Evaluation of Antioxidant and Protective Role of Hawthorn Fruit Lyophilized Extract Against Carbon Tetrachloride Toxicity in Rat

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Carbon tetrachloride, Hawthorn Fruit, Protective and Antioxidant role

Abstract: This manuscript has aimed to investigate the nephro-hepato protective potential and antioxidant role of lyophilized extract of hawthorn (Crataegus orientalis L) fruit (LEH) against carbon tetrachloride (CCl₄) toxicity. The experimental were conducted as; 0.5 ml/kg CCl₄ bw, 100 mg/kg LEH bw, 200 mg/kg LEH bw, CCl₄ 0.5 ml/kg + 100 mg/kg LEH bw and CCl₄ 0.5 ml/kg + 200 mg/kg LEH bw treated with the extract for 3 weeks. At the end of the experimental treatment, the nephro-hepato protective potential and antioxidant capacity of the LEH was evaluated measuring by liver and kidney damage serum biomarkers, antioxidant defense systems constituents (ADSCs) and malondialdehyde (MDA) content in the erythrocyte, brain, kidney and liver tissues of rats. According to results; liver damage serum enzymes activities and MDA content of the tissues were significantly higher in CCl₄ group compared to normal control (NC) whereas; these parameters were significantly lower in extract supplemented groups compared to CCl₄ group. According to the results, the study results suggest that the LEH supplementations diet restored most of the parameters towards the NC values with fluctuations in the ADSCs. Therefore, it is thought that the extract of hawthorn has antioxidant capacity and hepatoprotective effects against in CCl₄-intoxicated rats.

Alıç Meyvesi Liyofilize Ekstraktının Sıçanlarda Karbon Tetraklorür Toksisitesine Karşı Antioksidan ve Koruyucu Rolünün Değerlendirilmesi

Anahtar Kelimeler: Karbon tetraklorür, Alıç meyvesi, Koruyucu ve antioksidan rolü

Öz: Bu makale, karbon tetraklorür (CCl₄) toksisitesine karşı alıç (Crataegus orientalis L.) meyve liyofilize ekstrakti (LEH), böbrek ve karaciğer koruyucu potansiyelli ve antioksidan rolünü araştırmayı amaçlamaktadır. DeneySEL dizayn; Normal kontrol (NK) CCl₄ grubu (0,5 ml / kg CCl₄, vücut ağırlığı (wb)), LEH1grubu (100 mg / kg LEH bw), LEH4 grubu (200 mg / kg LEH bw), CCl₄+LEH1 grubu (0,5 ml / kg + 100 mg / kg LEH bw) ve CCl₄+LEH4 grubu (0,5 ml / kg + 200 mg / kg LEH bw) şeklinde 3 haftalık ekstre muamelesi ile yapılmıştır. DeneySEL muamelenin sonunda: LEH'in böbrek ve karaciğer koruyucu potansiyeli ve antioksidan rolünü, karaciğer ve böbrek hasarı serum biyobelirteçleri, eritrosit, beyin, börek ve karaciğerdeki antioksidan savunma sistemleri bileşenleri (ASSB) ile malondialdehit (MDA) içeriği ölçülerek değerlendirildi. Sonuçlara göre; karaciğer hasarı serum enzim aktiviteleri ve dokuların MDA içeriği CCl₄ grubundaki NK göre anlamlı olarak daha yüksekti. Bu parametreler, ekstre takviyeli gruplarda CCl₄ grubuna kıyasla önemli ölçüde daha düşüktü. Sonuçlara göre, LEH takviyeli diyetinin ASSB'lerdeki dalgalanmaları NK değerlerine doğru parametrelerin çoğunun geri çektiğini göstermektedir. Bu nedenle, CCl₄ ile toksisite oluşturan sıçanlarda alıç bitkisi meyve ekstresinin antioksidan kapasiteye ve karaciğer koruyucu etkilere sahip olabileceğini düşünülmektedir.
1. INTRODUCTION

