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ORIGINAL RESEARCH

Effect of Coumarin and Its Derivatives on the Protein Profiles in CCl_4-Treated Rats

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Abstract

Objective:

This study aimed to evaluate the effects of coumarin and its derivatives esculetin, scoparone, and 4-methylumbelliferone on serum protein profiles in male Sprague-Dawley rats with carbon tetrachloride (CCl_4)-induced hepatic injury. As the liver is essential for the synthesis of major plasma proteins such as albumin and globulin, disruptions in liver function can significantly alter these parameters. CCl_4, a commonly used industrial chemical, is known for its pronounced hepatotoxic and nephrotoxic effects.

Method:

Male Sprague-Dawley rats weighing between 150–200 grams were randomly assigned to six groups: a control group, a group receiving CCl_4 to induce hepatotoxicity, and four treatment groups that received oral doses of coumarin (30 mg/kg), esculetin (31.15 mg/kg), scoparone (35 mg/kg), or 4-methylumbelliferone (35 mg/kg). Hepatic injury was induced by administering CCl_4 (1.25 mL/kg) diluted in corn oil. Serum levels of total protein, albumin, and globulin were measured using standard biochemical techniques. Statistical analyses were performed using SPSS software, with p-values less than 0.05 considered statistically significant.

Results:

Oral administration of CCl_4 (1.25 mL/kg) resulted in a marked decrease in serum total protein and albumin concentrations, accompanied by a lowered albumin/globulin (A/G) ratio and elevated globulin levels. Conversely, treatment with coumarin and its derivatives significantly reversed the CCl_4-induced reductions in albumin and the A/G ratio. These compounds also effectively reduced the elevated globulin levels observed following CCl_4 exposure.

Conclusion:

The results suggest that the hepatoprotective effects of coumarin derivatives are closely related to their molecular structures, which appear to influence the regulation of hepatic protein synthesis. By modulating key enzymes involved in protein metabolism, these compounds may help preserve liver function and support the synthesis of essential proteins required for cellular regeneration and physiological balance. Coumarin based compounds hold potential as therapeutic agents for the prevention or mitigation of liver damage.

Keywords: *Hepatotoxicity, protein levels, carbon tetrachloride (**CCl_4**), coumarin, antioxidant*

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Introduction

The liver is the primary target for the harmful effects of toxic agents due to its central role in metabolizing and detoxifying xenobiotics. It is also a key organ in protein metabolism, being responsible for the synthesis, modification, and breakdown of various plasma proteins[1]. In recent years, interest has increasingly shifted toward complementary and alternative therapeutic approaches for managing liver related disorders.

Coumarins represent a large class of naturally occurring compounds, with approximately 1,300 known derivatives identified in dietary sources such as citrus fruits and green tea. In addition to their natural occurrence, several coumarins can also be chemically synthesized. Their rising popularity is largely attributed to a broad spectrum of pharmacological activities, including antioxidant, anti-inflammatory, hepatoprotective, and antifibrotic effects[2-4].

Carbon tetrachloride (CCl_4) is a commonly utilized industrial solvent and a recognized hepatotoxin. It is frequently employed in experimental models to induce oxidative stress, lipid peroxidation, and liver damage, mimicking human liver diseases. Hepatotoxicity caused by CCl_4 results in changes to the hepatic protein profile[5,6], triggers inflammatory responses, and promotes fibrosis, making it a valuable model for investigating liver injury and potential therapeutic approaches.

In addition to CCl_4, several other agents are commonly used to induce experimental hepatotoxicity, such as thioacetamide (TAA), acetaminophen, and aflatoxins. These agents cause liver damage in a way similar to CCl_4[7-9].

Albumin, a key protein produced by the liver, is essential for regulating the osmotic pressure of blood[10]. Hepatocyte damage caused by CCl_4 reduces albumin synthesis, leading to hypoalbuminemia. Coumarins may help restore albumin levels by reducing oxidative stress and promoting liver cell regeneration[11]. Similarly, globulins play a key role in immune response and may fluctuate due to inflammatory processes triggered by CCl_4 exposure. As liver damage progresses, globulin levels increase[12]. The anti-inflammatory properties of coumarins may help regulate globulin synthesis and immune balance.

