

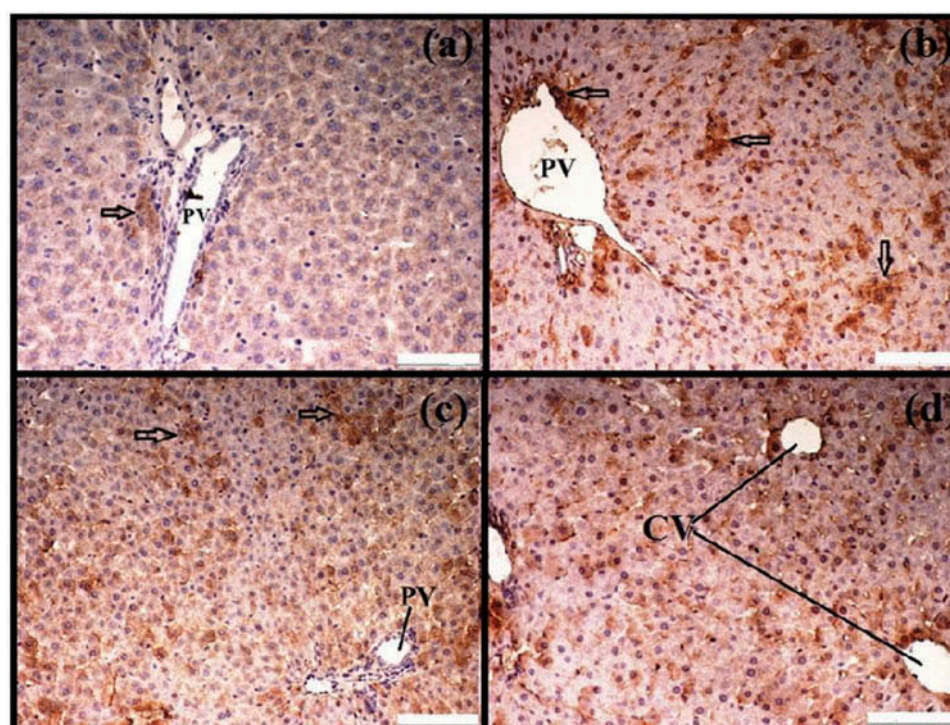


Fig. 7. Desmin immunoreactivity. a) Control group (G1). Poor desmin immunoreactivity (arrow). PV, portal vein. b) G2 rats (CCl₄ treated, 10 weeks). Strong desmin immunoreactivity in the parenchyma and around the portal vein (arrows). c) G3 rats (CCl₄ plus vitamin D for 10 weeks). Weak desmin immunoreactivity (arrows). d) G4 rats (given CCl₄ for 10 weeks and vitamin D for 12 weeks). Weak desmin immunoreactivity in the liver lobule. CV, central vein. DAB staining. Scale bars = 100 μm.

Previous studies have shown that desmin immunoreactivity is increased by fibrosis of the liver (Knežević et al. 2009, Fujii et al. 2010, Fan et al. 2013). Similarly, we found strongly desmin positive cells around the central veins and portal areas as well as in the walls of the sinusoids in sections of liver with fibrosis that resulted from CCl₄ exposure. Desmin immunoreactivity was reduced by vitamin D treatment.

Overall, our findings provide clear biochemical, histochemical and immunohistochemical evidence that administration of vitamin D protects rat liver tissue from the toxic effects of CCl₄. Vitamin D reduces lipid oxidation and exerts a clear anti-fibrotic effect. Our findings confirm and complement reports in the literature, but we introduced novel elements into the design of the experiment. In addition to the dosage of CCl₄ and vitamin D, we added 10 and 12 week groups. The anti-hepatotoxic effects of vitamin D likely are due to two mechanisms: vitamin D acts as an antioxidant by scavenging active oxygen and free radicals, and by preventing lipid oxidation. Vitamin D also exhibits anti-fibrotic effects on liver fibrosis (Abramovitch et al. 2011). Administration of vitamin D, particularly in animals treated for 12 weeks, prevented CCl₄ induced liver damage. Application of our findings to a therapeutic context must be supported by clinical studies.

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