Clinical and Experimental Health Sciences

The Protective Effect of Rheum Ribes L., and Quercetin on Protein Carbonyl Levels Against Carbon Tetrachloride-Induced Liver and Kidney Damage in the Rats

Ahmet Bakir[1], Suat Ekin[1], Sevgi Oztas[2], Gokhan Oto[3]

[1]Van Yuzuncu Yıl University, Faculty of Science, Department of Chemistry, Van, Türkiye.
[2]Hakkari University, Vocational School of Health Services, Hakkari, Türkiye.
[3] Van Yuzuncu Yıl University, Faculty of Medicine, Department of Pharmacology, Van, Türkiye.

Correspondence Author: Ahmet Bakir
E-mail: ahmetcu@msn.com

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ABSTRACT

Objective: This study was designed to examine the potential protective effects of Rheum ribes L., and quercetin on protein carbonyl (PCO) in kidney and liver tissue, trace elements (Fe, Cu, Zn) and mineral (P) in serum samples in Wistar rats of carbon tetrachloride (CCl4)-induced oxidative damage.

Methods: The 2, 4-dinitrophenylhydrazine (DNPH) method is the most reliable method widely used to measure carbonyl levels in proteins. In this study, the effect of Rheum ribes L. (Rr) and quercetin on protein carbonyl, trace elements (Fe, Cu, Zn) and mineral (P) levels against carbon tetrachloride (CCl4) mediated liver and kidney damage was investigated. For this purpose, 56 Wistar albino female rats weighing 200 ± 220 g were used. Groups were designed as: controls, 0.3 ml DMSO, 1 ml/kg olive oil, 1 ml/kg CCl4, 100 mg/kg Rr, 100 mg/kg quercetin, 100 mg/kg Rr+1 ml/kg CCl4 and 100 mg/kg quercetin+1 ml/kg CCl4 groups.

Results: The results showed that the CCl4 group had significantly higher level of protein carbonyl (PCO) than the control, DMSO, olive oil, Rr and quercetin groups ($p<0.001$, $p<0.05$, $p<0.001$, $p<0.01$, and $p<0.01$, respectively). A significant elevation in the group of CCl4 + quercetin, compare to control, DMSO, olive oil, Rr and quercetin groups ($p<0.001$, $p<0.05$, $p<0.001$, $p<0.01$, and $p<0.05$, respectively) in the liver tissue. Additionally, the CCl4 group had significantly higher level of PCO than the control, DMSO, olive oil, Rr and quercetin groups ($p<0.001$, $p<0.01$, $p<0.001$, $p<0.001$, and $p<0.001$, respectively). Similarly, the CCl4 + Quercetin group had increased level of PCO compared to the control and Rr.groups ($p<0.05$ and $p<0.05$) in the kidney tissue.

Conclusion: In the study, it was seen that the bioactive substances in Rheum ribes L. (root) and quercetin, a standard antioxidant, could be an alternative against the toxic effect of CCl4.

Keywords: Carbon tetrachloride, protein carbonyl, quercetin, rheum ribes L., trace elements.

1. INTRODUCTION

Proteins, which are among the most common organic molecules in living things, are molecules with much different functionality, since they participate in the structure of enzymes and hormones in living systems. Proteins constitute the main “working force” for all biological processes (1). Although the folding pattern of proteins and their precise three-dimensional structure, their tight dependence on their activities and functions (1) make research difficult, they are at an important and critical point in the diagnosis of diseases. Almost all vital function depends on this macromolecule class (2). Because of all these functions of proteins and their abundance in biological systems and high rate constants for reactions, they are the main targets for oxidants (3). For all these reasons, the living organism is exposed to a system that produces reactive oxygen species (ROS) that can damage nucleic acids, lipids and proteins (4).

Although some signs of oxidative stress such as DNA damage and lipid peroxidation have been extensively evaluated and reliable biomarkers have been found to determine the degree of damage associated with it, the attack of ROS on proteins and protein carbonyl formation is a newly investigated topic (5,6).