Many plants components are used in medicinal purpose for along time. Plant flavonoids are natural molecules of polyphenolic compound of plants. Due to varied effective biological activities and theirs low toxicity the healings effects of these polyphenolic compounds have made be subject of many scientific study. Researcher expres the that the plant based rich diet has consistently reduced the risk of several chronic diseases. It is reported that the constituents such as fruit, leave and flower of plants, which are responsible for free radical scavenging role, are especially epicatechin, hyperoside and chlorogenic acid. Also, a lot of studies showed that many aromatic and medical herbs constituents synthesize phytochemicals possessing antioxidant capacity and have been used as a natural product of free radical scavenging molecules [1,2]. Also, it has been suggested that many aromatic and medicinal plant spices contain a lot of biological active chemical molecules showing antioxidant role and the properties of herbs are attributed to bioactive phytocompounds as flavonoids, vitamins, phenols, carotenoids, alkaloids, phenolic acids and terpenoids [3]. Many researches support that oxidative damage to proteins DNA and lipids may contribute to the resulting of many illnesses such neurodegenerative, cardiovascular and cancer [4]. So what, it has been declared that the diet based on free radical scavenging role may be important in protection against to much illness [4,5].

Crataegus orientalis is belongs to Rosaceae family and it is estimated that Crataegus spp include 150 to 1200 species [6]. It had been reported that Crataegus spp. fruit is a rich source of vitamin C, flavanoids, tannin, glycoside, anthocynaidin, saponin and natural antioxidants molecules [7]. Hawthorn fruits are usually eaten fresh by local peoples. Also, the native hawthorn species fruits of are often have been used for the phytotherapy of weak heart disorder, and especially if the illness is accompanied with high blood pressure [8]. Further, it has reported that the essential constituents of hawthorn are organic acids, proanthocyanidins, flavonoids and some amines. Also, it is speculates that some C. orientalis may be good antioxidants. They are also among the best anti lipoperoxidants [9-11]. However, it has been reported that the consumption of C. orientalis also cause a case of multisystem hypersensitivity reaction and progressive acute renal damage to proteins DNA and lipids may contribute to the resulting of many illnesses such neurodegenerative, cardiovascular and cancer [4]. So what, it has been declared that the diet based on free radical scavenging role may be important in protection against to much illness [4,5].

2. MATERIALS and METHODS

2.1. Chemicals

Technical greate of chemicals used during this study were supplied from Sigma Chemical Co. (St. Louis, MO, USA). Kits for superoxide dismutase (SOD) and glutathione peroxidase (GPx) analysis were supplied by Randox Laboratories Ltd and Paraoxonase (PO1) was supplied from Rel Assay Diagnostics kit.

2.2. Animals

Thirty six Wistar albino rats with aged 3-4 months and an average weighing 150-250 g were provided from Van Yüzüncü Yıl University Experimental Animal Research Center, and The rats were placed in standard plastic rat cages and were adapted to the laboratory conditions and kept at 22 ± 2 °C in a 12-hour photoperiod during the experiment. The authors decelerate that they have followed EU standards regulations for the protection of animals during experiment and approved by The Local Ethics Committee of Experimental Animal with 24.03.2016 and 2016/03 protocol number.

2.3. Preparation of lyophilized extract of C. orientalis fruit

Briefly, C. orientalis fruit was supplied from a local producer in Edremit producing province of Van Turkey. Freshly harvested hawthorn fruit was washed with distilled water, and then the fresh hawthorn fruit were dried at room temperature (26±2 °C). The fruit material was powdered using blender. The aqueous extract of the plant fruit was prepared using the method described by Dalar and Konczak [14]. To obtain the lyophilized plant extract, the fruit aqueous extract was put into falcon tube and freeze-dried under 0.030 mBar a vacuum at -54 °C for 3 days.

2.4. Experimental Design

The rats used in this experimental study were divided into 6 groups which is one containing 6 rats.

2.4.1. Normal control (NC)

Nothing was applied to the group rats. The rats were fed only with standard rat feed and water as ad libitum.

2.4.2. 0.5 mL Carbon tetrachloride (CCl4)

This group rats were received i.p. injection of CCl4 in olive oil (1:1) at a dose of 0.5 mL /kg CCl4 and fed with standard rat feed and water as ad libitum. The dose of CCl4 was selected on the basis of a 0.5 mL/kg bw at which caused nephrotic, hepatic toxicity and oxidative stress [15].