Changes in albumin and globulin levels directly affect total protein levels. CCl_4 toxicity impairs hepatic protein synthesis, leading to a decline in total protein levels. Coumarins may help preserve total protein levels by supporting liver function and reducing oxidative damage[13].

Enzymes responsible for drug metabolism play a crucial role in detoxifying various xenobiotic compounds; however, they can also activate certain substances, increasing their toxicity[14]. In such instances, the liver is the primary organ affected by these newly generated toxic metabolites. Hence, understanding the protective mechanisms of the liver is essential.

Although coumarin has been reported to exhibit hepatotoxic effects in rodents, primarily due to the production of coumarin 3,4-epoxide, which leads to elevated plasma alanine transaminase (ALT) and aspartate transaminase (AST) levels, other studies have highlighted its potential protective properties against toxicity[15-18].

Considering the conflicting findings in the literature, this study seeks to determine whether coumarin derivatives exert a protective influence against CCl_4-induced liver damage. Furthermore, we aim to explore how structural variations among different coumarin derivatives impact the protein profile in rats with experimentally induced hepatotoxicity.

Materials and Methods

In this study, male Sprague–Dawley rats weighing between 150 and 200 grams were utilized. Throughout the experimental period, the animals were housed in a controlled environment maintained at 21 ± 2°C with a 12-hour light/dark cycle. They were provided with unrestricted access to standard pellet feed and drinking water. The study protocol was reviewed and approved by the Animal Ethics Committee of Dicle University.

Chemicals: CCl_4, coumarin, and 4-methylumbelliferone were supplied by Sigma Chemicals Company. Scoparone and esculetin were purchased from Aldrich Chemicals Company (Figure 1). Corn oil was acquired from a local supplier.

Experimental Design and Treatment Protocol: The rats were randomly divided into six groups, each comprising six weight matched animals (150–200 g). Hepatic injury related hyperlipidemia was induced by administering carbon tetrachloride (CCl_4) at a dose of 1.25 ml/kg, dissolved in corn oil (20% v/v), through oral gavage.

The control group received four oral doses of saline (3 ml/kg) at 12-hour intervals over a period of 48 hours, followed by a single oral dose of corn oil one hour after the final saline administration. The CCl_4 group followed the same saline dosing schedule, but instead of corn oil, received CCl_4 one hour after the last saline dose. Treatment groups receiving coumarin or its derivatives were handled similarly to the CCl_4 group; however, esculetin (30 mg/kg), scoparone (35 mg/kg), 4-methylumbelliferone (35 mg/kg), or coumarin (35 mg/kg) were administered in place of the final saline dose.

Twenty-four hours following the final administration, the rats were anesthetized with ketamine, and blood samples were obtained through cardiac puncture. Serum levels of total protein, albumin, and

globulin were measured using standard automated techniques at the Biochemistry Laboratory of Dicle University Faculty of Medicine.

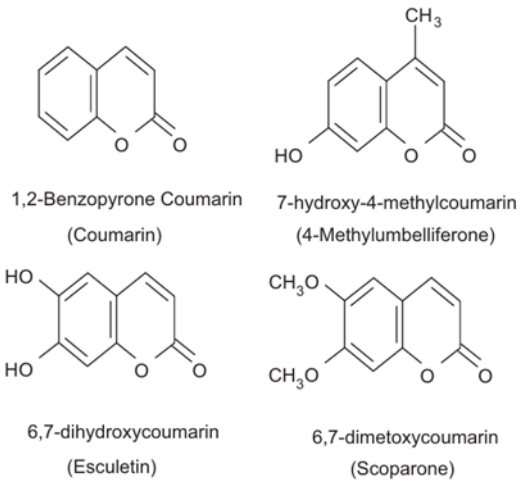

Figure 1. *Structural representations of coumarin and its derivatives.*

Statistics: All statistical evaluations were carried out using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA). The normality of distribution for continuous variables was assessed using the Kolmogorov-Smirnov or Shapiro-Wilk tests. For variables that did not exhibit normal distribution, the Kruskal-Wallis test was used to compare three or more groups. In cases where statistically significant differences were found, pairwise comparisons were conducted using the Dunn test with Bonferroni correction. Continuous data are presented as median values along with their minimum and maximum ranges. A p-value below 0.05 was considered indicative of statistical significance.

