Protective Effect of Silybin in Rats Liver Toxicity

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Abstract

Silybin is a flavonoid extracted from the herb Armurariu (Silybum marianum) and has the potential efficacy in the treatment of liver disease. The aims of this study were to investigate the effect of alcohol and CCl4 on liver histology and the capacity of silybin to ameliorate the hepatotoxicity. Thirty adult male Wistar rats were used in the study. Liver toxicity was induced by dietary alcohol administration and CCl4 intra-peritoneal injection. The protective effect of silybin was investigated by co-administration of silybin with these toxic agents. Hepatocellular and extracellular matrix integrity was determined by histopathological and immunohistochemical study. Hematoxylin-Eosin and trichrome stains sections were studied in each case. For immunohistochemistry we used monoclonal anti-collagen IV primary antibody. Light microscopic evaluation of liver tissues shows that control and silybin treated groups has normal liver structure. In the toxicity groups, HE and trichrome staining showed hepatocellular necrosis, inflammatory infiltrate and proliferating collagen fibers. Immunoreactivity of collagen IV was variable. In the control group, we found negative expression. Collagen IV displays positive immunoreaction in hepatotoxicity groups, at the level of the areas rich in inflammatory infiltrate and with degenerative aspect. After this study, we can conclude that silybin, in rats, has protective effects.

Keywords: silybin, hepatotoxicity, alcohol, CCl4

1. Introduction

Silybin is the main biologically active flavonolignan extracted from the dried seeds of the herb Armurariu (Silybum marianum). Silymarin is the biological extract of Armurariu and silybin is the major active constituent of silymarin [1]. Various studies have evidenced the positive biological effects of silybin, which has anti-inflammatory, antioxidative and anticarcinogenic properties in vivo animal models [2,3,4]. It has been used in the treatment of acute poisoning by the mushroom Amanita phalloides and a variety illness of different organs as liver, prostate, lungs, CNS, kidneys, pancreas, skin [5,6,7].

The aim of this study was to investigate the effect hepatoprotective effects of silybin in rats treated with two different toxic agents: alcohol and CCl4. The alcohol and CCl4 are a potent hepatotoxicity and carcinogenetic effect and induced liver injury in rats experimental models.

2. Materials and methods

Drug Administration. Liver toxicity was induced by dietary alcohol administration and CCl4 intra-peritoneal injection. The protective effect of silybin was investigated by co-administration of silybin with these toxic agents. Silybin was purchased from Aldrich Chemical Co. The required dose was dissolved in 0.2ml propylene glycol in salin (1:1, v/v) [1]. Silybin
was intraperitoneal (i.p.) injected at a dose of 200 mg/kg/day, 5 days/week, 12 week. CCl4 (Sigma Aldrich Chemical Co) was administrated at a dose of 1.0 ml/kg body weight, three times a week for 12 wk, dissolved in olive oil [8]. Alcohol 40% was given by dietary administration 9 g/kg per day, 12 weeks.

**Animals.** Adult Wistar male rats (200-250g) were obtained from the Experimental Animal Center of Timisoara Medical University. The animals were kept at 25°C with enough humidity and under controlled lighting (12-hours light/12-hours dark) periods. The rats were fed standard diets and allowed food and water *ad libitum*. Animals were randomly divided into five groups containing six animals in each group. Each group was kept in a separate cage. The first group: rats were administered propylene glycol in saline (1:1, v/v), this group served as a control. The second group: rats were i.p. injected with 10% CCl4. The third group: rats were i.p. injected with silybin and 10% CCl4. The fourth group: rats received 40% alcohol. The fifth group: rats received silymarin by i.p. injection and alcohol.

**Histopathological and immunohistochemistry examination**

Liver biopsy specimens was fixed in 10% neutral buffered formalin, dehydrated in ethanol, cleared in xylene, embedded in paraffin and cut into 5 μm thick sections which were placed on plain glass slides. Hematoxylin-Eosin (HE), Masson and Goldner trichrome stained sections were studied in each case. Liver sections were examined under light microscope and graded as normal, minimal, mild or severe lesions. For immunohistochemistry visualization, liver sections was placed on polylysine-coated glass slides and transferred into 3% H2O2 in methanol for 10 minutes; then washed with PBS containing 0.03% non-fat milk and 0.01% Tween 20 and immunostained with primary antibodies for collagen IV (monoclonal mouse anti-body, Dako, used at a 1:25 dilution). For secondary antibodies, we used the system-HRP. The section were counterstained with Mayer’s hematoxylin and the color reaction was developed with DAB (diaminobenzidine tetrahydrochloride).

