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Analyzing the Possible Hepatic Protection of Ursodeoxycholic Acid in Rat Models of Carbon Tetrachloride-Induced Hepatotoxicity

Ayala Ya seen Ali Bey
Department of Pharmacology and Toxicology, College of Medicine, University of Babylon, Iraq, med739.alyaa.yaseen@student.uobabylon.edu.iq

Maj id Radium Barabbas
Department of Pharmacology and Toxicology, College of Medicine, University of Babylon, Iraq, majid.bashara@uobabylon.edu.iq

Ahmed Raj
Department of Pathology, College of Medicine, University of Babylon, Iraq, dr.ahmedraji@uobabylon.edu.iq

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ORIGINAL STUDY

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Analyzing the Possible Hepatic Protection of Ursodeoxycholic Acid in Rat Models of Carbon Tetrachloride-Induced Hepatotoxicity

Ayala Ya seen Ali Bey [a,*], Maj id Radium Barabbas [b], Ahmed Raj [c]

[a] Department of Pharmacology and Toxicology, College of Medicine, University of Babylon, Iraq
[b] Department of Pharmacology and Toxicology, College of Medicine, University of Babylon, Iraq
[c] Department of Pathology, College of Medicine, University of Babylon, Iraq

Abstract

Background: The liver is essential for the detoxification of toxic substances as well as for metabolism, storage, and secretion. The liver is the primary organ for the metabolism of proteins, lipids, and carbohydrates. Exposure to certain xenobiotics can cause oxidative stress, which can impair liver function.

Objectives: The current study aimed to evaluate the possible protective effect of ursodeoxycholic acid against the hepatotoxicity induced by carbon tetrachloride administration.

Materials and Methods: Thirty adult male albino rats weighing between 250 and 300 g and aged between 10 and 12 weeks were included in the study. Three groups were included; group A received only normal saline intraperitoneal (I.P.) for 14 days. Group B received 0.5ml/kg carbon tetrachloride (CCL4) I.P. on day 6 of the experiment, twice a week, and Group C received 50 mg/kg ursodeoxycholic acid for 14 days + CCL4 was given I.P. on day 6 of the experiment, twice a week.

Results: In contrast to the positive control group (induction by carbon tetrachloride), the results showed that ursodeoxycholic acid preserved the liver in the treated group. In comparison to the positive control group, the Ursodeoxycholic acid-treated group showed significantly higher levels of glutathione (GSH), significantly lower levels of TNF-α, and lower levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). These results proved that ursodeoxycholic acid has a hepatoprotective effect on male albino rats.

Conclusion: These findings highlight ursodeoxycholic acid's capacity to protect the liver.

Keywords: Ursodeoxycholic acid, Carbon tetrachloride (CCL4), Hepatotoxicity, Oxidative stress, Inflammation

1. Introduction

The detoxification of drugs and other xenobiotics is handled by the liver. The metabolism and detoxification of drugs and xenobiotics are significantly influenced by phase I and phase II enzymes. Drug consumption, metabolism, and excretion alter dynamic equilibrium, causing a shift towards the production of free radicals and oxidative stress, both of which are detrimental to normal liver function [1]. Hepatic stellate, endothelial, parenchymal, and kupffer cells are important targets for oxidative radicals produced by toxins and drugs. Excessive production of oxidative radicals damages hepatocytes and initiates a cascade mediated by reactive oxygen species (ROS) that causes hepatocyte death and either acute or chronic liver damage [2].

Viral infections, drug irritations, chemical toxicants, alcohol-induced oxidative stress, inflammation, and immunological responses are the main causes of liver

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* Corresponding author.
E-mail addresses: med739.alyaa.yaseen@student.uobabylon.edu.iq (A. Ya seen Ali Bey), majid.bashara@uobabylon.edu.iq (M. id Radium Barabbas), dr.ahmedraji@uobabylon.edu.iq (A. Raj).