Carbonyl groups are introduced into proteins by various oxidative means, particularly by metal catalyzed oxidation (MCO) of specific protein amino acid side chains or the addition of carbonyl-containing oxidized lipids (4-hydroxynonenal, malondialdehyde) or sugars (7). Protein oxidation is expressed as covalent change of proteins induced directly by free radicals such as hydrogen peroxide (H_2O_2) and hydroxyl (OH^-) and/or indirectly induced by the reaction with secondary products of oxidative stress (8). Protein carbonyl (PCO) products are formed as a result of damage to the amino acid residue or peptide backbone such

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as histidine (His), proline (Pro), arginine (Arg) and lysine (Lys) as a result of the interaction of reactive oxygen species (ROT) mentioned above with proteins. This damage is irreversible and responsible for cell death (8).

It is a fact that protein oxidation that occurs in certain situations will be protected by the existing environmental, genetic and dietary factors, as well as affect the balance between antioxidant and peroxidant activities (4).

The antioxidant activities of natural products are generally observed in phenolic compounds. Quercetin is a common ingredient found in many fruits and vegetables. Quercetin, a plant-derived flavonoid, is thought to have a multifunctional molecular structure and may act partially through a mitochondrial mechanism, although its biological properties and underlying mechanisms of action are not fully known (9). Recent studies have shown that quercetin exhibits antioxidative, anti-inflammatory and anti-apoptosis properties, and also protects the liver from damage caused by hepatoxins (10). *Rheum ribes L.,* belonging to the Polygonacea family, is a perennial herbaceous plant that grows in the Eastern Anatolia Region in Turkey. It has been reported that the leaves and body of the plant are sour and strengthens the stomach (11). It has been shown in studies that the content of the Rheum ribes L. plant contains high phenolic content and is rich in Fe, Zn and vitamin C (12, 13). Chemical components of anthraquinone and stilbene have been identified in the roots of *Rheum ribes L.* (14). Anthraquinones stimulate fluid balance, electrolyte exchange and colon smooth muscle movement.

The aim of this study is to determine the effects of quercetin and *Rheum ribes L*., which have important antioxidant effects, on protein carbonyl in liver and kidney tissues damaged with carbon tetrachloride (CCl_4).

2. METHODS

2.1. Chemical Reagents

All chemicals and reagents used were of analytical grade. Quercetin (sigma, USA), 10% DMSO, (sigma, USA), CCl_4 (sigma-Aldrich, USA), Guanidin-HCl (sigma, USA), Olive oil (Turkiye)

2.2. Plant Material and Extraction

Rheum ribes L. plant used in the study was collected from the Van region in May – June 2017. Since the ingredients of the product in the spring are at the highest level, the products grown in the spring were selected. The sample material was recorded by performing the necessary identification procedures in the herbarium of *Rheum ribes L*. (root), Van YYU Faculty of Science, Department of Biology.

The water extraction of *Rheum ribes* L. roots was performed by modifying the decoction method used by Eddouks et al. (15). 10 grams of ground *Rheum ribes L.* roots were boiled in 100 ml distilled water at 250 rpm for 10 minutes and then allowed to cool for 15 minutes. Then, the water of the extract, which was filtered through Watman filter paper, was placed in 50 mm falcon tubes (not exceeding 1/3 when tilted) and kept at – 80 °C for a week, and then lyophilized in a – 80 °C lyophilizer for 48 hours. The yield of the extract obtained was calculated to be 5.184%. After determining the yield, dose calculations were made in the rats according to their weights and the amount of plant to be given daily was determined. The plant extracts prepared daily by decoction method for 7 days were dissolved in 15 ml of distilled water and vortexed sufficiently and then kept in closed glass bottles until the time of application. It was given to rats by gavage at 10.00 every day throughout the study. *Rheum ribes L.,* plant was administered to the fifth and seventh groups throughout the study at a dose of 100 mg/kg.