Results

Albumin Levels:

Compared to the control group, the CCl_4-treated group showed a marked reduction in serum albumin levels ($p < 0.001$). Treatment with each of the coumarin derivatives led to a significant elevation in albumin concentrations, indicating a potential restorative effect. However, coumarin and 4-metyl umbelliferone exhibited a weaker effect compared to esculetin and scoparone, as their levels

did not completely return to control values (Figure 2).

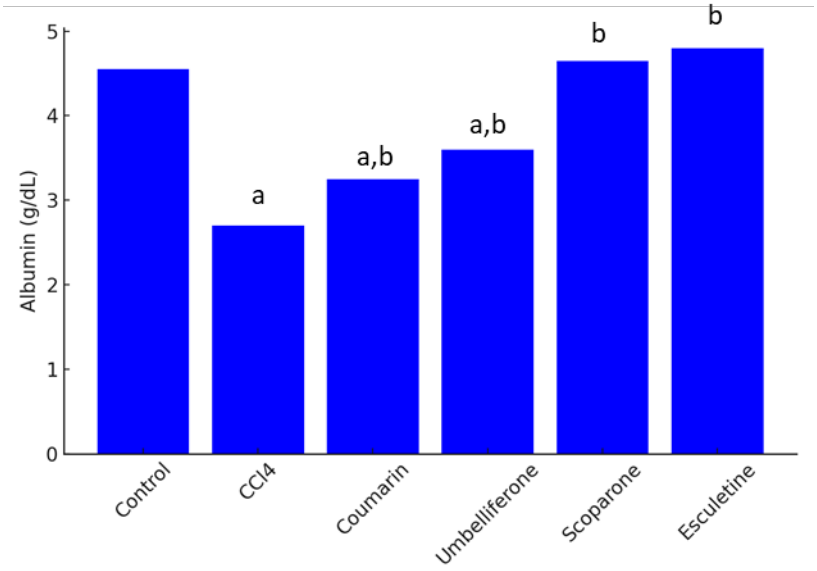

Figure 2. *Serum albumin levels. a: Significantly different from the control group (p < 0.001). b: Significantly different from the CCl_4-treated group (p < 0.001).*

Globulin Levels

CCl_4 exposure led to a significant increase in globulin levels ($p < 0.001$). The administration of all coumarin derivatives resulted in a statistically significant reduction in globulin levels, among the applied coumarins, esculetin administration exhibited the strongest effect, indicating a potential hepatoprotective effect ($p < 0.001$) (Figure 3).