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injury. oxidative stress and inflammation are the primary causes of liver damage [3]. Hepatic stellate cells are activated by chronic inflammation and subsequently develop into myofibroblasts. The majority of the extracellular matrix in the liver is produced by these myofibroblasts. Liver fibrosis develops over time as a result of the extracellular matrix's growing production [4]. More than a thousand drugs have been linked to drug-induced liver injury (DILI) [5]. It's commonly thought that carbon tetrachloride (CCl4) causes liver damage by increasing lipid peroxidation, lowering antioxidant enzyme activity, and creating free radicals; however, this is true of almost all toxic substances. Chronic liver injury may develop as a result of prolonged carbon tetrachloride (CCl4) dosage. The liver is an amazing organ for regeneration; however, persistent damage to the liver can cause cirrhosis, fibrosis, and eventually liver failure [6]. Lipid peroxidation is the oxidative damage process by which CCl4 damages tissues. It entails the use of the cytochrome P450 enzyme to convert CCl4 to free radicals of extremely harmful trichloromethyl radicals ($\bullet CCl_3$) and trichloromethyl peroxyl radical ($\bullet CCl_3$ O2) [7].

Human bile contains trace amounts of ursodeoxycholic acid (UDCA), a naturally occurring bile acid. Ursodeoxycholic acid decreases the amount of cholesterol produced and released by the liver as well as blocking its absorption through the intestines. UDCA is the most widely prescribed drug for hepatopathies; however, it differs from other bile acids due to its distinct physiological and physicochemical properties, which include anti-inflammatory and protective effects [8].

The current study aimed to evaluate the possible protective effect of ursodeoxycholic acid against the hepatotoxicity induced by carbon tetrachloride administration.

2. Materials and methods

Thirty adult male albino rats weighing between 250 and 300 g and aged between 10 and 12 weeks were included in the study. These rats had to adjust to living in an animal house with 25-degree Celsius temperatures, 60 to 65 percent relative humidity, light and dark cycles lasting 14 hours, and a specific brand of commercial water and food. After ten days of acclimatization, the chosen rats were split into three groups at random. In every study, ten rats were utilized. From December 18, 2023, to January 1, 2024, this study was carried out at the College of Medicine at the Animal House/University of Babylon.

The selected rats were divided randomly into three groups, with ten rats in each group as follows:

- Group A received only Normal saline intraperitoneal (I.P.) for 14 days.
- Group B received 0.5 ml/kg Bwt of carbon tetrachloride (CCL4) (India) I.P. on day 6 of the experiment, twice a week [9].
- Group C is the treated group and received 50 mg/kg body weight (Bwt) of ursodeoxycholic acid (Bioneer/ Iraq) dissolved in normal saline orally by gavage for 14 days + CCL4 (India) was administered intraperitoneally.P. on day 6 of the experiment, twice a week [8].

On day 15 of the experiment, the rats were euthanize using xylazine (10 mg/kg) and ketamine (75 mg/kg). The diaphragm and rib cage were removed, the rat's abdomen was dissected, and serum samples were obtained from the heart so that blood could be extracted from it. Then, in order to avoid hemolysis, the blood was carefully poured into a gel tube after being extracted straight from the heart using a 5 cc syringe. The gel tube containing all of the blood was then centrifuged for ten minutes at 3000 RPM in order to produce a clear serum. Following that, each serum sample was moved to a plain tube and kept in the refrigerator, where it was kept between 2 and 8 degrees. Celsius, in order for them to be biochemically investigated using an ELISA kit (BT LAB /China) for glutathione (GSH), aspartate aminotransferase(AST), alanine aminotransferase(ALT) and tumor necrosis factor alpha (TNF-a).

3. Statistical analysis

Data was expressed as the mean ±SEM, and the statistical significance of the differences between various groups was determined by one-way analysis of variance (ANOVA). Differences were considered statistically significant for (P-value) less than (0.05).

