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Protective Effects Of Silymarin Co-Administered with CCl4 On Hepatotoxicity Of Female Albino Rats

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Abstract

This study explained the protective effects of silymarin against liver toxicity induced by carbon tetrachloride (CCl4) in female rats. Silymarin, a natural herbal compound extracted from the Milk thistle (*Silybum marianum*) plant. The experiment included three groups, each consisting of six rats. The first group served as the negative control. The second group was exposed to CCl4 (CCl4 30 % dissolved in olive oil intraperitoneally (i.p) 3ml/kg body weight twice a week for four weeks to induce toxicity. The third group received a combination of silymarin100 mg/kg b.w and the CCl4 3ml/.kg b.w daily for Three weeks to assess its protective effects. In the conducted experiment, rats that received silymarin alongside CCl4 exhibited significant improvements(reduction) in liver enzyme toward the normal values (Alanine Aminotransferase ALT, Aspartate Aminotransferase AST, Alkaline Phosphatase ALP, and Gamma-Glutamyl Transferase GGT) levels and also exhibited significant improvements(reduction)in lipid profiles (Total Cholesterol (TC), Triglycerides (TG), Low-Density Lipoprotein (LDL) and Very Low-Density Lipoprotein (VLDL) and elevation in High-Density Lipoprotein (HDL), compared to those treated only with CCl4. While, they were significantly elevated in the CCl4 group compared to the control group, indicating the presence of liver injury. These findings indicate that silymarin may serve as a potential preventive option for reducing liver damage caused by CCl4. Conclusion: The silymarin demonstrates significant potential in protecting against CCl4-

induced liver damage, making it a viable candidate for further investigation and potential therapeutic use in liver disease management Additional research is needed to comprehensively understand silymarin's impact on lipid metabolism and liver injury.

Keywords: Silymarin, CCl4, hepatotoxicity, Milk Thistle, Liver function, lipid profile.

1. Introduction

The liver plays a central role in metabolism, detoxification, and secretion within the body. It is affected by numerous disorders for which effective remedies are currently lacking, though ongoing efforts are focused on discovering new treatments. Traditional plant-based folk remedies have long been employed to treat liver diseases. Responsible for regulating crucial metabolic processes, any damage to the liver disrupts these functions. Liver disease persists as a significant global health concern. (Prakash *et al*.,2008). Hepatotoxicity, or liver toxicity, refers to liver damage caused by exposure to various substances, including drugs, chemicals, and environmental toxins. The liver, being the primary organ responsible for detoxifying harmful substances, is particularly vulnerable to damage (Mansour *et al*.,2006 and Sivakrishnan and Pharm, 2019). Hepatotoxicity can lead to a range of liver diseases, from mild dysfunction to severe liver failure, posing significant health risks. Carbon tetrachloride (CCl4) is a well-known hepatotoxin used extensively in research to induce liver damage in experimental models(Zarezad *et al*.,2018). When metabolized by liver enzymes, CCl4 generates reactive free radicals that cause oxidative stress, lipid peroxidation, and cellular damage. This cascade of events leads to inflammation, necrosis, and fibrosis in the liver, mimicking conditions seen in human liver diseases such as hepatitis and cirrhosis (Weber *et al*.,2003). Silymarin is a natural compound extracted from the seeds of the milk thistle plant (Silybum marianum). It has been used for centuries as an herbal remedy for liver disorders. Silymarin is composed of several flavonolignans, including silibinin, which is its most active component. Known for its antioxidant, anti-inflammatory, and anti-fibrotic properties, silymarin helps protect liver cells from damage and supports liver regeneration (Baradaran *et al*.,2019). Previous studies have demonstrated its efficacy in treating various liver diseases, making it a promising candidate for mitigating CCl4-induced hepatotoxicity. Given the increasing prevalence of liver diseases and the limitations of current treatment options, there is a need to explore effective and safe hepatoprotective agents. Aims of this study to investigate the protective effects of silymarin against CCl4 in female rats by evaluating its impact on liver Enzymes (ALT, AST, ALP, and GGT) and lipid profile (total cholesterol, triglycerides, HDL, VLDL and LDL).