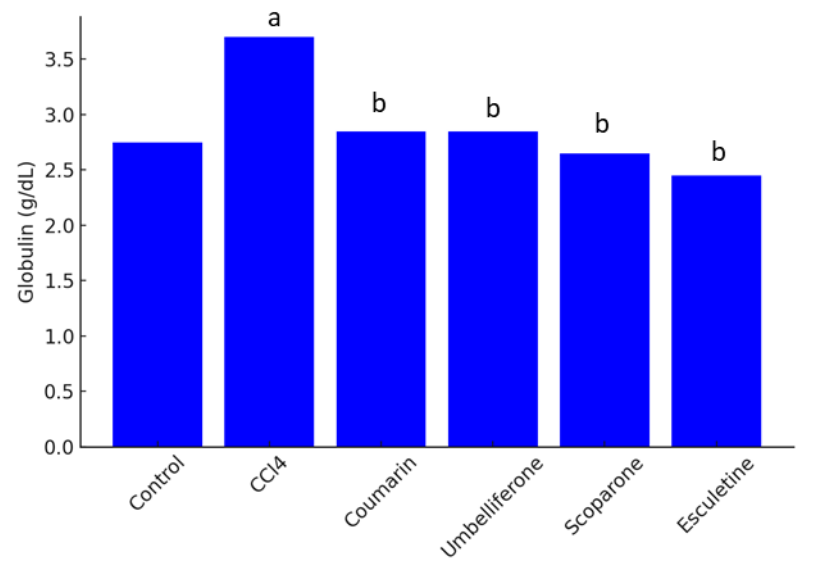

Figure 3. *Serum globulin levels a: Statistically significant difference compared to the control group (p<0.001. b: Statistically significant difference compared to the CCl_4 group (p<0.001).*

Albumin/Globulin Ratio (A/G)

The albumin/globulin (A/G) ratio was significantly lower in the CCl_4 treated group compared to the control group ($p < 0.001$). The administration of all coumarin derivatives led to a significant increase in the A/G ratio relative to the CCl_4 group, Even though all coumarins have significant effect on A/G ratio. It has been observed that esculetin and scopoletin preserve protein balance more effectively and show a greater hepatoprotective effect compared to coumarin and 4-metylumbelliferone ($p < 0.001$) (Figure 4).

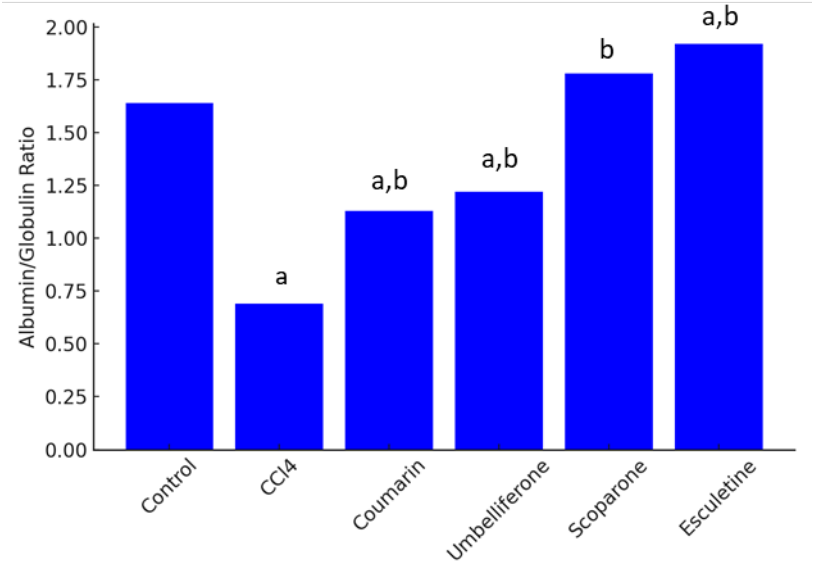

Figure 4. *Albumin/Globulin ratio. a: Statistically significant difference compared to the control group ($p<0.001$). b: Statistically significant difference compared to the CCl_4 group ($p<0.001$).*

Total Protein Levels

In the CCl_4 induced hepatotoxicity group, a significant reduction in total protein levels was observed compared to the control group ($p < 0.001$). The administration of coumarin and umbelliferone did not lead to a significant change in total protein levels, indicating a lack of protective effect in this parameter. However, both esculetin and scoparone significantly increased total protein levels, restoring them to control levels ($p < 0.001$), suggesting a potential hepatoprotective effect (Figure 5.).