4. Results

The results of the three parameters are described in Table 1.

4.1. Alanine aminotransferase biomarker

Results (n = 10) are expressed as the mean (± SD). This figure shows a highly significant increase in ALT level in Group B group in comparison to Group A. Meanwhile, in Group C, the ALT level decreased significantly compared to Group B.

4.2. Aspartate aminotransferase biomarker

Results (n = 10) are expressed as the mean (± SD). This figure shows a highly significant increase in AST levels in group B compared to group A. However, in

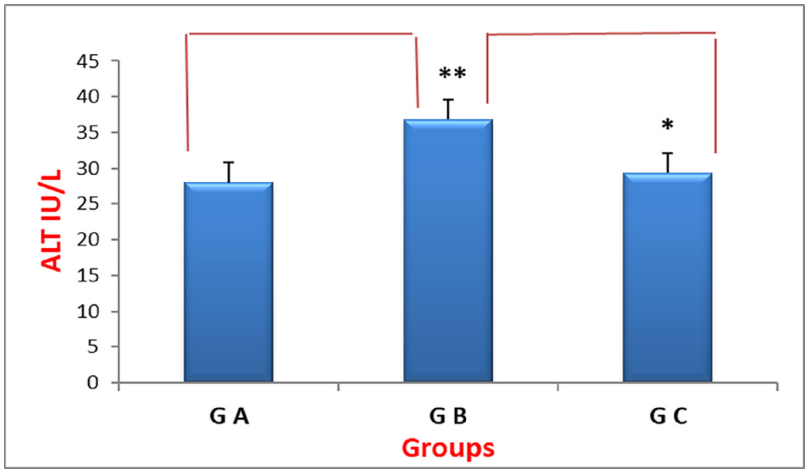

*Fig. 1. Group A received only normal saline for 14 days; Group B received 0.5 ml/kg of carbon tetrachloride two doses weekly for 2 weeks; Group C is the treated group (50 mg/kg of ursodeoxycholic acid was given for 14 days + carbon tetrachloride was given 2dose/week for 2 weeks). (*p < 0.05), (**p < 0.001).*

Table 1. Comparison of the studied biomarkers in all the studied groups.

Dependent variable	Group A N = 10	Group B N = 10	Group C N = 10
ALT IU/L	27.99 ± 3.79	36.82 ± 8.3**	29.3 ± 5.62 α
AST IU/L	38.27 ± 6.5	109.16 ± 21.67**	37.98 ± 14.65 α
GSH mg/L	92.96 ± 13.44	59 ± 4.97***	95.28 ±13.44*
TNF-α ng/l	120.32 ±15.74	133.14 ±11.86**	118.69 ± 7.85 α

* Significant increase compared to group B; ** Significant increase compared to group A; *** Significant decrease compared to group A; α significant decrease compared to group B. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GSH: Glutathione; TNF-a: Tumor necrosis factor alpha. Group A received only normal saline for 14 days; Group B received 0.5 ml/kg of carbon tetrachloride two doses weekly for 2 weeks); Group C is the treated group (50 mg/kg of ursodeoxycholic acid was given for 14 days + carbon tetrachloride was given 2dose/week for 2 weeks.

group C, AST levels decreased significantly compared to the B group.

4.3. Glutathione biomarker

Results (n = 10) are expressed as the mean (± SD). This figure shows a highly significant decrease in GSH levels in group B compared to group A. On the other hand, group C shows a highly significant increase in GSH levels compared to the B group.

4.4. TNF-α biomarker

Results (n = 10) are expressed as the mean (± SD). It has been shown that TNF-α levels increased significantly in group B compared to group A. Meanwhile, TNF-α levels detected a significant decrease in group C compared to group B.