2. Materials and Methods

The Silymarin (Milk Thistle P.E.) utilized in this study was procured from Xi'an Natural Field Bio-Technique Co., Ltd, China. Sodium Carboxymethylcellulose powder (CMC-Na) was obtained from BDH Chemical Limited, Poole, England. Carbon Tetrachloride (CCl4) standarad pure solution (HPLC 99.0%) was supplied by Shanghai Macklin Biochemical Co., Ltd, China. Diagnostic kits for assessing serum levels of various parameters were sourced from FUJIFILM, Japan.

2.1. Experimental Animals

The research was carried out at the College of Veterinary Medicine, University of Basrah, in the animal house of the Department of Physiology. The study included 18 adult female albino rats (Rattus rattus). weighing approximately 200 ± 20g and aged between 8 to 10 weeks, were used. The rats underwent a two-week adaptation period before the experiment, housed in three groups, with six rats per group, each in individual plastic cages measuring 15x35x50 cm. They were fed a standard pellet diet ad libitum from Institute for Public Accuracy and had unrestricted access to water. The rats were kept under controlled conditions of temperature (22-25°C) and light, with a 12-hour light-dark cycle.

2.2. Experimental Design

The study includes three groups of female albino rats (each group consists of 6 rats) a First group (Negative Control group receiving neither CCl4 nor silymarin), a second group (CCl4 group receiving CCl4 30 % dissolved in olive oil intraperitoneally (i.p) 3ml/kg b.w twice a week for four weeks according to method of (Khan *et al*.,2012) and the Third group (CCl4 + Silymarin) receiving 3ml /kg b.w CCl4 i.p. combined with silymarin dissolve it in a 0.5% CMC_Na solution orally via gavage at 100 mg/kg b.w according to (Wang *et al*.,2018) daily for three weeks. All rats are housed under controlled conditions with ad libitum access to water and standard diet, maintained at a temperature of 22-25°C with a 12-hour light-dark cycle.

2.3. Blood samples collection

At the end of the experiment, 24 hours after the last dose administration, the rats were anesthetized with chloroform. Blood was collected by cardiac puncture using a 5 ml disposable syringe according to the method of Hoff and Ralatg (2000). The collected blood

was placed in gel tubes and centrifuged for 20 minutes at 1000 rpm to extract the serum then moved to Eppendorf tubes and kept at -20°C for biochemical analysis.

2.4. Biochemical Analysis

Liver Enzymes (ALP, ALT, AST and GGT) and The lipid profile Total Cholesterol (TC), Triglycerides (TG), Low-Density Lipoprotein (LDL), High-Density Lipoprotein (HDL), and Very Low-Density Lipoprotein (VLDL) were estimated by Using the Auto Analyzer, the DRI-CHEM system from FUJIFILM, following the manufacturer's guidelines

2.4.1. Principle: DRI-CHEM NX500:

DRI-CHEM from FUJIFILM was a dry chemistry analyzer that can perform multiple test parameters of Clinical Chemistry. It had a built-in auto-pipetting system that requires no calibration and no water, providing easy preparation and maintenance. The new DRI-CHEM NX500 delivers results using a simple 3-steps procedure. It's a quick and easy operation. The main principle of dry chemistry is based upon the reflectance spectrophotometry. In dry chemistry, slides are dry, multilayered analytical elements coated on polyester supports. A small amount of sample is deposited onto the slide and evenly distributed to all of the layers. The spreading layer contains the appropriate substrate and other components needed for the reaction. The analyte in the sample catalyzes the reaction sequence to yield products which absorb light at wavelengths in various regions (340 – 680nm), diffuses into the underlying layer, was monitored by reflectance spectrophotometry. The test types were colorimetric, enzymatic endpoint, two-point or multi-point rate, or potentiometric. The rate of change in reflection density is converted to the enzymatic activity or the amount of colored complex formed which is proportional to the analytic concentration in the sample.

