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Protective effects of saffron, safranal and crocin administration on vitamins (A, D, E, K) and protein carbonyl levels against CCI4-induced oxidative damage in rats

Ahmet BAKIR[1], Damla YILDIZ[1], Suat EKIN[1], Gokhan OTO[2], Ibrahim ARAS[3], Irfan BAYRAM[3]

[1] Department of Biochemistry, Faculty of Science, Van Yuzuncu Yil University, Van, Turkey
[2] Department of Pharmacology, Faculty of Medicine, Van Yuzuncu Yil University, Van, Turkey
[3] Department of Pathology, Faculty of Medicine, Van Yuzuncu Yil University, Van, Turkey

Corresponding Author: Ahmet BAKIR
E-mail: ahmetbakir@yyu.edu.tr

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ABSTRACT

Objective: The possible effects of saffron and its active components on oxidative stress are known. Protein carbonyls (PCO), formed due to protein exposure to oxidizing agents, are a newly researched topic. In this study, it was aimed to determine, antioxidant fat-soluble vitamins (A, D, E, K) and PCO values after saffron, safranal and crocin administration with carbon tetrachloride (CCl4) in rats.

Materials and Methods: Fifty-four Wistar albino male rats were randomly selected, and 9 groups of n=6 were formed. Vitamin levels in rat serum were determined by HPLC and PCO levels were determined by spectrophotometric method.

Results: A significant difference ($p<0.01$) was found between the CCl4 and the saffron, safranal and crocin groups. A significant decrease was observed in retinol and cholecalciferol values between CCl4 and saffron group ($p<0.05$, $p<0.001$), and a significant decrease in cholecalciferol and phylloquinone levels between CCl4 and safranal groups ($p<0.01$, $p<0.05$). Moreover, a decrease in cholecalciferol level ($p<0.05$) was determined between the olive oil, saffron and CCl4+crocin groups.

Conclusion: As a result, saffron and safranal have a protective effect against CCl4-induced oxidative damage to PCO, retinol, phylloquinone and cholecalciferol, and this effect may be due to the potent antioxidative effects of saffron and safranal.

Keywords: Carbon tetrachloride, Protein carbonyl, Saffron, Safranal, Vitamin

1. INTRODUCTION

Carbon tetrachloride (CCl_4) is a chemical that causes tissue damage by producing free radicals [1]. Cytochrome P450 metabolises CCl4-induced damage. Free radicals formed due to this metabolism cause oxidative stress on DNA, proteins, lipids and generally other components of the cell [2, 4]. Oxidative stress is a condition that manifests itself when certain chemicals or drugs are taken or when the antioxidant level in the organism decreases. CCl_4 is widely used as a model for screening the effects of drugs or plant extracts [5, 6]. Unraveling the mechanism of cellular metabolism is one of the topics that scientists have been researching for a long time [7]. This is because everything a living organism does occurs at the cellular level. Oxygen-breathing organisms are exposed to various reactive oxygen species (ROS) throughout their lives, which can directly or indirectly damage molecules such as DNA, lipids, and proteins [8]. ROS cause oxidative stress, which triggers the survival button in the cell by undergoing a series of reactions. Following this process, a balance was established with free radicals and various enzymatic or non-enzymatic structures in living cells, and various comments were made on the direction of this balance, which is still being made.

Protein carbonyls are elevated under various oxidative stress conditions. Amino acids, which are the main mechanisms of proteins, may undergo some deterioration in their structure under various oxidative stress conditions. These modifications, called carbonyl formation, may be an early sign of protein oxidation [9]. Oxidative damage occurs in proteins with reactive oxygen species (ROS) formed by a series of reactions by metal-ion catalyzed reactions (MCO), photochemical processes, ionizing radiation and enzyme-catalyzed redox reactions, accompanied

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by increased protein carbonyl levels [10, 11]. Due to the vital functions of proteins at the cellular level, their structures are damaged over time. It is essential to strictly control this damage and to maintain order [12].

Vitamins are organic compounds required in trace amounts for the body's basic functions. They are catalysts for reactions involving energy metabolism. Various studies have defined the main functions of fat-soluble vitamins (retinol, cholecalciferol, α-tocopherol, phylloquinone) in the organism, such as antioxidants, bone health, immune system, blood clotting and vision [13-16]. Plants make important contribution to the protection of our health. The main reason for this is the active substances in the content of the plants. Naturally found in various plants, these active substances have been defined as free radical inhibitors, oxygen scavengers or reducing agents [17].