2.3. Preparation of Quercetin

Quercetin was administered daily at a dose of 100 mg / kg to the sixth and eighth groups by oral gavage.

2.4. Preparation of Dimethyl Sulfoxide (DMSO)

DMSO (% 10) was added daily to 5 ml of distilled water and 25 µl was administered to the second group as 0.3 ml / kg by oral gavage.

2.5. Preparation of CCl_4

CCl4 was mixed with olive oil at a ratio of 1:1 and administered intraperitoneally as a single dose to the fourth, seventh and eighth groups on the 7th day as 1ml / kg.

2.6. Animals

In this study, 56 female Wistar albino rats were used. Rats at Van Yüzüncü Yıl University Experimental Medicine Application and Research Center; it was fed with standard pellet at room temperature set to 22 ± 2 °C, illuminated at a rhythm of 12 hours light – 12 hours dark. The rats were kept in standard plastic cages with free feed and water intake. Before starting the study, the study approval (decision dated 27.02.2020 and numbered 2020/02) was obtained from Van Yüzüncü Yıl University, Animal Experiments Local Ethics Committee.

2.7. Establishment of Experimental Groups and Experiment Plan

In the study, 8 groups were formed with 7 rats in each group.

1. Group (n= 7): It was determined as the control group and fed with normal water and standard pellet feed for 7 days.

2. Group (n=7): DMSO group (0.3 ml intragastrically) was determined and administered for 7 days.

3. Group (n=7): Olive oil group (1 ml/kg intraperitoneal) was determined and a single dose was administered on the 7th day.

4. Group (n=7): Carbon tetrachloride group (1 ml/kg intraperitoneal, dissolved in olive oil 1:1 ratio) was determined and a single dose was administered on the 7th day.

5. Group (n=7): *Rheum ribes L.* (root) water extract group (100 mg/kg intragastrically) was determined and administered for 7 days.

6. Group (n=7): The quercetin group was determined (100 mg/kg intragastrically) and administered for 7 days.

7. Group (n=7): *Rheum ribes L.* (root) water extract (100 mg/kg intragastrically) was administered for 7 days and carbon tetrachloride (1 ml/kg in intraperitoneal olive oil at a ratio of 1:1) as a single dose on the 7th day.

8. Group (n=7): Quercetin was administered (100 mg/kg intragastrically) for 7 days and carbon tetrachloride (1 ml/kg intraperitoneal, 1:1 in olive oil) as a single dose on the 7th day.

2.8. *Performing Tissue Homogenate Processes*

Liver and kidney tissues taken from rats were kept at – 65 ºC and gradually dissolved until they reached room temperature. Tissue extraction process for protein carbonyl determination method in tissues was performed as follows. For the extraction, 1 mL of buffer containing 6.8 g of KH_2PO_4 and 2.92 g of EDTA was prepared. 0.5 g of kidney and liver tissue was weighed in a precision balance (Denver instrument SI-234) and put into tubes and 2 mL of buffer was added. Tissues were homogenized on ice until disintegrated and a further 3 mL buffer was added again. Then it was centrifuged at +4 ºC in a cooled centrifuge (EBA 20 Hettich) device at 9500 rpm for 10 minutes. Clear supernatants obtained from liver and kidney tissue were made ready for analysis (16).

2.9. Protein Carbonyl Determination Method

PCO content in liver and kidney tissues were determined according to the method described by Reznick and Packer (17). The basic principle protein carbonyl derivatives are one of the most used markers in the detection of oxidative protein damage. The formation of derivatives of carbonyl groups with 2-4 dinitrophenylhydrazine (DNPH) is followed by the formation of dinitrophenylhydrozane (DNP) products. Finally, the DNP formation was determined at 360 nm at UV. The value found was expressed as nanomole protein carbonyl per mg.