5. Discussion

Fatty liver disease is often associated with inflammation, oxidative stress, and hepatocyte death. Examining the potential advantages of ursodeoxycholic acid (UDCA) for the treatment of non-alcoholic fatty liver disease (NAFLD) was the aim of this study. UDCA treatment was observed to significantly reduce edema, cellular necrosis, fatty degeneration, and immune cell infiltration when compared to rats with NAFLD-induced edema. Reduced antioxidant defenses and elevated reactive oxygen species production are the causes of oxidative stress in non-alcoholic fatty liver disease (NAFLD).

An increased rate of hepatocyte apoptosis is associated with elevated levels of oxidative stress. The degenerative effects of NAFLD in the animals' livers were dramatically decreased by UDCA treatment, which also decreased the inflammatory response and increased enzymatic antioxidation [10]. This study uses indicators such as glutathione, TNF-alpha, aspartate aminotransferase (AST), and alanine transaminase enzyme (ALT) to measure and evaluate the effect of ursodeoxycholic acid on the hepatotoxicity of carbon tetrachloride in male rats. The most visible indicator of liver damage is the release of cellular enzymes into the bloodstream, such as alanine transaminase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), due to impaired transport functions in liver cells. The ALT test provides a more accurate assessment of hepatic damage [11]. Due to its primary concentration in the liver, ALT is more susceptible to liver damage [12].

Numerous endogenous and exogenous environmental stressors have made the liver more vulnerable to damage in recent years, increasing the risk of acute

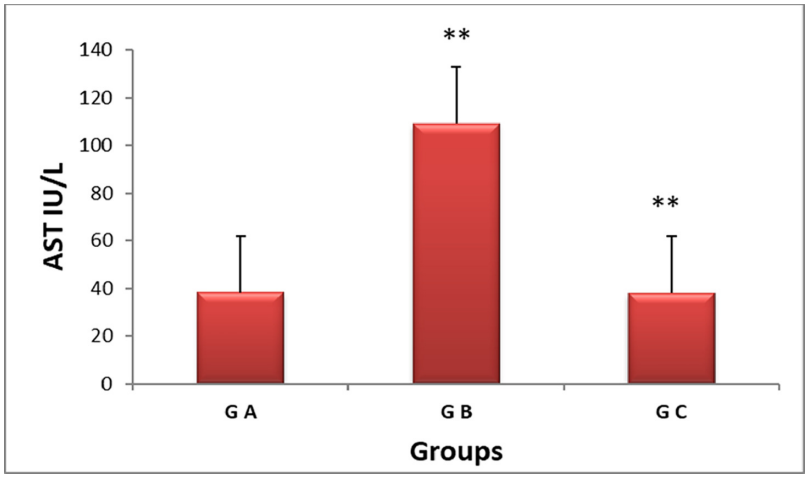

*Fig. 2. Group A received only normal saline for 14 days; Group B received 0.5 ml/kg of carbon tetrachloride two doses weekly for 2 weeks; Group C is the treated group (50 mg/kg of ursodeoxycholic acid was given for 14 days + carbon tetrachloride was given 2 dose/week for 2 weeks). (**p < 0.001).*