Saffron (*Crocus sativus L.*) is a perennial, sedentary plant of the Iridaceae family. Saffron is mainly used to impart color, flavor and aroma to foods, and certain chemical components have been identified. Due to the unique properties of saffron and its components, it has attracted the attention of many researchers. The part of the saffron plant responsible for its color is crocin, while safranal and picrocrocrocin are responsible for its bitter taste and aroma [18]. Saffron is used both in folk and modern medicine for therapeutic purposes [19]. Numerous studies have revealed that saffron has cytotoxic, anticarcinogenic and antitumor properties [20].

Fat-soluble vitamins (A, D, E, K) work most safely and effectively in the context of nutrition when they are taken into living cells in appropriate doses through a rich diet. In addition, there are many studies in the literature that state that protein carbonyls are associated with excessive production of ROS species. From this perspective, this study focused on PCO and vitamins between the groups as a result of treatment with saffron and its active ingredients, safranal and crocin, by creating oxidative stress in rats due to CCl_4 application. In summary, this study aims to investigate the effects of CCl_4-triggered oxidative stress on vitamins and protein carbonyl levels in the serum of rats and whether saffron and its active components modulate these effects. In addition, this study will be important in contributing to the literature, guiding researchers who will conduct other studies, and having the potential to create intellectual resources.

2. MATERIAL and METHOD

Chemical Substances Used

Ethanol, Methanol, n-Hexane, Tetrahydrofuran (THF), Monopotassium hydrogen phosphate, Trichloroacetic acid, Hydrochloric acid, 2,4-Dinitrophenylhydrazine, Ethyl acetate, Guanidine-HCl, Carbon tetrachloride (CCl_4-simya, aldrich, catalogue No: 289116), Saffron (sigma, aldrich, catalogue No: S8381), Safranal (sigma, aldrich, catalogue No: W338907), Crocin (sigma, aldrich, catalogue No: 17304), Olive oil (sigma, aldrich, catalogue No:O1514), Ketamine hydrochloride.

Experimental Procedure

The rats used in the study were 54 Wistar albino breeds, weighing between 180 and 250 g. The experiment was carried out at Van Yüzüncü Yıl University, Experimental Medicine Research and Application Center Directorate. Rats provided in the same place were fed with standard pellet feed throughout the experimental period. The room temperature was adjusted to 22 ± 2 °C and the environment was adjusted to a 12-hour light-12-hour dark rhythm. Rats were kept in standard plastic cages with free food and water until the end of the experiment. Before starting the research, the study was approved by the Van Yuzuncu Yil University Ethics Committee with the decision dated 25.12.2015 and numbered 2015/560.

Establishment of experimental groups and experiment plan

Carbon tetrachloride was mixed with olive oil at a ratio of 1:1 and administered intraperitoneally as a single dose to the third, seventh, eighth and ninth groups on the 7th day as 1ml/kg. Safron was administred as oral gavage to the fourth and seventh groups at a daily dose of 100 mg/kg. Safranal was administered to the fifth and eighth groups and crocin intraperitoneally to the sixth and ninth groups at a daily dose of 100 mg/kg. The application in the groups created is stated below. Blood samples were taken from rats 24 hours after CCl_4 administration (day 8). The preparation method of CCl_4, saffron, safranal, and crocin substances, as well as the subsequent application procedure to rat groups, are stated above.

In the study, 9 groups were formed, with 6 rats in each group. 1) The control group was given saline (0.9% NaCl) orally for 7 days, 2) the olive oil group was administered (1 ml/kg i.p. olive oil) for 7 days, 3) the CCl_4 group was administered a single dose on the 7th day (1 ml/kg i.p.), 4) saffron group was applied for 7 days (100 mg/kg by orogastric gavage), 5) safranal group was applied for 7 days (100 mg/kg i.p.), 6) crocin group was administered for 7 days (100 mg/kg i.p.), 7) CCl_4 + saffron group (CCl_4 was administered as a single dose 1 ml/kg 1:1 on the 7th day; saffron was administered at 100 mg/kg by orogastric gavage for 7 days), 8) CCl_4 + safranal group (CCl_4 was administered as a single dose, 1 ml/kg i.p. on the 7th day; safranal was administered 100 mg/kg i.p. for 7 days), 9) CCl_4 + crocin group (CCl_4 was administered as a single dose, 1 ml/kg i.p. on the 7th day; crocin was administered at 100 mg/kg i.p. for 7 days).