2.4.2 Assay Procedure:

1-The slide was prepared (dry slide reagents).
2-The sample was set.
3-The start bottom was pressed.

3. Statistical analysis

The data were analyzed using the Statistical Package for the Social Sciences (SPSS, version 11.0). A one-way ANOVA with LSD post hoc analysis was employed to identify significant differences in mean values between groups.

4. Results

4.1. Liver enzymes

The data in Table (4.1) indicate that CCl4 administration leads to significant increases($P<0.05$) in ALT, AST, ALP, and GGT levels compared to the negative control group. However, treatment with Silymarin (100mg/kg B.W) combined with CCl4 appears to diminish these elevations, bringing them closer to the levels observed in the negative control group. These findings indicate a potential protective effect of Silymarin against toxicity in liver.

Table (4.1) The protective effect of silymarin on liver enzymes against CCl4 in female rats

Groups	parameters (Mean ± SD) No=6			
	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)
The first group (control)	46.50±12.30B	116.16±2.71C	34.16±10.94B	3.75±0.30B
The second group CCl4 (3ml/kg b.w)	88.66±5.39A	181.16±5.77A	71.16±7.13A	14.63±0.42A
The Third group (CCl4+Silymarin100mg/kg b.w)	53.83±5.52B	146.16±11.54B	39.33±4.08B	3.73±0.30B
LSD	7.33	11.66	10.45	2.4

* A, B, C The different letters a show significant differences at ($P<0.05$)

4.2. Lipid profile

Total cholesterol, Triglyceride and LDL levels are significantly elevated ($P<0.05$) in the CCl4 group compared to both the negative control and CCl4 + Silymarin groups as in the result in Table (4.2). LDL levels show a marked increase in the CCl4 group compared to the negative control, with a reduction observed in the CCl4 + Silymarin group. HDL levels

are significantly decrease in the CCl4 group compared to the negative control and CCl4 + Silymarin groups, indicating a negative impact of CCl4 on HDL levels, which is increased by Silymarin when given as combination with CCl4. VLDL levels are significantly higher in the CCl4 group compared to the negative control, with a slight reduction observed in the CCl4 + Silymarin group.

Table (4.2) The protective effect of silymarin on lipid profile against CCl4 in female rats

Groups	parameters (Mean ± SD) No=6				
	T. Cholesterol (mg/dl)	T.G. (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)
The first group (control)	68.50±5.24C	50.83±10.98B	38.66±3.73C	20.33±1.03A	10.16±2.19B
The second group CCl4(3ml/kg b.w)	123.33±9.41A	86.50±5.16A	101.86±7.71A	4.16±1.47C	17.30±1.03A
The Third group (CCl4+Silymarin100mg/kg b.w)	84.83±5.49B	56.00±7.87B	62.66±4.12B	13.50±2.81B	10.60±1.64B
LSD	6.00	9.00	6.66	3.83	1.73

* A, B, C The different letters a show significant differences at (P<0.05)

5. Discussion

The data indicate a notable increase in liver enzyme levels (ALT, AST, ALP, GGT) in CCl4 Group compared to the negative control group, demonstrating significant liver damage caused by CCl4. Elevated levels of these enzymes indicate cellular leakage and a loss of cell membrane integrity in the liver. CCl4, once an inactive metabolite, is converted into a free radical by the microsomal cytochrome P-450-dependent enzyme, leading to the activation of CCl4 toxicity. (Al-Qarawi *et al.*,2006 and Parmar *et al.*,2010). Given of CCl4 + Silymarin significantly reduced these enzyme levels to word the normal values compared

