Original Article

Nephroprotective Hepatoprotective Potential and Antioxidant Role of Carob Pods (*Cerotonia siliqua* L.) against Carbon Tetrachloride-induced Toxicity in Rats

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ABSTRACT

Objective: The aims of current study are the evaluation of the protective effect and antioxidant role of carob pods against carbon tetrachloride (CCl_4)-induced oxidative stress, hepatotoxicity and nephropathy. **Material and Methods:** The present experiment was designed as I (control), II (0.5 ml/kg CCl_4), III (%10 CP), IV (CCl_4 0.5 ml/kg + %10 CP) groups. While rats in group I and III were fed with a diet without CCl_4, II, and IV groups received twice 0.5 ml/kg/week, where IV group additionally received %10 CP supplementation for 50 days. The protective roles and antioxidant activity of the CP supplementation feed against CCl_4-induced oxidative stress and toxicity were evaluated by histopathological changes, measuring hepatic and renal damage biomarkers (HRDB), antioxidant defence system constituents (ADSC) and malondialdehyde (MDA) parameters in the erythrocyte, liver, brain, kidney and spleen tissues of rats. **Results:** According to the results, the biochemical analysis showed a considerable increase in the serum AST (aspartate aminotransferase), ALT (alanin aminotransferase), GGT (gamma glutamyl transpeptidase) and LDH (lactate dehydrogenase) enzymes, creatinine and urea, and decrease in the group II as compared to that of I group. On contrary, such parameters were decreased in IV group as compared to that of group II. In addition, the results showed that CP supplementation diet restored the CCl_4 induced MDA (malondialdehyde) and ADSC towards to control. The hepatoprotection of CP is further substantiated by the almost normal histological findings in IV group against degenerative changes in II group. Protective effects by CP are further substantiated by the almost normal HRDB for kidney and liver in IV treated group as against degenerative changes in the II treated rats. **Conclusion:** The results indicated that CP could be as an important as diet-derived antioxidants in preventing oxidative damage in the tissues by reducing the MDA or inhibiting the production of CCl_4-induced free radicals and liver and kidney destruction.

Key words: Carbon tetrachloride, Carob pods, Protective potential, Antioxidant role.

INTRODUCTION

There is a growing interest of natural products in human diet, both due to the possible negative effects of synthetic food additives on human health and the increased consumer perception of this problem in recent years.[1] Epidemiological studies consistently show that increased consumption of plant-based, antioxidant-rich foods, i.e., fruits, vegetables, whole grains, and nuts, is associated with the reduced risk for several chronic diseases.[2] Biomolecules from plants have attracted a great deal of attention, mainly concentrated on their role in preventing diseases. In addition, epidemiological studies have consistently shown that there is a clear significant positive association between intake of these

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natural products and reduced rate of heart disease mortalities, common cancers and other degenerative diseases.[3] The carob tree, an evergreen plant, is generally grown at Mediterranean region. Per year, about 13500 Tons CP are produced in Turkey.[4] The carob includes pod and seed. Carob pods have been generally consumed as human food and animal feed. The importance of carob arises from its seed. Nowadays, carob seeds have been paid attention on and they have acted an important role economically since they are used as food additives, in pharmaceutical and cosmetic industries.[5-7] *In vitro* antioxidant activities of CP have been examined by Fe^{+3} reducing, radical scavenging power activity[8] and by oxidation of linoleic acid.[9] Carob seeds are a particularly rich source of complex polymers of flavonoids such as proanthocyanidin, ellagitannin, and gallotannin. These phytochemicals have been used in medicine for its pharmacological properties against numerous diseases and have free radical scavenging activities.[10] Further, it was concluded that carob seeds has a hepatoprotective effect and antioxidant capacity in rats with ethanol toxicity, probably acting by promoting the antioxidative defense systems.[1] Carob pulp increases the blood glucose values after digestion in healthy subjects. Specially, it has reported that 5 h after consumption of 20 g carob flour decreased serum triacylglycerol levels.[11] In addition, Carob fibers extract inhibiting DNA synthesis inhibits cell proliferation (*in vitro*) by adenoma suppressing to adenocarsinoma.[12]