Collecting Serum Samples

At the end of the study, the rats were anesthetized with 10% ketamine/Xylazine. The blood taken from the hearts of the rats with the help of injectors was put into gel biochemistry tubes. Serums were centrifuged at room temperature and 2500 rpm for 10 minutes to ensure appropriate separation and then stored at −65°C until the study began.

Protein Carbonyl Determination Method

The PCO content in the serum of rats was determined according to the method described by Reznick and Packer [9]. Briefly, 0.01M 2,4 – dinitrophenylhydrazine in HCl was added to rat

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serum. The resulting mixture was incubated for 120 minutes at room temperature, and then 1 ml of tricyclic antidepressants (TCA) was added. The samples were then incubated for 6 minutes and centrifuged for 15 minutes. The resulting mixture was washed with ethanol: ethyl acetate and dissolved in 0.02M phosphate buffer (pH=6.8). Each sample was scanned on Shimadzu UV-1800 spectrophotometer against a replica. Peak absorbance between 360-370 nm was used to quantify PCO and final data were recorded.

Determination of Vitamins (retinol, cholecalciferol, α-tocopherol and phylloquinone)

Preparing standard solutions for vitamins

Vitamins stock solutions were prepared at 500 µg/mL. The solutions were appropriately diluted with methanol to match the standard solution. Calibration was calculated using linear regression analysis of peak area to standard solution concentrations.

Extraction process

To prevent the samples from deteriorating against UV rays, they were thawed at ambient temperature under fluorescent lights and covered with plastic caps. The vitamins examined in the serum were extracted by modifying the method determined by Su et al. [21]. A 150 µL serum with 0.025% BHT was added to the extraction solution. Then, it was deproteinized by adding 150 µL EtOH and butylated hydroxytoluene (BHT), and the vortex mixed the samples. Samples were extracted twice with 800 µL *n*-hexane. The prepared samples were vortexed for 5 seconds and then centrifuged at 6000 rpm for 15 minutes. The hexane formed in the standard tube was evaporated to dryness under a stream of nitrogen at 36 °C. The residue formed at the bottom of the samples was dissolved in 0.05 mL of THF, and then 0.15 mL of methanol was added. After vortexing, the 0.1 mL samples for 1 minute, the samples were transferred to amber glass bottles.

Chromatographic conditions

The chromatographic system consisted of HP Agillent 1100 with a G-1328 Diode Array Detector (DAD) and G1329 ALS autosampler (-8 °C). Agilent Technologies HP software was preferred to process the data. 5 µm Gl Science C_{18} reverse phase column (250 × 4.6 mm ID) was used for separation. Then, the mobile phase of the MeOH-THF mixture (80:20, v/v) was made by modifying the method of Siluk et al. [22]. The pump used for vitamin analysis was set at a flow rate of 1.5 mL/min. Chromatographic analysis was made at 45°C using isocratic elution. The chromatogram was monitored with DAD array detection at 325, 265, 290 and 248 nm (simultaneous measurement of retinol, cholecalciferol, α-tocopherol and phylloquinone, respectively).

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 done using SPSS®, version 23.0 statistical software (SPSS Inc. Chicago Ill, USA).

3. RESULTS

The PCO, retinol, cholecalciferol, α-tocopherol and phylloquinone data analyzed in rat serum within the scope of our study are shown in Table I. A comparison of PCO levels between groups is given in Figure 1. Considering the PCO data in the rat's serum, a statistically considerable ($p<0.001$) relationship was determined between the control and olive oil groups and the group administered 1 ml/kg CCl_4 (Table I). In addition, a significant relation was determined between the groups administered 100 mg/kg saffron, safranal and crocin, and the group administered 1 ml/kg CCl_4 ($p<0.01$) (Table I and Figure 1). The significant difference in PCO values between rat groups showed that the toxic effect of CCl_4 caused protein oxidation by creating oxidative stress in rats. It was observed that the application of saffron (100 mg/kg, oragastric gavage), safranal (100 mg/kg, i.p.) and crocin (100 mg/kg, i.p.) together with CCl_4 was significant on this oxidative stress (Figure 1).

Within the scope of our study, the levels of retinol, cholecalciferol, α-tocopherol and phylloquinone vitamins in serum between groups are shown in figures 2, 4, 6, and 8, and their chromatograms are shown in figures 3, 5, 7 and 9, respectively. It was determined that the vitamins in rat serum had significant content within the groups (except for the 3rd group, CCl_4 1 ml/kg i.p.) (Figures 2, 4, 6, 8). When the retinol vitamin was examined, a significant relationship was determined between the control, saffron (100 mg/kg, oragastric gavage) and olive oil (1 ml/kg i.p) groups and the group administered CCl_4 (1 ml/kg i.p), ($p<0.05$, $p<0.05$ and $p<0.01$, respectively) (Table I and Figure 2).