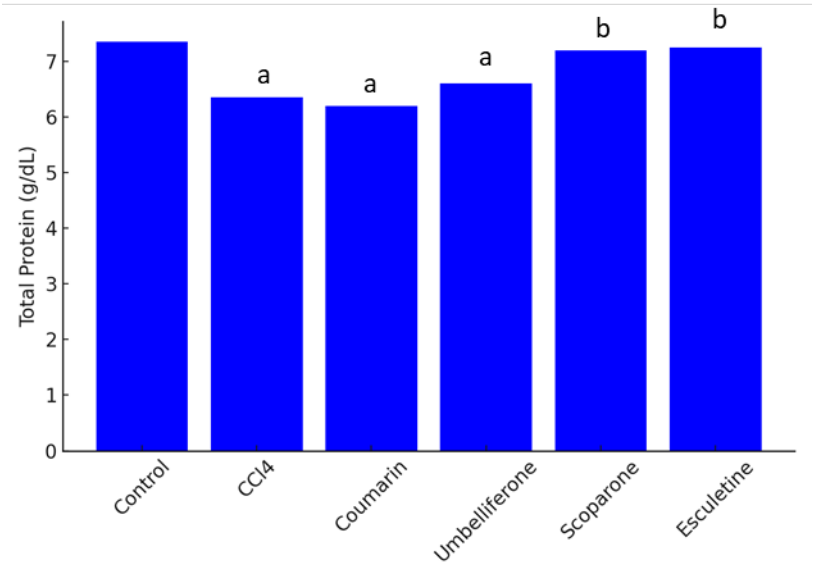

Figure 5. *Serum total protein levels. a: Statistically significant difference compared to the control group (p<0.001). b: Statistically significant difference compared to the CCl_4 group (p<0.001).*

Discussion:

The liver is the largest internal organ and a vital metabolic hub in the human body. It plays a crucial role in detoxification, metabolism, protein synthesis, and immune system regulation. Assessing liver function in experimental hepatotoxicity models involves measuring biochemical parameters such as albumin, globulin, total protein and A/G ratio[10]. Albumin is a major plasma protein synthesized by the liver, reflecting its synthetic capacity. A decline in albumin levels suggests hepatic dysfunction. Globulin is a group of proteins involved in immune responses. Elevated globulin levels may indicate chronic inflammation or liver disease. Total Protein is the sum of albumin and globulin, serving as an indicator of nutritional status and liver function. Albumin/Globulin (A/G) ratio is a sensitive marker of liver health. A decreased A/G ratio is associated with hepatic impairment and systemic inflammation.

Given the central role of oxidative stress in liver injury, antioxidants are widely studied for their hepatoprotective effects. Many natural compounds, including coumarins, exhibit antioxidant, anti-inflammatory, and free radical scavenging properties, potentially mitigating liver damage by reducing lipid peroxidation and enhancing endogenous antioxidant defenses (e.g., glutathione and superoxide dismutase).

Coumarins have distinctive chemical structure serves as a foundation for numerous synthetic derivatives with enhanced pharmacological properties[19]. Coumarins act as potent free radical scavengers, reducing oxidative stress and preventing lipid peroxidation, which is implicated in various chronic diseases[20].

Carbon tetrachloride (CCl_4)-induced liver injury is one of the most extensively utilized experimental models for investigating hepatotoxicity and assessing the protective or therapeutic potential of various agents[21,22]. CCl_4 causes hepatic damage primarily through the induction of oxidative stress, which disrupts cellular integrity. In the current study, CCl_4 administration resulted in significant hepatic injury in rats, as evidenced by reduced serum levels of total protein, albumin, and the albumin/globulin (A/G) ratio, alongside elevated globulin levels. Consistent with these findings, Ali *et al*. also reported that CCl_4 exposure led to decreased serum concentrations of total protein and albumin, while globulin, ALT, ALP and AST levels were markedly increased[23].

Coumarin and some coumarin analogs such as esculetin and scoparone have been shown to protect hepatocytes against CCl_4 induced toxicity, primarily through antioxidant and anti-inflammatory mechanisms[24].

Our previous studies indicate that chemical structure of coumarins plays an important role in the prevention of oxidative stress, regulation of serum lipid profiles and prevention of liver toxicity against CCl_4 induced acute hepatotoxicity in rats[25,26].