**3. Results and discussion**

Light microscopic evaluation of liver tissues shows that control group has normal liver architecture (figure 1). In the toxicity groups, normal structure of lobules was destroyed. HE and trichromic staining showed hepatocellular necrosis, vacuolization, steatosis, inflammatory infiltrate and proliferating collagen fibers. In rats livers treated only with alcohol, we found microvesicular steatosis and spotty hepatocellular necrosis (figure 2). The infiltrative inflammatory process is made up of lymphocytes, plasmocytes but also of fibroblasts and fibrocytes (figure 3). In contrast to the control group, Masson and Goldner trichrome staining revealed an increased number of collagenous fibers, perivenular fibrosis and collagenosis of the Disse spaces (figure 4,5).

![Figure 1](image1.png)
**Figure 1.** Normal histological structure of liver in control group, HE, 100x

![Figure 2](image2.png)
**Figure 2.** Steatosis, vacuolisation and hepatocellular necrosis in alcohol-treated group, HE, 200x

![Figure 3](image3.png)
**Figure 3.** Inflammatory infiltrate, in alcohol-treated group, HE, 200x
Centrilobular necrosis, moderate macrovesicular and microvesicular steatosis of liver were found in the CCl4-only-treated group (figure 6). Liver cells around central veins showed relatively a high number of necrosis and apoptosis. The perilobular inflammatory process turns into an intralobular one on some preparations, the inflammatory infiltrate having the same structure as the one present at the level of the port area (figure 7).

In few cases, we noticed lesions at the label of the bile ducts, which were signaled by the vacuolarisation of the cytoplasm and by its acidity. In this group, there was collagen accumulation in the centrilobular area (figure 8).

In silybin-treated rats, the liver structures were not different from the experimental control. HE staining showed normal central veins, hepatic cells and hepatic plates in the control group. Masson trichrome revealed few fibrous tissues around the central vein and septa.

Immunoeexpression of collagen IV was variable. Liver section from the control group showing negative expression. Collagen IV displays positive
immunoreaction in hepatotoxicity groups, at the level of the areas rich in inflammatory infiltrate and with degenerative aspect (figure 10).

**Figure 10.** Immunostaining for collagen IV antibody, DAB, 200x

After repeated injury, the extracellular matrix of basement membrane including type I, III, and IV collagen is over-deposited in perisinusoid. Deposition of type I, III, IV collagen and LN reduces hepatic sinusoidal penetration and causes sinusoidal capillarization [9].

In this study, in toxicity groups, we noticed an unspecific lesion pattern, with variable histological aspects both at the level of the parenchyma and at the level of the extracellular matrix. This results agreed with previous studies witch have demonstrated that alcohol induced liver damage in animals models. When treated with alcohol by gavages three times a day for 12 weeks, the model exhibited hepatocellular steatosis, necrosis and inflammation; fibrosis occurred around the central vein and perisinusoidal area [10,11,12,13]. CCl4 has been used as a tool induced hepatotoxicity in animals models [14,15]. In the administration of CCl4 to the animals, the normal structure of lobules was destroyed, and pseudolobules formed. Infiltration of inflammatory cells was found around the portal area and central vein. In liver tissues from rats with hepatic fibrosis, hyperplasia of the collagenous fibers was observed in portal area and extended outwards. The hepatic lobules were encysted and separated by collagen bundles [8, 14, 16].

The available literature shows that the extracts obtained from several plants, including Silybum marianum, have hepatoprotective activities [17,18,19,20]. Ahmed et al., reported that, silymarin has antihepatotoxic activity against carbon tetrachloride induced hepatotoxicity in albino rats [21]. Lee et al., reported that, administration of silymarin (100 mg/kg) by gavage twice a day for 2 consecutive days resulted in an elevation of hepatic superoxide dismutase levels. It also resulted in a decrease in hepatic inducible nitric oxide protein in liver homogenate after 24 hr after CCl4 intoxication [22]. Necrotic cells were observed very rarely in the Silybum marianum and thioacetamid treated rats [23]. Silymarin is known to have hepatoprotective and anticarcinogenic effects [6,24,25,26,27]. The use of silymarin/silybin may make a breakthrough as a new approach to protect other organs in addition to liver. Nomura et al also suggest that silybin modulated the insulin actions, including glucose uptake in adipocytes [28]. Oral silybin has been shown to inhibit skin carcinogenesis in mice [20,29] and human bladder tumor [30].

**4. Conclusions**

In summary, after this study, we can conclude that silybin, in rats, has protective effects against alcohol and CCl4 induced liver toxicity. Silybin administration significantly reduced the number of animals with liver alterations. In near future new derivatives of silybin open new ways to its therapeutic applications.

**References**