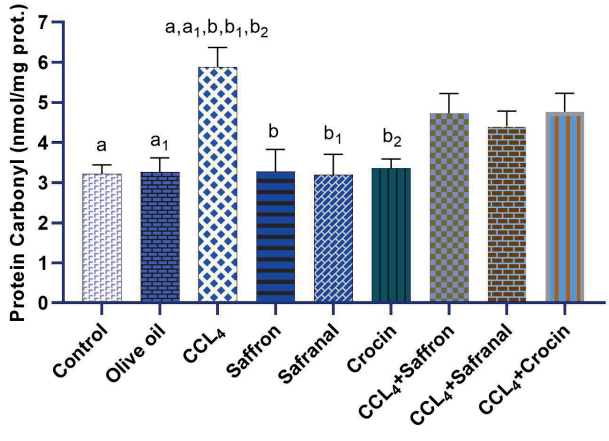

***Figure 1**. Comparison of PCO levels between rat serum of control, olive oil, CCl_4, saffron, safranal, crocin, CCl_4 + saffron, CCl_4 + safranal and CCl_4 + crocin groups.*

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***Table I**. PCO, retinol, cholecalciphenol, α-tocopherol, and phylloquinone findings in rat serum of control, olive oil, CCl_4, saffron, safranal, crocin, CCl_4 + saffron, CCl_4 + safranal and CCl_4 + crocin groups.*

Groups	PCO X ± SEM	Retinol X ± SEM	Cholecalciferol X ± SEM	α-tocopherol X ± SEM	Phylloquinone X ± SEM
Control	3.22±0.23^{a}	3.15±0,14^{c}	0.10±0.01b	1.30±0.23	0.23±0.03^{c}
Olive oil	3.27±0.35^{a1}	3.34±0,23^{b}	0.11±0.01^{c,a}	1.44±0.12	0.24±0.01^{c1}
CCl_4	5.89±0.48$^{a, a1,b,b1,b2}$	2.14±0.14^{c,b,c1}	0.06±0.00^{b,b1,a,a1}	0.89±0.13	0.12±0.00^{c,c1,c2}
Saffron	3.28±0.54^{b}	3.10±0.17^{c1}	0.11±0.00^{c1,a1}	1.28±0.13	0.22±0.03
Safranal	3.19±0.51^{b1}	3.04±0.22	0.10±0.00^{b1}	1.41±0.24	0.23±0.02^{c2}
Crocin	3.36±0.24^{b2}	2.99±0.35	0.10±0.00	1.35±0.26	0.21±0.04
CCl_4 + Saffron	4.73±0.49	2.76±0.19	0.08±0.00	1.00±1.16	0.17±0.00
CCl_4 + Safranal	4.41±0.38	2.86±0.15	0.09±0.00	1.13±0.21	0.18±0.02
CCl_4 + Crocin	4.77±0.46	2.54±0.17	0.07±0.00^{c,c1}	0.97±0.09	0.16±0.03

Different letters: significant, differences between groups (a: p<0.001, b: p<0.01, c: p<0.05). PCO (nmol/mg prot.), retinol, cholecalciferol, α-tocopherol and phylloquinone (μmol/L).

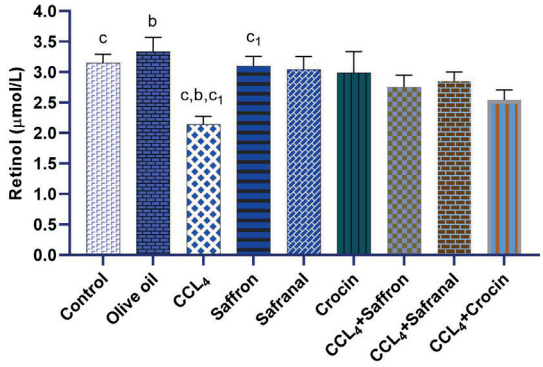

***Figure 2**. Comparison of retinol levels between rat serum of control, olive oil, CCl_4, saffron, safranal, crocin, CCl_4 + saffron, CCl_4 + safranal and CCl_4 + crocin groups.*