2.10. Trace Element and Mineral Determination

Trace element (Cu, Zn, Fe) and mineral (P) analyzes measured in serum were performed using inductively coupled plasma-optical emission spectrometry (ICP-OES).

2.11. Statistical Analysis

The results are presented as means ± the standard error of the mean (X±SEM). Variance analysis (ANOVA) was applied. Tukey's test was applied for post hoc comparison. Statistical significance was considered as p<0.05. The statistical analysis was carried out using SPSS®, version 23.0 statistical software (SPSS Inc. Chicago Ill, USA).

3. RESULTS

Within the scope of our study, the protein carbonyl (PCO) data in the liver and kidney tissues of the rats and the comparison of the liver PCO levels between the groups are given in table 1, and the comparison of the PCO levels in the kidney tissue is given in the Table 2.

Statistical analysis showed that in the CCl_4 group was significantly higher than the control, DMSO, Olive oil, *Rheum ribes L.*, and Quercetin groups in the PCO levels (p<0.00, p<0.05, p<0.001, p<0.01, and p<0.01, respectively). However, CCl_4 + Quercetin group was also significantly higher than control, DMSO, Olive oil, *Rheum ribes L.*, and Quercetin groups regarding PCO levels (p<0.001, p<0.05, p<0.001, p<0.01, and p<0.05, respectively) in the liver. (Table 1 and Figure 1).

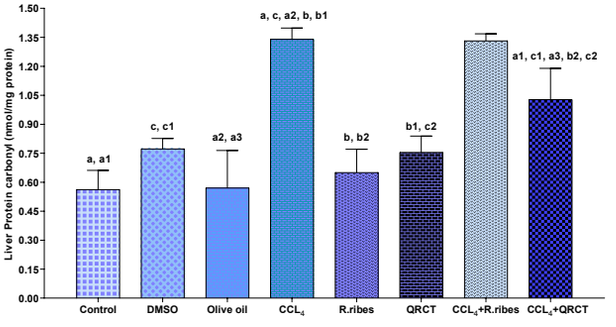

Fig 1. *Comparison of protein carbonyl (PCO) levels between liver tissue control, DMSO, olive oil, CCl4, Rheum ribes L., quercetin, CCl4 + Rheum Ribes L.ve CCl4 + quercetin groups. (Different letters on the error bars represent mean significant difference).*

Table 1 demonstrates Protein carbonyl (PCO) values in control, DMSO, olive oil, CCl_4, *Rheum ribes L.*, quercetin, CCl_4 + *Rheum Ribes L.*ve CCl_4 + quercetin groups in the liver and kidney tissues.

Table 1. *Protein carbonyl (PCO) findings of control, DMSO, olive oil, CCl4, Rheum ribes L., quercetin, CCl4 + Rheum Ribes L.ve CCl4 + quercetin groups in the liver and kidney tissues.*

Groups/Parameters	Liver PCO (nmol/mg prot.)	Kidney PCO (nmol/mg prot.)
Control	$0,56 \pm 0,10^{a,a1}$	$1,05 \pm 0,06^{a,c}$
DMSO	$0,77 \pm 0,05^{c,c1}$	$1,15 \pm 0,11^{b}$
Olive oil	$0,57 \pm 0,19^{a2,a3}$	$1,09 \pm 0.09^{a1}$
CCl_4	$1,34 \pm 0,06^{a,c,a2,b,b1}$	$1,74 \pm 0,08^{a,b,a1,a2,a3}$
Rheum ribes L.	$0,65 \pm 0,12^{b,b2}$	$1,08 \pm 0,10^{a2,c1}$
Quercetin	$0,75 \pm 0,08^{b1,c2}$	$1,07 \pm 0,09^{a3}$
CCl_4 + *Rheum ribes L.*	$1,03 \pm 0,16$	$1,46 \pm 0,16$
CCl_4 + Quercetin	$1,33 \pm 0,04^{a1,c1,a3,b2,c2}$	$1,53 \pm 0,04^{c,c1}$

a,a_1,a_2,a_3:p<0.001, b,b_1,b_2: p<0,01, c,c_1,c_2: p<0.05 (different letters in superscripts represent statistically significant differences between groups).