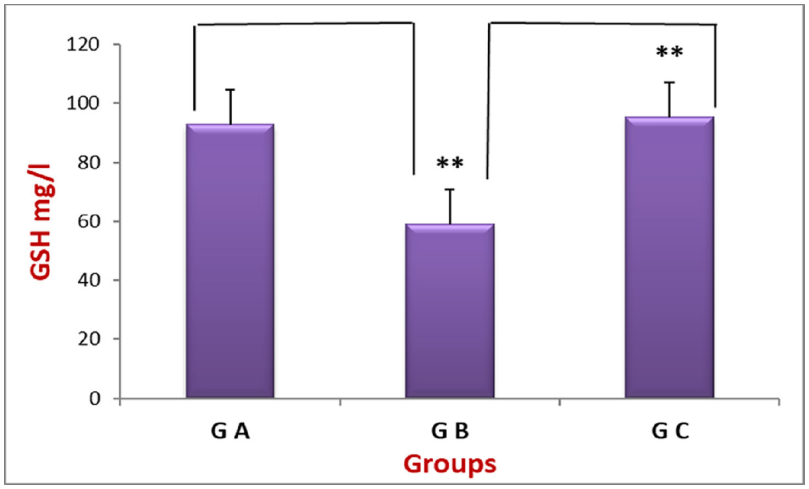

*Fig. 3. Group A received only normal saline for 14 days; Group B received 0.5 ml/kg of carbon tetrachloride two doses weekly for 2 weeks; Group C is the treated group (50 mg/kg of ursodeoxycholic acid was given for 14 days + carbon tetrachloride was given 2dose/week for 2 weeks). (**p < 0.001).*

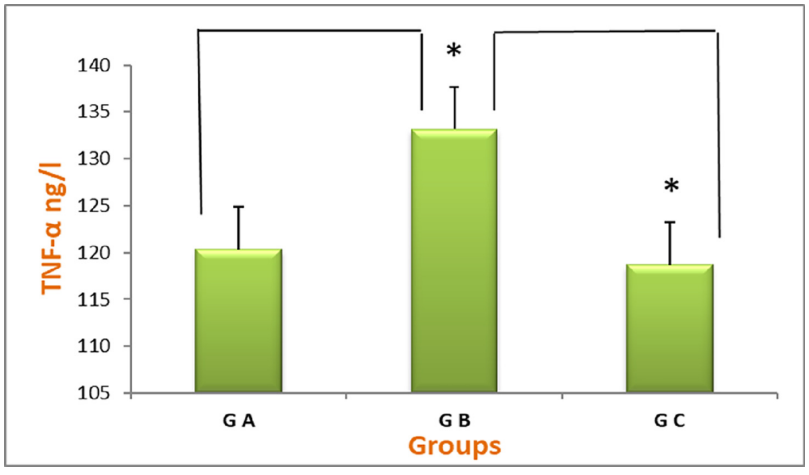

*Fig. 4. Group A received only normal saline for 14 days; Group B received 0.5 ml/kg of carbon tetrachloride two doses weekly for 2 weeks; Group C is the treated group (50 mg/kg of ursodeoxycholic acid was given for 14 days + carbon tetrachloride was given 2dose/week for 2 weeks). (*p < 0.05).*

or chronic liver disorders. One of the most potent poisons, carbon tetrachloride (CCL4) cause reactive oxygen species (ROS) to be produced in all body tissues when it is administered to a person or animal, either in small doses over time or all at once. This causes tissue damage [9]. When the production of free radicals surpasses the ability of cells to scavenge radicals, hepatocellular damage may result [13]. When CCl4 was given, there was a significant increase in serum ALT, ALP, and AST levels. This may be the result of damage to the cells and a rupture of the plasma membrane, which releases these enzymes quickly into the bloodstream from the cytoplasm of the cell [13].

Alternatively, the data demonstrated that ursodeoxycholic acid treatment, either alone or in conjunction with CCL4, significantly reduced all liver marker enzymes. This study demonstrated that the metabolic functions of the liver are impacted by the histological alterations introduced by carbon tetrachloride. The hepatocytes in the second group that received injections of carbon tetrachloride necrosed and were replaced by other inflammatory cells. This might have aided in the production of free radicals, which might have led to variations in the levels of liver enzymes [14]. According to this study, the GSH level in the B group decreased highly significant than that of the A group. In the liver, glutathione (GSH) is essential for eliminating toxins brought on by oxidative stress. Reactive oxygen species (ROS) may accumulate in liver cells when GSH levels drop, endangering the antioxidant defense system.