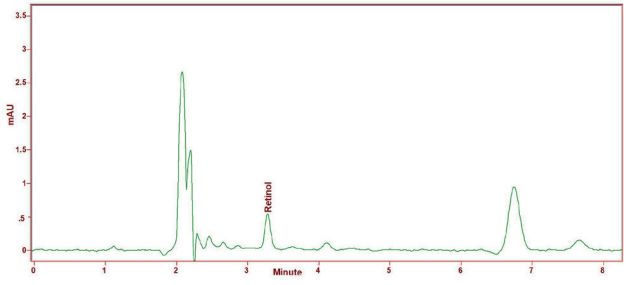

***Figure 3**. Chromatogram of the retinol (vitamin A) [(mobile phase: methanol/ tetrahydrofuran (20/80 v/v)], flow rate 1,5 mL min^{-1}. Clomn: GI science C_{18} 5μL (250/4,6 mm), wavelength: 325 nm.*

Considering the cholecalciferol data, a statistically significant relationship (p<0.05 and p<0.05) was determined between saffron and olive oil and the group treated with CCl_4 + crocin. On the other hand, a significant relation was found between the control, saffron, safranal and olive oil and 1 ml/kg CCl_4 administered group (p<0.01, p<0.01, p<0.001 and p<0.001, in order of) (Table I and Figure 4).

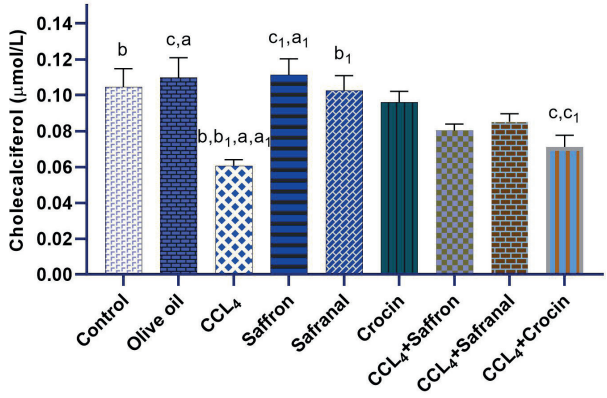

***Figure 4**. Comparison of colecalciferol levels between rat serum of control, olive oil, CCl_4, saffron, safranal, crocin, CCl_4 + saffron, CCl_4 + safranal and CCl_4 + crocin groups.*

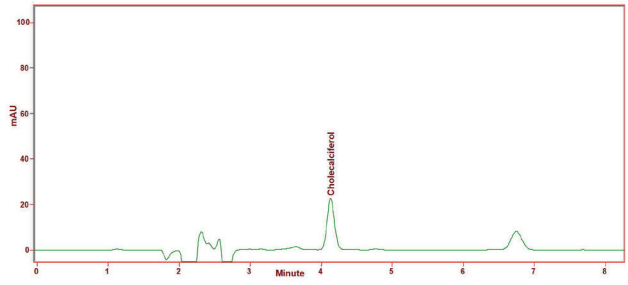

***Figure 5**. Chromatogram of the colecalciferol (vitamin D) [(mobile phase: methanol/ tetrahydrofuran (20/80 v/v)], flow rate 1,5 mL min^{-1}. Clomn: GI science C_{18} 5μL (250/4,6 mm), wavelength: 265 nm.*

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Within the study's scope, no statistically significant relationship was found between the groups in the level of α-tocopherol (p>0.05, data not shown, Table I and Figure 6).

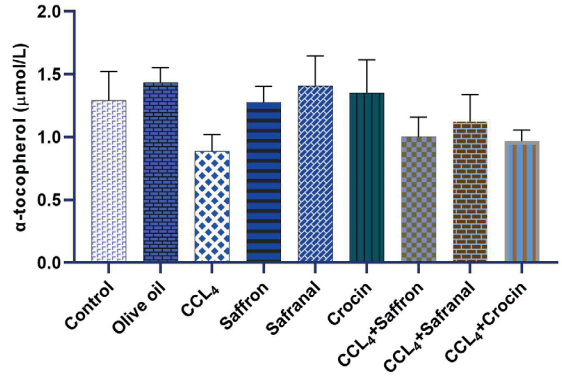

***Figure 6**. Comparison of α-tocopherol levels between rat serum of control, olive oil, CCl_4, saffron, safranal, crocin, CCl_4 + saffron, CCl_4 + safranal and CCl_4 + crocin groups.*