According to the statistical analysis in kidney tissue, PCO levels were significantly higher in the CCl_4 group than the control, DMSO, Olive oil, *Rheum ribes L.,* Quercetin groups (p<0.001, p<0.01, p<0.001, p<0.001 and p<0.001, respectively). Moreover, PCO were increased in CCl_4 + Quercetin group compared to control and *Rheum ribes L.* groups (p<0.05, and, p<0.05) (Table 1, Figure 2).

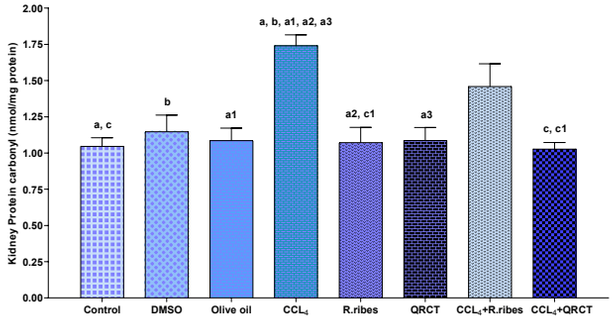

Fig 2. *Comparison of protein carbonyl (PCO) levels between kidney tissue control, DMSO, olive oil, CCl4, Rheum ribes L., quercetin, CCl4 + Rheum Ribes L.ve CCl4 + quercetin groups. (Different letters on the error bars represent mean significant difference).*

Table 2 shows the trace element (Cu, Zn, Fe) and mineral (P) levels between control**,** DMSO, olive oil, CCl_4, *Rheum ribes L.*, quercetin, CCl_4 + *Rheum Ribes L.*ve CCl_4 + quercetin group.

Table 2. *Trace element (Cu, Zn, Fe) and mineral (P) levels between control, DMSO, olive oil, CCl4, Rheum ribes L., quercetin, CCl4 + Rheum Ribes L.ve CCl4 + quercetin group*

Groups/ Parameters	Cu (µmol/L)	Zn (µmol/L)	Fe (mmol/L)	P (mmol/L)
Control	0,95 ± 0,11	3,15 ± 0,06^{a}	0,118 ± 0,0001	6,34± 0,43^{c,b}
DMSO	1,16 ± 0,16	3,58 ± 0,07^{a,a1,c,b,c1,b1}	0,116 ± 0,0007	4,47± 0,25^{c,b1}
Olive oil	1,04 ± 0,10	3,15 ± 0,05^{a1}	0,118 ± 0,0004	6,32± 0,33^{b1,b2}
CCl_4	1,32 ± 0,13	3,39 ± 0,06	0,117 ± 0,0009	4,29± 0,30^{b,b2,c1}
Rheum ribes L.	0,99 ± 0,09	3,26 ± 0,07^{c}	0,118 ± 0,0004	6,07± 0,26^{c1}
Quercetin	1,03 ± 0,11	3,22 ± 0,06^{b}	0,117 ± 0,0003	5,55 ± 0,38
CCl_4 + *Rheum ribes L.*	1,11 ± 0,14	3,25 ± 0,04^{c1}	0,118 ± 0,0006	5,44 ± 0,42
CCl_4 + Quercetin	1,17 ± 0,17	3,22 ± 0,08^{b1}	0,117 ± 0,0009	5,50 ± 0,42

a,a_1:p<0.001, b,b_1,b_2: p<0,01, c,c_1,c_2: p<0.05 (different letters in superscripts represent statistically significant differences between groups).