This research investigated the effects of coumarin and its derivatives—esculetin, scoparone, and 4-methylumbelliferone—on CCl_4-induced alterations in protein profiles in male Sprague-Dawley rats. The dosages of coumarins were determined based on previous research findings[27,28].

The hepatotoxicity of CCl_4 primarily occurs through reductive dehalogenation catalyzed by cytochrome P4502E1 (CYP2E1), leading to the formation of the highly reactive trichloromethyl free radical. These radical initiates biochemical reactions that result in liver cell damage[29,30]. It can covalently bind to proteins and lipids, interact with oxygen to generate trichloromethyl peroxy radicals, or undergo hydrogen abstraction, leading to the formation of chloroform[31].

Inhibiting CYP2E1 can reduce the levels of reactive metabolites and, consequently, minimize tissue injury. Therefore, considering the central role of reactive oxygen species and free radicals, dietary antioxidants such as coumarins have been explored as potential therapeutic agents to mitigate liver damage[32-34].

The current investigation revealed that animals treated with esculetin and scoparone exhibited a notable decline in the CCl_4-induced increase in serum globulin levels, the decreased albumin levels and A/G ratio were restored. The effects of scoparone and esculetin were comparable, showing similar protective properties in the study. Compared to esculetin and scoparon, coumarin and another coumarin derivative, 4-methylumbelliferone, have been observed to exhibit weaker hepatoprotective effects against CCl_4-induced liver damage. The present study demonstrated that another coumarin

derivative, scoparone, appeared to effectively preserve liver architecture and counteract the CCl_4-induced reduction in serum albumin, total protein levels, and the A/G ratio.

According to Lin et al. and Okamoto et al., esculetin (6,7-dihydroxycoumarin), which contains two hydroxyl groups, exhibits potent antioxidant properties and effectively neutralizes free radicals[28,35]. This effect is attributed to the molecular structure of coumarins. Studies have demonstrated that an increased number of hydroxyl groups improves free radical neutralization. In contrast, coumarin, which lacks hydroxyl or methoxy groups, was ineffective in preventing hepatic damage induced by CCl_4 [16].

Conversely, pretreatment with 4-methylumbelliferone (7-hydroxy-4-methyl) and coumarin (1,2-benzopyrone) exhibited a weaker protective effect against CCl_4-induced liver toxicity. This result aligns with previous studies suggesting that the hydroxylation pattern is a key determinant of coumarins' antioxidant and hepatoprotective properties[27,28].

In our study, esculetin (6,7-dihydroxycoumarin) provided the most effective protection against CCl_4 induced hepatotoxicity, while its 6,7-methoxy derivative, scoparone, showed a weaker compared to esculetin, but still protective effect. These findings are consistent with previous studies indicating that hydroxyl groups enhance the protective effects of coumarins[24,26].

In our earlier research, the suppression of CCl_4-induced increases in AST, ALT, and ALP levels was observed as follows: esculetin (approximately 90–94%), scoparone (63–78%), 4-methylumbelliferone (10–49%), and coumarin (18–20%). These results are in agreement with histological findings, which demonstrated reduced liver damage. The outcomes of this study align closely with our previous investigations and are further supported by various reports in the literature[26,36,37].

Conclusion

Based on the findings of this study and existing literature, it can be inferred that the hepatoprotective effects of coumarins against CCl_4-induced liver damage may be attributed to their antioxidant properties, particularly their capacity to neutralize free radicals and enhance serum protein profiles. Among the tested compounds, esculetin and scoparone demonstrated the most pronounced effects in regulating albumin, globulin, total protein levels, and the albumin/globulin ratio within this hepatotoxicity model. These results indicate that hydroxy-substituted and dimethoxy coumarins hold promise as potential hepatoprotective agents, likely through mechanisms involving antioxidant, anti-inflammatory, and radical scavenging activities. However, further research is essential to elucidate their exact modes of action and assess their applicability in clinical settings. Should future studies

confirm their therapeutic efficacy and safety, coumarins could be considered for development as dietary supplements aimed at liver disease prevention.

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