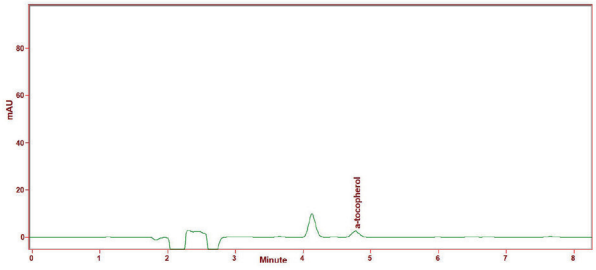

***Figure 7**. Chromatogram of the α-tocopherol (vitamin E) [(mobile phase: methanol/ tetrahydrofuran (20/80 v/v)], flow rate 1,5 mL min^{-1}. Clomn: GI science C_{18} 5µL (250/4,6 mm), wavelength: 290 nm.*

When the phylloquinone data were examined, a significant relationship was determined between the control (p>0.05), safranal (p>0.05), olive oil groups (p>0.05) and the CCl_4 group (1 ml/kg i.p) (Table I and Figure 8).

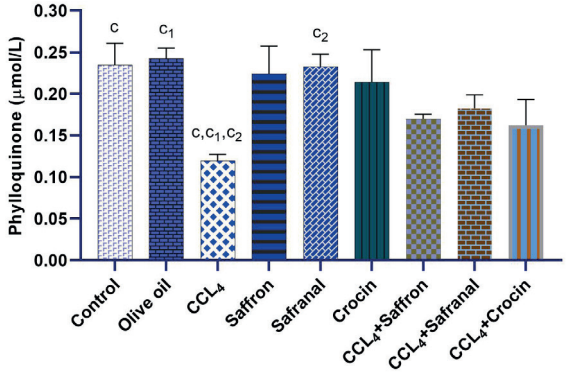

***Figure 8**. Comparison of phylloquinone levels between rat serum of control, olive oil, CCl_4, saffron, safranal, crocin, CCl_4 + saffron, CCl_4 + safranal and CCl_4 + crocin groups.*

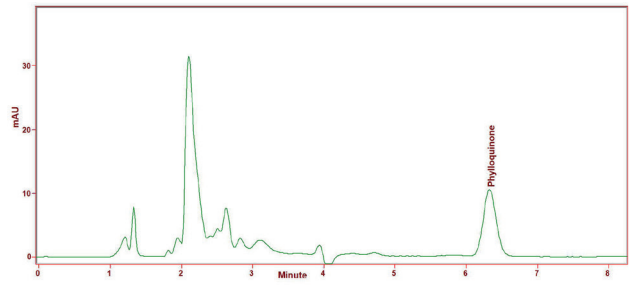

***Figure 9**. Chromatogram of the phylloquinone (vitamin K) [(mobile phase: methanol/ tetrahydrofuran (20/80 v/v)], flow rate 1,5 mL min^{-1}. Clomn: GI science C_{18} 5µL (250/4,6 mm), wavelength: 248 nm.*

4. DISCUSSION

Toxic effect of CCl_4: It is the result of activation of trichloromethyl (-CCl3) by cytochrome P450, which easily reacts with oxygen to form trichloromethylperoxy radical (CCl3OO.) These free radicals initiate cell damage through two main mechanisms: covalent bonding and lipid peroxidation [23]. Protein carbonyls levels increase when cells are exposed to various oxidative stress conditions [8]. CCl_4 is known in the literature to be a toxic chemical that causes cell and tissue damage in animals [1]. PCO is widely used as an early marker of protein oxidation in cells and tissues. One of the techniques used to detect proteins is the reaction of carbonyls with 2,4-denitrophenylhydrazine (DNPH) to form stable dinitrophenyl (DNP). Because DNP absorbs light at 370 nm, carbonyl groups can be measured spectrophotometrically.

Carbonyl groups enter proteins with various oxidative groups, especially metal ion-catalyzed variants of specific protein amino acid side chains. On the other hand, they are also incorporated by removing carbonyl-containing oxidized lipids (MDA, HNE) or sugars [24]. 4-hydroxyonenal (HNE) is in higher amounts in lipid peroxidation chain reactions due to increased oxidative stress. Although, protein oxidation's mechanism, pathways and products differ, factors that cause lipid oxidation can also initiate protein oxidation. Functional groups on the side chains of amino acid residues and the peptide backbone are targets for ROS. In this respect, PCO levels in the experimental groups focused on detecting carbonyl groups as markers of oxidative protein modification.