The trace element (Cu, Zn, Fe) and mineral (P) levels were measured in serum in our study and the proportional findings between these elements are shown in table 2. Regarding the zinc (Zn) trace element findings, between the group treated with 0.3 ml DMSO and the control group and the group treated with 1 ml kg^{-1} olive oil (p<0.001), between the group administered 100 mg kg^{-1} quercetin and the group treated with CCl_4 + quercetin (p<0.01) and between the group administered 100 mg kg^{-1} *Rheum ribes L.* and the group treated with CCl_4 + quercetin (p <0.05), a significant relationship was found (Table 2). No significant relationship was found between the control group and other groups in terms of Cu and Fe trace element levels (Table 2).

The CCl_4 group was also significantly lower than control, Olive oil and *Rheum ribes L.* groups regarding P level (p<0.01, p<0.01 and p<0.05, respectively), whereas the DMSO group had decreased level of P comparing with control and Olive oil groups (p<0.05 and p<0.01).

The mean ratio trace element and mineral Zn/Cu, P/Zn, P/Fe and Zn/Fe in the control**,** DMSO, olive oil, CCl_4, *Rheum ribes L.*, quercetin, CCl_4 + *Rheum Ribes L.*ve CCl_4 + quercetin groups are shown in Table 3 (Figure 3).

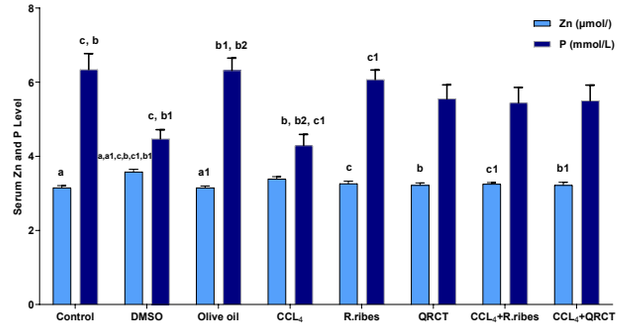

Fig 3. *Comparison of zinc (Zn) element and phosphorus (P) mineral levels in serum control, DMSO, olive oil, CCl4, Rheum ribes L., quercetin, CCl4 + Rheum Ribes L.ve CCl4 + quercetin groups. (Different letters on the error bars represent mean significant difference).*

Table 3. *Findings of the ratio trace element and mineral Zn/Cu, P/Zn, P/Fe and Zn/Fe in the control, DMSO, olive oil, CCl4, Rheum ribes L., quercetin, CCl4 + Rheum Ribes L.ve CCl4 + quercetin groups*

Groups/ Parameters	Zn/Cu	P/Zn	P/Fe	Zn/Fe
Control	3,55 ± 0,38	2,02 ± 0,13^{a,b}	54,01 ± 3,93^{b,c}	26,80±0,73^{b}
DMSO	3,81 ± 1,02	1,25 ± 0,06^{a,a1,c}	38,75 ± 2,22^{c,c1}	30,98 ± 0,58^{a,b,b1,c,c1,c2}
Olive oil	3,25 ± 0,36	2,01 ± 0,13^{a1,b1}	53,67 ± 2,71^{b1,c1}	26,8 ± 0,49^{a}
CCl_4	2,75 ± 0,35	1,27 ± 0,11^{b,b1,c1}	36,76 ± 2,43^{b,b1,c2}	28,93 ± 0,73
Rheum ribes L.	3,47 ± 0,36	1,86 ± 0,10^{c,c1}	51,77 ± 2,22^{c2}	27,71 ± 0,70^{c}
Quercetin	3,38 ± 0,39	1,73 ± 0,12	47,49 ± 3,30	27,53 ± 0,56^{c1}
CCl_4+*Rheum ribes L.*	3,25 ± 0,44	1,65 ± 0,11	46,17 ± 3,66	27,6 ± 0,44^{c2}
CCl_4+Quercetin	3,23 ± 0,54	1,72 ± 0,14	46,86 ± 3,68	27,43 ± 0,90^{b1}

a,a_1:p<0.001, b,b_1: p<0,01, c,c_1,c_2: p<0.05 (different letters in superscripts represent statistically significant differences between groups).