Many plants flavonoids and natural polyphenolic components are currently used in medicinal treatment. Since little is known relatively about the therapeutic of the plant's molecules used in different purpose they have been studied extensively to determinate varied biological activities and their toxicity effect in the last time. The aim of this study was to elevate healings potential of C. orientalis against induced nephrotic and hepatic toxicity with CCl4 by evaluating protective capacity as liver and kidney damage serum biomarkers and ADSCs in the brain, kidney and liver tissues of rats.
2.4.3. 100 mg/kg bw, LEH (LEH1)

This group rats were fed only with standard rat feed and water as ad libitum and were received with the extract supplementation (100 mg/kg, bw) by an oral gastric gavage per day for 21 days.

2.4.4. 200 mg/kg bw LEH (LEH2)

This group rats were fed only with standard rat feed and water as ad libitum and were treated with the extract supplementation (200 mg/kg, bw) by an oral gastric gavage per day during 21 days.

2.4.5. 0.5 mL CCl4 /kg bw + 100 mg/kg bw LEH (CCl4+ LEH1)

This group rats were received i.p. injection of CCl4 in olive oil (1:1) at a dose of 0.5 mL/kg CCl4 and were treated with the extract supplementation (100 mg/kg, bw) by an oral gastric gavage per day during 21 days.

2.4.6. 0.5 mL CCl4 /kg bw + 200 mg/kg bw LEH (CCl4+LEH2)

This group rats were received i.p. injection of CCl4 in olive oil (1:1) at a dose of 0.5 mL/kg CCl4 and were treated with the extract supplementation (200 mg/kg, bw) by an oral gastric gavage per day during 21 days.

2.5. Preparation of Tissues Supernatant and Erythrocyte Pellets

The rats knocked out by injection of ketamine (10 mg/100 g, bw) intraperitoneally at the end of the 21 days experiment and sacrificed after the necessary samples are taken. Blood were taken from a cardiac puncture using syringe to determine of serum biomarkers as serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) enzymes, total cholesterol (TC), total protein (TP) creatinine (CRE) and urea levels. For preparation of erythrocytes packet: 1 mL of blood wit EDTA was taken to another test tube and 2 mL saline (0.9% NaCl) was added. Then they were centrifuged at 3000 rpm at +4 °C in a cooled centrifuge for 15 minutes to separate. After centrifugation, the resulted plasma was discarded from the erythrocytes packet, and each time an equal amount of saline (0.9% NaCl) was added to the remaining volume and this process was repeated three times. GSH level and MDA content was performed immediately in the erythrocyte packet. The remaining erythrocyte pellet was stored in the deep freeze at -80 °C until analysis. Meanwhile, the brain, kidney and liver tissues of rats washing the tissues with physiological saline (0.9% NaCl) were dissected and put in storage falcon tube at kept at -80°C during the analysis.

For preparation of supernatant extraction of tissues; briefly, 500 mg the tissue sample were weighed and 5 ml cold buffer containing 0.32 mol/L sucrose, 1mmol/L EDTA, 10nm/L Tris HCl (pH 7.4) was added. The tissues were thoroughly crushed with glass baguette and homogenized for 3 minutes in ultrasonic homogenizer. The homogenate was immediately centrifuged for 30 minutes at 9500 rpm at +4 °C. The clear supernatants from obtained the tissue were used for analysis of ADSCs such as Paraoxonase (PON1) Catalase (CAT) Superoxide dismutase (SOD) Glutathione peroxidase (GPx) Glutathione S-transferase (GST) activities, Reduced glutathione (GSH) level and Malondialdehyde (MDA) content [16-18].

2.6. Biochemical Analysis

MDA contents was determined using the method described by Jain et al [19] based on TBA reactivity. GSH levels were measured using the method described by Beutler et al [20]. GST was assayed as described by Mannervik & Guthenberg [21]. GPx was assayed based on that of the rate of the oxidation of glutathione by cumene hydroperoxide [22]. SOD activity was measured by calculating inhibition percentage of formazan dye formation at 505 nm [23]. CAT activity was measured using the method described by Aebi [24]. PON1 activity was measured by reading the absorbances with a spectrophotometer (Genesys 10 UV/Vis Spectrophotometer; Shimadzu) using kits (Rel Assay Diagnostics kit; Mega Tıp) [25]. On the other hands, serum biomarkers releate to liver and kidney damage as AST, ALT, LDH enzyme activities, TC, TP CREA and urea levels were measured by an auto analyzer (BM/HITACHI-911) using the kits.