In the study, a single dose of 1 ml/kg CCl_4 was administered to the rats in the 3rd, 7th, 8th, and 9th groups on the 7th day and were sacrificed under anesthesia on the 8th day of the application. The purpose of this study was to determine the levels of PCO, retinol, α-tocopherol, cholecalciferol and phylloquinone in the serum of rats treated with CCl_4, saffron and saffron components (safranal and crocin), and to determine which of the important components of saffron and its components are more effective. The PCO, retinol, α-tocopherol, cholecalciferol and phylloquinone data measured in serum are given in Table I. According to the statistical analysis results in the study, a significant increase

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($p<0.001$) was detected in PCO levels between the control and olive oil groups and the CCl_4 group. On the other hand, there was a significant difference ($p<0.01$) between the CCl_4 group and the saffron, safranal and crocin groups. PCO results showed that CCl_4 caused oxidative damage, and the effect in the groups treated with carbon tetrachloride 100 mg/kg in saffron, safranal and, crocin was as close as in the control and olive oil groups. The presence of PCO, a high level of oxidative biomarker in CCl_4 administration, was evident in rats; CCl_4 caused, most likely, oxidative stress. From this point of view, it is evident that saffron protects against CCl_4-induced oxidative stress in rats, as assessed by the reduction in the formation of safranal and crocin protein carbonyl. On the other hand, no study has been found in the literature on the effect of saffron and its active ingredients, safranal and crocin compounds, on PCO in CCl_4 rats. From this perspective, it can be thought that these data will be a reference for similar studies.

It is impossible to have a life completely devoid of the oxidative stress that occurs in the cell due to free radicals. Ultimately, there will be a loss in all cells that produce energy, and minimizing the damage caused by this loss is a situation that the cell will overcome. So, how will cells do this? At this point, endogenous and exogenous antioxidants come into play in response to reactive oxygen species (ROS) [25]. People have been looking for medicine in nature to treat their diseases in the past, and this process continues. Due to the low side effects, difficulty of administration, and prices of synthetic drugs, it has been widely used worldwide [26]. The mechanisms of antioxidants, proteins and amino acids in foods have been associated with their ability to chelate pro-oxidant metals [27].

The saffron plant has been found to contain more than 150 volatile and aromatic compounds, including pharmacologically active and important components such as safranal, crocin, picrocrocin and crocetin [28-30]. Research showed that this plant has antioxidant potential, as shown by the results of in-vitro analysis performed on different parts of saffron flowers [31]. On the other hand, the literature reveals that saffron is used to treat diabetes, bronchitis, asthma, coronary artery diseases and neurodegenerative disorders [31-32]. Safranal, an important component of the essential oil contained in saffron, has been used in different scientific studies to evaluate pharmacological and biological activities since its discovery [26]. For example, in their study on mice in 2015 by Zanjani et al., it was found that safranal is safe for the immune system and has no toxicity on cellular immune responses. On the other hand, another study conducted by Boskabady et al., in 2014 found that safranal has therapeutic values in treating asthma with its immunoregulatory effect that reduces airway sensitivity [33-34]. Crocins are bioactive compounds and water-soluble carotenoids found in the stigmas of saffron, composed of their glycosides [28-30, 35]. This substance is the main factor for the bitter taste of bile and may be involved in the production of safranal [36].

Monaghan and Schmitt first described the antioxidant potential of vitamin A and carotenoids [37]. This is important, since, carotenoids are the precursors of vitamin A. It is thought that some of their specific functions, closely related to their functions in plants, are also effective in mammalian tissues [38]. α-tocopherol is a potent peroxyl (ROO-) radical scavenger, a chain-breaking antioxidant that prevents the propagation of free radical damage in biological membranes [39]. Due to this and similar studies, it is now a fact that vitamins such as α-tocopherol and carotenoids (including ß-carotenoid, the precursor of vitamin A) have antioxidant capacity. Although, the possible effects of vitamin K and D on oxidative stress have received little attention, their roles or deficiencies in the antioxidant defense system are poorly understood [40]. However, studies have identified vitamin D receptors in various tissues. Therefore, it has been suggested that vitamin D may play a role in cardiovascular, multiple sclerosis, hypertension, colorectal and prostate cancers, diabetes risk and cancer prevention [41, 42]. On the other hand, vitamin K is fat-soluble compound required for the post-translational conversion of protein-bound glutamates to γ-carboxyglutamates in various proteins, apart from its function in coagulation. Studies on the effect of vitamin K on protein carbonyl have been reported [35]. The 1,4-naphthoquinone structure of vitamin K is similar to the benzoquinone structure, and therefore, these vitamins may also contain antioxidant properties [43]. Conversely, dietary vitamin deficiency or insufficiency affects changes in serum protein levels. Serum vitamin levels may provide important parameters in neutralizing free radicals. When living tissue is under stress, PCO can be found in higher amounts due to the increase in the chain reaction [44]. The decrease or increase in both PCO and vitamin levels may give clues about the effect of antioxidants.