The results showed that the CCl_4 group had significantly lower levels of P/Zn ratio than the control, Olive oil and *Rheum ribes L.*groups (p<0.01, p<0.01, and, p<0.05, respectively). Additionally, the DMSO group had significantly lower levels of P/Zn than the control, Olive oil and *Rheum ribes L.*groups (p<0.001, p<0.001, and p<0.05, respectively). The CCl_4 group had increased levels of P/Fe compared to the control, Olive oil and *Rheum ribes L.* groups (p<0.01, p<0.01 and p<0.05, respectively). Similarly, DMSO group had significantly lower levels of P/Fe than the control and Olive oil group (p<0.05 and p<0.05). However, the DMSO group had a significantly higher level of Zn/Fe ratio than the control, Olive oil, *Rheum ribes L.,* Quercetin, CCl_4 + *Rheum ribes L.* and CCl_4 + Quercetin groups (p<0.01, p<0.001, p<0.05, p<0.05, p<0.05, and p<0.01, respectively). (Table 3, Figure 4).

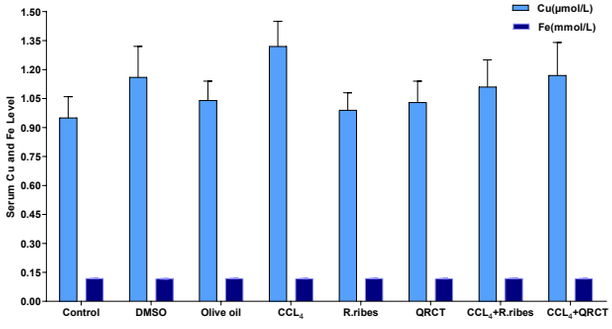

Fig 4. *Comparison of copper (Cu) and iron (Fe) element levels in serum control, DMSO, olive oil, CCl4, Rheum ribes L., quercetin, CCl4 + Rheum Ribes L.ve CCl4 + quercetin groups*

4. DISCUSSION

CCl_4 is known as hepatotoxin and nephrotoxin (18, 19). In experimental studies, the mechanism of CCl_4 induced damage is that cytochrome P450 transforms into free radicals trichloromethyl (CCl_3^-) and trichloromethyl peroxy ($CCl_3O_2^-$) and increases lipid peroxidation and protein oxidation in the liver, as well as the heart, kidney, brain, lung and testis. It has been reported to cause damage in many organs (19, 20, 21, 22). CCl_3 – radical undergoes both oxidative and reductive biotransformation and initiates biochemical events that lead to liver cell necrosis (18, 23). On the other hand, oxidative damage of proteins is also caused by the fenton-type reaction of free radicals and trace elements such as Fe^{+2}, Cu^{+2}. Conditions leading to the mobilization of Fe or Cu to redox active forms lead to high levels of protein carbonyls (24). As the carbonyl groups of proteins increase, they become more susceptible to oxidative damage (25). Symptoms such as increases in carbonyl levels, rheumatoid arthritis, ischemia-reperfusion damage to the heart muscles, and muscle damage caused by strenuous exercise are also examined (17).

In our study, a non-lethal dose of CCl_4 (1 ml/kg i.p) was used and the rats were decapitated 24 hours after CCl_4 treatment. This period has been reported in the literature review to be the most appropriate time for inducing liver damage (26, 27). In this study, the effect of these antioxidant substances on protein carbonyl in the presence of *Rheum ribes L.* and quercetin in CCl_4-induced liver and kidney damage was investigated. When table. 1 was examined, it was observed that the PCO levels of 1 ml kg^{-1} CCl_4 administered in both liver and kidney tissue groups increased compared to the control group (p<0.001, p<0.05). It was determined that the group given CCl_4 with 100 mg kg^{-1} quercetin also decreased the PCO levels compared to the CCl_4 group (p<0.01; p<0.05), but increased compared to the 100 mg kg^{-1} *Rheum ribes L.* group. These results show that CCl_4 causes oxidative damage on liver and kidney tissues, *Rheum ribes L.* and quercetin have been found to reduce this damage. In CCl_4 application, it can be thought that the presence of PCO, which is a high level of oxidative biomarker in kidney tissue parallel to the liver tissue, is most likely caused by the application of CCl_4 oxidative stress.