to the CCl4 group alone, indicating the protective effect of silymarin versus CCl4-induced liver damage. Saller *etal.*, (2001) present in the review section of their study on the effect of silymarin as a treatment for liver diseases who noticed AST, ALT, and GGT levels all fell significantly with silymarin therapy. The mechanism of Silymarin involves preserving the hepatocellular membrane's integrity and inhibiting the penetration of toxins into the liver's interior, thereby preventing additional cellular damage. Additionally, Silymarin enhances ribosomal protein synthesis by activating nucleolar polymerase A (NPA), which initiates hepatocyte synthesis and promotes liver regeneration Pradhan and Girish, (2006). This suggests that silymarin helped maintain the structural integrity of hepatocyte membranes, thereby preventing the leakage of these enzymes into the plasma. This finding is consistent with a previous report on the hepatoprotective effect of silymarin in isotretinoin-induced liver damage (Kumas *et al.*, 2018).

On the other hand, the Lipid Profile showed there was a clearly increase in total cholesterol, triglycerides, LDL, and VLDL levels, and a decrease in HDL levels in the CCl4 Group compared to the negative control indicating a substantial negative impact of CCl4 on the lipid profile due to the effect of CCl4 on liver which lead to disturbance liver functions these findings agreed with (Yang *et al*.,2011) they reported that the liver is the major site for the synthesis and metabolism of cholesterol .Distinct alterations in lipid metabolism have been reported in CCl4 induced hepatotoxicity in rats (Singhal *et al*.,2012). Generally, in cases of hepatotoxicity, liver damage can lead to alterations in lipid metabolism in the body. These alterations may include accumulation of fat within the liver itself and increased release of fats into the bloodstream, resulting in elevated levels of total fats in the blood, including total cholesterol and triglycerides.

The rise in cholesterol levels could result from enhanced fatty acid esterification, inhibition of fatty acid β-oxidation, and reduced cellular lipid excretion (Srivastava and Shivanandappa, 2010). CCl4 promotes the uptake of acetate into liver cells, contributing to increased cholesterol synthesis. Additionally, it boosts fatty acid and triglyceride synthesis from acetate and stimulates lipid esterification. (Kamalakkannan *et al*.,2005). Moreover, the findings of (Kamalakkannan *et al.*,2005) indicated that CCl_4 inhibits the synthesis of apo-lipoprotein thus reducing the synthesis of lipoproteins. The results in Table (4-2) showed a notable increase($P<0.05$)in total cholesterol, triglyceride, LDL and VLDL) concentrations and this elevation was associated with a significant decrease in high-density lipoprotein (HDL) concentration in the treated group with CCl4 in comparison to control group, due to the effect of CCl4 on liver which lead to disturbance liver functions these findings agreed with (Khan *et al.*,2023) they reported that the exposure to CCl4 in Albino Rats disrupts normal lipid levels, resulting in toxicity andCCl4-treated rats exhibit significantly elevated serum levels of total cholesterol, triglycerides and LDL compared to

normal rats. This lipid profile imbalance is attributed to CCl4-induced liver damage, impacting lipid metabolism and transportation. Increased oxidative stress from CCl4 exposure contributes to heightened cholesterol and triglyceride levels in the bloodstream and tissues. The abnormal lipid profile observed also aligns Uddin *et al.*, (2015) indicating compromised hepatic function due to CCl4 toxicity. While CCl4 + Silymarin Group showed significantly lowered total cholesterol, triglycerides, LDL, and VLDL levels, and increased HDL levels, compared to CCl4 group indicating a protective effect on the lipid profile This supports the role of silymarin in improving blood lipid levels and this agreement with (Lien *et al.*,2016) suggest that silymarin, extracted from milk thistle, may help improve blood lipid levels.

Conclusion

The results of this study demonstrated that the use of silymarin as a protective agent, when administered in combination with carbon tetrachloride (CCl4), provided significant hepatoprotective effects. This indicates that silymarin has effective protective properties that protect the liver against the toxic effects of CCl4, making it a potential option for preventing liver damage caused by toxic substances.

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