As far as our literature survey could ascertain, little studies have so far been reported on the hepatoprotective and nephroprotective role and antioxidant capacity of the CP supplementation. The objective of this study was to determine healthful potentials of CP against CCl_4-induced oxidative stress and toxicity by evaluating their *in vivo* hepatoprotective and nephroprotective role and antioxidant role. Thus, in the present study, it was extensively studied the antioxidant activity chemopreventive of CP using *in vivo* models. For this aim, the treatment of CP was done orally as food containing CP 10% because the effect of the functional plant represents a well characterized in nutrition and widely used as consumption by human in our country Turkish folk medicine. The serum HRDB such as AST (aspartate aminotransferase), ALT (alanin aminotransferase) GGT (gamma glutamyl transpeptidase) LDH (lactate dehydrogenase) TP (total protein) TC (total cholesterol) and histopathological changes were chosen due to their importance as index of hepatotoxicity and nephropathy. In addition, It was determined the effects of carbon tetrachloride (CCl_4) and CP supplementation on some phase II detoxification ADS (antioxidant defence systems) such as GSH (reduced glutathione), GR (glutathione reductase), SOD (superoxide dismutase), GST (glutathione *S*-transferase), CAT (catalase), GPx (glutathione peroxidise) and MDA (malondialdehyde) parameters in the erythrocyte, liver, brain, kidney and spleen tissues of rats 50 days during experiment.

MATERIALS AND METHODS

Chemicals

Thiobarbituric acid (TBA), butylated hydroxytoluene (BHT), trichloroacetic acid (TCA), ethylenediaminetetraacetic acid (EDTA), reduced glutathione (GSH), metphosphoric acid, 5,5' dithiobis-(2-nitrobenzoic acid) (DTNB), trihydroxymethyl aminomethane (Tris), 1-chloro-2,4-dinitrobenzene (CDNB), oxidized glutathione (GSSG), β-Nicotinamide adenine dinucleotide phosphate (NADPH), potassium dihydrogen phosphate (KH_2PO_4) and sodium chloride (NaCl) of technical grade used in this study were supplied by Sigma Chemical Co. (St. Louis, MO, USA). Kits for antioxidant enzymes analysis were supplied by Randox Laboratories ltd.

Animals

Rats (*Wistar albino*) with an average weighing 200-300 g were provided from the Experimental Animal Research Centre, Yuzuncu Yil University, and were housed in 4 groups, each group containing 6 rats. The animals were housed at 20°±2°C in a daily light/dark (~16/8) cycle. All animals were fed a group wheat-soybean-meal-based diet and water *ad libitum* in stainless cages, and received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institutes of Health. The ethic regulations were followed in accordance with national and institutional guidelines for the protection of animal welfare during experiments. This study was approved by The Ethic Committee of the Yuzuncu Yıl University.

Preparation of food

CP (*Cerotonia siliqua* L.) was provided from a tree local producer in Fethiye, Köycegiz, and a major CP producing province of Muğla-Turkey. The natural CP is a seller local market. CP was ground into powder and then the amount of powdered CP was adjusted to 10% of the rat food.

Experimental design

The rats were randomly divided into four groups each containing six rats.

Group I (Control): the rats received tab water and fed with standard pellet diet as *ad libitum*.

Group II (CCl_4): the rats received 0.5 ml CCl_4/kg rat weight intraperitonally and fed with standard pellet diet as *ad libitum*. Dose of CCl_4 was selected on the basis of a 0.5 ml CCl_4/kg rat weight diluted in olive oil (1:1 dilution) intraperitonally injection twice per week concentration at which caused oxidative stress and hepatotoxicity administered.[13]

Group III (10% CP): the rats received tab water and fed with 10% CP containing diet supplementation.

Group IV (0.5 ml CCl4/kg BW + 10% CP): the rats received 0.5 ml CCl_4/kg rat weight intraperitonally injection twice per week and fed with 10% CP containing diet supplementation.

Preparation of tissues supernatant and erythrocyte pellets

At the end of the 50 days experiments, the rats were anesthetized by injection of ketamine (5 mg/100 g body weight) intraperitonally. The blood samples were obtained from a cardiac puncture using syringe for the determination of serum HRDB levels and biochemical analysis. The serum samples were obtained by centrifuging blood samples at 4000xg for 15 min at 4°C, and enzyme levels were measured in these serum samples. For biochemical analysis, blood samples were immediately put into silicon disposable glass tubes with EDTA as an anticoagulant and were centrifuged at 4000xg for 15 min at 4°C and erythrocyte pellets were obtained. Then, the pellets were washed tree times with physiological saline (0.9 % NaCl).