Cu, Fe and Zn are trace elements that have important roles in fulfilling the functions of many enzymes and protein metabolism in the organism (2, 28, 29). Cu is an essential trace mineral essential for many biological processes. This requirement is thought to be due to the Cu element acting as a cofactor for proteins involved in various biological reactions such as photosynthesis, respiration, free radical reactions, connective tissue formation, Fe metabolism, and neurological function (30). Iron is an essential element required as a cofactor for proteins that govern oxygen transport, such as hemoglobin and myoglobin (31). The antioxidant effect of Zn is due to its role in preventing the formation of free radicals and protecting against oxidative stress (32). Phosphates are the central building blocks in nucleic acids. P is an important mineral that regulates the fat content in the blood by taking part in carbohydrate and fat metabolism, apart from being in a critical position in important physiological functions including energy production, cellular replication and bone mineral metabolism (2, 29, 33). In our study, when trace elements (Cu, Fe, Zn) and mineral (P) levels were evaluated, the values of P, Zn/Cu, P/Zn and P/Fe were statistically significant between the control group and the group administered 1 ml kg^{-1} CCl_4 (p<0.05; p<0.001; p<0.001) a decrease was detected. Significant decrease in P level (p<0.01) between the 1 ml kg^{-1} CCl_4 applied group and the control group indicates that there is a sign of muscle damage. Because it has been understood that deficiency of P mineral causes muscle weakness (2), and one of the side effects of CCl_4 to the organism is muscle weakness (34). Therefore, it can be interpreted that the increases in protein carbonyl levels in the group administered 1 ml kg^{-1} CCl_4 may cause muscle damage in the organism.

The hepatoprotective herbal preparations recommended as an alternative treatment method for the elimination and prevention of hepatic disorders have been shown to be antioxidant in clinical and experimental studies (35) and black seed (36), nettle seeds studies have been included in the literature. In addition, there are studies in the literature in which CCl_4 and various antioxidants are used both single and multiple times. It has been shown that antioxidants such as ascorbic acid (37), selenium (38), a-tocopherol (39) combined

with CCl_4 prevented or partially protected hepatotoxicity in rats. In the literature review, no study was found on the effects of *Rheum ribes L.* and quercetin on protein carbonyl on liver and kidney tissue damage by causing oxidative stress with CCl_4 in rats. However, when looking at similar studies obtained in our study, Sundari et al. (40) found that oxidative protein accumulation in liver damage by causing oxidative stress with CCl_4 in rats is an early finding in CCl_4-mediated liver damage. Kılıçgün and Altıner (41) investigated the inhibition effect of Rosa canina (rosehip) protein oxidation in rats with liver damage with CCl_4 and found that the plant in question reduced liver protein oxidation.

5. CONCLUSION

Throughout our investigations and analyses, it was observed that oxidative stress increased in rats exposed to CCl_4 and the associated protein carbonyl levels in liver and kidney tissues increased. Considering the liver and kidney tissues between the groups, it was observed that the protective effect of the group administered with 100 mg kg-1 Rheum ribes L on PCO is higher than the group administered with100 mg kg-1 quercetin. These findings show that the protective effect of Rheum ribes L plant on CCl4-induced liver and kidney toxicities. In addition, it can be considered that in further studies, *Rheum ribes L.,* active ingredient dosage and method will be a preliminary study in terms of determining protein oxidation.

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