It has been found that applying CCl4 therapy to severe stress injuries inhibits the body's ability to eliminate antioxidants [15]. A decrease in NADPH or the use of GSH to rule out peroxides could be the cause of this drop in GSH [16]. The detoxification of the reactive, toxic CCl4 metabolites requires glutathione (GSH) depletion, and the fibrotic process begins when GSH stores are significantly lowered [13].

Glutathione (GSH) is an essential component of the immune system that is made in the liver from three amino acids: glutamate, cysteine, and glycine. Specifically, GSH has been shown to safeguard host immune cells and is necessary for the immune system to operate at its best [16]. The inflammatory cytokine production, including tumour necrosis factor-α (TNF-α), increases noticeably as liver injury progresses pathologically. Under normal physiological conditions, these cytokines are essential for stimulating inflammation and aiding in the healing process. Many processes, including inflammation, cellular death, coagulation, metabolism, insulin sensitivity, tumor development and invasion, and vascular functions, are regulated by tumour necrosis factor-α (TNF-α).

TNF-α also has a deleterious effect on liver damage. It starts a chain reaction in cells that triggers apoptosis and accelerates the liver's natural cell-death process when damaged. An adverse prognosis is linked to elevated TNF-α levels in the blood of patients suffering from liver damage [17]. Tumor necrosis factor-alpha converting enzyme [TACE] is the enzyme that distinguishes between transmembrane and soluble tumor necrosis factor. Two different receptors—TNF receptor 1 [TNFR1] and TNF receptor 2 [TNF2]—are activated when TNF performs its actions. Biologics that block TNF-α and related cytokine pathways have been used to treat a range of inflammatory and autoimmune disorders due to TNF-α's extensive role as a pro-inflammatory agent [18]. Nevertheless, compared to Group B, pre-treatment with 50 mg/kg Bwt of ursodeoxycholic acid dissolved in normal saline administered orally by gavage resulted in a significant decrease in TNF-a levels. Reduced capacity of the antioxidant system to scavenge free radicals leads to oxidative stress and liver damage. Enhancing the antioxidant defense system and, subsequently, the removal of free radicals can have a positive treatment effect on CCl4-induced liver damage [19].

UDCA has been demonstrated to protect hepatocytes from damaging attacks, such as chemical injury with CCL4, in a number of experimental models of liver injury. UDCA pretreatment significantly corrected all assessed inflammatory biomarkers. UDCA's anti-inflammatory features have been shown in a number of animal models. The activation of Kupffer cells and neutrophils damages the hepatocellular and endothelium by releasing ROS and proteases, which exacerbates the liver's structural damage and functional impairment [20]. Furthermore, it has been shown that UDCA increases the number of proteins that contain thiol and glutathione, protecting hepatic mitochondria and hepatocytes from oxidative stress. Additionally, UDCA reduces oxidative stress and fibrosis by blocking cell activation and glutathione synthesis in hepatic stellate cells. Given that the liver is particularly unique in terms of regeneration, more laboratory and clinical research is needed to clarify the protective effect of UDCA treatment on the activated stem cell activity in hepatic damage [21].

6. Conclusion

This study showed that when CCl4 is metabolized in the body, it produces extremely reactive free radicals that damage the liver by altering enzyme activity

and producing reactive oxygen species during the process. The intraperitoneal injection of CCl4 causes an increase in the activity of ALT and AST, a decrease in the levels of GSH, and an increase in the level of TNF-a. The discoveries of this study revealed that ursodeoxycholic acid improves CCl4-prompted hepatotoxicity and oxidative stress in rats. However, the oral administration of ursodeoxycholic acid prevents all of these negative effects from occurring in the livers of rats. It diminishes oxidative stress, suppresses inflammatory cell infiltration, increments the regenerative capacity of damaged tissues, and decreases liver apoptosis. However, because of its antioxidant properties, ursodeoxycholic acid reduces the hepatocellular damage brought on by CCl4. It is advised that further research be done on this medication at various doses and for a longer duration.

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Conflict of interest

No conflict of interest.

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