2.7. Analysis of Data

The statistical analyses were made using the Minitab 13 program for MS Windows. One-way analysis of variance (ANOVA) statistical test was used to determine the differences between means of the experimental groups accepting the significance level at \( p \leq 0.05 \). All statistical data were expressed as mean ± standard deviation (SD).

3. RESULTS and DISCUSSION

Following the experimental treatment, nephro-hepato protective capacity and antioxidant activity of the LEH supplemented diet against to the toxicity of CCl4 were evaluated by liver and kidney damage index of serum biomarkers, ADSCs and MDA content of the rat tissues. According to the obtained data, liver damage index of serum biomarkers such as AST, ALT and LDH levels of CCl4 group were significantly increased as compared with the control group whereas these biomarkers levels of LEH supplementation groups resulted in a signifanse decrease (Table I). With regard to ADSCs changes, while CCl4 caused fluctuations in ADSCs by oxidative stress condition in the rats, the treatment of the extract supplementation restored the CCl4 induced and fluctuated antioxidant system towards near normal particularly in the tissues of rats. In addition, the imbalance between increased MDA content of tissues due to oxidative stress induced by CCl4 in the all tissues was found to be decreasing in the tissues the extract treated groups (Table II).
Today, many natural products molecules of plants have been using rather than synthetic drugs as medicinal treatment agents. It is thought that the preventive molecules in plants protect against harmful physical and chemical environmental affects by via strengthen the body's defense system due to have mainly properties of antioxidant, antioxidant and anticancer substances. Therefore, to changing of the synthetic food additives with natural antioxidants is also increasing the recent efforts towards plant treatments. Also, this situation is resulting from functional foods content through their specific components and therapeutic effect. Hence, as C. orientalis prevention role, under the hepatoprotective effects and chemopreventive, can be considered as liver and kidney damage serum biomarkers as serum AST, ALT and LDH enzymes activities, TP, TC, CRE and uric acid levels. With regard to antioxidant capacity, it can

<table>
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<th>Table I: Effect of CCl4 and C. orientalis fruit aqueous supplementations on body weight, food and water intake and serum biomarkers</th>
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<td>Parameters</td>
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| Food and water intake (Day)                   |         |           |           |           |                |                |
| Food intake (g)                               | 19.2±1.9 | 15.7±2.7  | 18.6±1.5  | 17.8±1.6   | 13.7±2.8       | 14.8±3     |
| Water intake (mL)                             | 31.4±3.3 | 23.5±3.5  | 33.2±3.4  | 31.7±3.8   | 24.3±4.5       | 25.4±6     |

| ALT (U/L)                                    | 27.50±2.42 | 31.50±2.34 | 25.33±4.84 | 28.50±1.87 | 25.83±2.31     | 28.67±13    |
| AST (U/L)                                    | 143.83±12.29 | 189.33±3.01 | 143.33±7.38 | 169.17±2.79 | 140.50±3.62   | 122.17±3.06  |
| UREA (mg/dL)                                 | 53.17±5.19 | 48.67±4.1  | 41.2±6.1  | 43.17±2.40 | 46±16   | 45.50±3.73    |
| CRE (mg/dL)                                  | 0.49±0.05 | 0.50±0.08  | 0.46±0.05 | 0.50±0.07  | 0.43±0.04  | 0.49±0.04    |
| LDH (U/L)                                    | 128.83±35.72 | 2508±99.30 | 1646.67±54.87 | 1521.67±163.58 | 2275±270.71  | 1835±52.44   |

Significantly different from beginning. *: Groups are different significantly from control. **: Groups are different significantly from CCl4

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<th>Table II: Effect of CCl4 and C. orientalis fruit aqueous extract supplementations on lipid peroxidation and antioxidant defense systems constituents</th>
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Significantly different from beginning. *: Groups are different significantly from control. **: Groups are different significantly from CCl4

*: Groups are different significantly from control. **: Groups are different significantly from CCl4
consider as ADSCs and MDA content of the various tissues samples.