When the retinol, cholecalciferol, α-tocopherol, and phylloquinone levels were examined according to the results of the statistical analysis in the study, retinol, cholecalciferol and phylloquinone levels, a significant decrease was determined between CCl_4 and control group ($p<0.05$, $p<0.01$, $p<0.05$) and between CCl_4 and olive oil group ($p<0.01$, $p<0.001$, $p<0.05$), respectively. In addition, a significant decrease was observed in retinol and cholecalciferol values between CCl_4 and saffron group ($p<0.05$, $p<0.001$), and a significant decrease in cholecalciferol and phylloquinone levels between CCl_4 and safranal groups ($p<0.01$, $p<0.05$). However, there was a significant decrease ($p<0.05$, $p<0.05$) in cholecalciferol levels between olive oil and CCl_4 + crocin and saffron and CCl_4 + crocin groups. As it is known, vitamins A, D, E and K are stored in the liver and fatty tissues. It allows the body to benefit from these stored reserves when insufficient dietary intake. These vitamins are essential for antioxidant defense and many other functions [45]. The study conducted by Ynaci et al., determined that α-tocopherol was effective in healing liver damage due to CCl_4 application to rats [46]. In addition, against the liver damage caused by the increased release of ROS, Elsisi et al., in their study, found that liver damage was blocked in rats treated with 250,000 IU/kg/day retinol against 0.15 or 2 ml/kg CCl_4 challenge [47]. On the other hand, in the study conducted by Forbes and Taliaferro, they concluded that diet-fed rats were more resistant to the hepatotoxic effects of carbon tetrachloride than animals fed a well-balanced stock diet or a low vitamin D but otherwise balanced diet [48]. Our study evaluated the vitamins and PCO levels of saffron and its active ingredients against CCl_4

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challenge without any external vitamin supplements in rats. It was observed that oxidative stress increased in rats exposed to CCl_4 and, in parallel, PCO, retinol, cholecalciferol, α-tocopherol and phylloquinone levels increased. The increased presence of vitamins along with PCO, a protein oxidation marker, indicates that these vitamins have antioxidant properties. However, more studies are needed on the antioxidant properties of these vitamins and their presence in oxidative stress. On the other hand, when the levels between the groups were examined, it was determined that 1 ml/kg CCl_4 + 100 mg/kg safranal group showed significant difference compared to the saffron and crocin groups. It can be said that this is due to the bitter taste and aroma of safranal and its positive benefits to the vitamins in the antioxidant defense system. Recent studies have shown that safranal is the part of saffron that determines its most important characteristic flavour [49]. It has been shown to have antioxidant effect due to its high radical scavenging activity [50].

Conclusion

Our study observed that oxidative stress increased in rats exposed to CCl_4 and, in parallel, PCO, retinol, cholecalciferol, α-tocopherol and phylloquinone levels increased. When the levels between the groups were examined, it was determined that 1 ml/kg CCl_4 + 100 mg/kg safranal group showed significant difference compared to the saffron and crocin groups. In this case, it can be said that the bitter taste and aroma of safranal are effective. As a result, saffron and safranal have a protective effect against the oxidative damage caused by CCl_4 on protein carbonyl, retinol, phylloquinone and cholecalciferol, and this effect may be due to the strong antioxidative effects of saffron and safranal. Saffron is an important plant that has been subject to application on laboratory animals. Its effective application in both medicine and alternative medicine has attracted the attention of many researchers. This attention is mostly due to the reporting of the effects of saffron on antitumor and anticancer. However, studies in which this plant and its active ingredients are applied together are limited. No study in the literature is close to the vitamins (A, D, E, K) and PCO values between the groups in our experimental study. In this respect, it can be said that this experimental study will contribute to the literature and attract attention.

Compliance with Ethical Standards

Ethical approval: This study was approved by Van Yuzuncu Yil University Animal Research Ethics Committee with the decision dated 25.12.2015 and numbered 2015/560.

Conflict of interest: The authors declare that there is no conflict of interest.

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Authors contributions: IA, IB and GO: Collected the material, AB and DY: Performed the experiments, AB: Analyzed and interpreted the results and wrote the paper, SE: performed the statistical analysis and supervised the manuscript. All authors approved the final version of the manuscript.

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