As shown in the Table I, CCl₄ caused significantly an increase in the levels of AST, ALT, and LDH activities as compared with the control rats whereas the plant fruit extract supplementations diets caused a significant decrease in these biomarkers in comparison to CCl₄ group rats. The reasons for such effect of CCl₄ and the plant fruit extract were not certainly understood based on the present data. However, it is known that liver damage biomarkers such as AST, ALT and LDH have been considered as indicators of the hepatic cell dysfunction and degeneration. Further, the increase of LDH, AST and ALT activities in serum is estimate mainly due to the permeate of these enzymes from the hepatocytes cytosol into the blood stream by hepatocellular destruction or necrosis occured in liver [26]. Therefore, these data indicate that while CCl₄ might have lead to the inducing of the enzymes into plasma because of autolytic breakdown or hepatic necrosis, the plant fruit extract supplementation posses protection against CCl₄ induced liver injury that may result in development of liver damage.

On the other hands, the tissue samples as antioxidant capacity as efficiency indicator can be considered from the antioxidant enzymes GST, PON1, CAT, SOD, GPx, activities and GSH and MDA content. The present study demonstrated that the extract could have antioxidative role in rats. This was resulted from that the obvious of the MDA concentration in the tissues of the hawthorn fruit extract supplemented groups lower than CCl₄ group. According to the obtained results, the MDA content in the tissues of CCl₄ was a significant as competed to control group whereas the MDA contents of the plant fruit extract supplementation group significantly decreased in compared to that of CCl₄ group (Table II). The obvious such effect of CCl₄ and the hawthorn extract additions diet are not exacty understood at the moment. However, it can be say that the increasement of MDA content in the CCl₄ group might have a resulting of the increased reactive oxygen system (ROS) as a result of oxidative stress condition caused by CCl₄ intoxication whereas the hawthorn extract supplementation diet have posses protection the role against CCl₄ induced ROS. Previous studies accordance with our results had been showed that CCl₄ is hepatocellular destruction or necrosis occured in liver or damage causing in vital organs like liver [27-30]. The protective and antioxidantant properties of the plant fruit extract may be attribute to active phytochemicals such as terpenoids, flavonoids, vitamins, simple phenols, carotenoids, lignans, alkaloids and phenolic acids [3]. Also, the excessive production of ROS causing by CCl₄ on liver intoxication might have been provoke a severe increase of MDA content in the tissues. [31]. It have been reported that the serious over production of ROS such as singlet oxygen and H₂O₂ as a resulting liver intoxication of consumption of some xenobiotics can be easily converted to reactive 'OH radical by different mechanisam. Furthermore, it is known that severe reactive 'OH radicals can initiate lipid peroxidation in tissues and MDA, which is a major peroxidized product of polysaturated fatty acids [4]. On the other hands, it is known that the increasement of MDA level, final product of lipid peroxidation, is an major indicator of lipid peroxidation [32]. With regard to antioxidant capacity of the plant fruit extract, the enzymes activity and GSH level were observed as fluctuate at significant levels in the CCl₄ group whereas the administration of the extract supplementations also restored the the fluctuated ADSCs in the tissues to near normal levels. The such effect of functions of plant fruit extract supplemented diet are not exactly state of putting forward at the now. But, ROS as a result of oxidative stress condition caused by CCl₄ intoxication might have been induced the the ADSCs in the rats tissues during exposure to CCl₄. Further, the fluctuation of ADSCs may reflect an adaptive change against CCl₄ induced ROS toxicity [31]. The increase of ADSCs are known to serve as protective responses to eliminate xenobiotics intoxication too [33]. Therefore, the existence of induce of ADSCs might have been a result of an adaptation of organisms too. Also, the reasons for such effect might have been due to antioxidant capacity of the plant fruit extract supplementations [7,9-11].

4. CONCLUSION

The study showed that the CCl₄ exposition gave rise to a significant increase of serum liver damage biomarkers, lipid peroxidation and fluctuate ADSCs in rat. But, the administration of the supplemented extract diet restored to normal the levels of serum enzymes, the fluctuated ADSCs and the increased MDA content. The data obtained by this survey may be concluded that there is a protective feature, and has antioxidant activity of the plant fruit extract nutrition in the rats. In spite of everything, the results recommends that systematic intake of the functional food may be useful for the prevention of chronic degenerative liver diseases.

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