The tissues (brain, kidney, spleen and liver) were dissected and put in Petri dishes. After washing the tissues with physiological saline (0.9% NaCl), samples were taken and kept at -78°C during the analysis. The tissues were homogenized for 5 min. in 50 mM ice-cold KH_2PO_4 solution (1:5 w/v) using stainless steel probe homogenizer (20 KHz frequency ultrasonic, Jencons Scientific Co.) for 5 min. and then centrifuged at 7000xg for 15 min. All processes were carried out at 4°C. Supernatants and erythrocyte pellets were used to determine ADS constituents and MDA contents.[14,15,16]

Biochemical analysis

The erythrocyte and tissues MDA (Malondialdehyde) concentration were determined using the method described by Jain *et al.*[36] based on TBA reactivity. The erythrocyte and tissues GSH (reduced glutathione) concentration was measured using the method described by Beutler *et al.*[17] GST (glutathione *S*-transferase) was assayed by following the conjugation of glutathione with 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm as described by Mannervik and Guthenberg.[18] GR (glutathione reductase) activity was assayed according to Carlberg and Mannervik[19] as the decrease in absorbance of NADPH at 340 nm. GPx (glutathione peroxidise) activity was assayed according to Paglia and Valentine[20] based on that the GPx catalyses the oxidation of GSH by cumene hydroperoxide. SOD (superoxide dismutase) activity was measured at 505 nm by calculating inhibition percentage of formazan dye formation.[21] CAT (catalase) activity was determined using the method described by Aebi[22] based on that of the rate of H_2O_2 consumption and as the decrease in absorbance at 240 nm.

Measurement of enzyme levels

Hepatic and renal damage biomarkers levels such as such as AST, ALT, GGT, LDH, total cholesterol (TC), total protein (TP) creatinine and urea levels were measured by an auto analyzer (COBAS 8000/ROCHE/ Germany/Serial No 1296-08) using the Roche kits.

Analysis of data

All data were expressed as mean ± standard deviation (SD). The statistical analyses were made using the Minitab 13 for windows packet program. Means and Standard deviations were calculated according to the standard methods for all parameters. 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 $p \leq 0.05$.

Histopathological examination

Tissue samples from organs were collected in 10% neutralized formaldehyde after sacrification. After fixation, samples were dehydrated in alcohol, cleared in xylene and embedded in paraffin wax. Sections were cut at 5µm and stained with haematoxylin and eosin (Thermo Shandon, 15275, USA). Microscopically, degenerations in livers and kidneys were graded. Hepatocellular degeneration in livers as follows; Slight (degree 1): Mild hepatocellular swelling due to hydropic degeneration and fatty changes only in centrilobular areas. Moderate (degree 2): Clear hepatocellular swelling in both centrilobular and midzonal areas. Severe (degree 3): Diffuse and severe hepatocellular swelling, cytoplasmic paleness and rupture.[23]

RESULTS

Effects of carob pods supplementation on body weights and hepatic and renal damage biomarkers

Hepatic and renal damage biomarkers levels such as AST, ALT, GGT, LDH, TC, TP, creatinine and urea levels as

well as body weight of control, CCl_4, CP-treated and CCl_4+CP-treated groups rats were summarized in Table 1. The dosage of CCl_4 and CP supplementation did not show any mortality in all groups during experimental period. Body weights of the rats increased in all groups. Present results showed that levels of hepatic and renal damage biomarkers except for TC and TP were markedly elevated in CCl_4-treated group in comparison to control group. By contrast, levels of these biomarkers were prominently dropped in CCl_4+CP-treated group with respect to CCl_4-treated group. In addition, there was a significant difference in GGT and LDH levels between control and CCl_4+CP-treated groups (Table 1).

Effects of carob pods supplementation on antioxidant defence system constituents and malondialdehyde

Following the treatment of experimental groups, the effect of CCl_4 and the CP (carob pods) supplemented diet on oxidative stress were evaluated as ADS (antioxidant defense system) constituents and MDA content of blood and erythrocyte, liver, brain, kidney and spleen tissues of rats. The study results showed that CCl_4 significantly increased MDA content of the tissues in comparison to control whereas, MDA contents of the tissues significantly decreased in the dried CP supplementation group in comparison to CCl_4 group. On the other hand, the results of experiment showed that the treatment of rats with CCl_4 and CCl_4+CP supplementation caused changes in ADS constituents of the erythrocyte, liver, brain, kidney and spleen tissues in comparison to those of control rats. Namely, while CCl_4 caused fluctuation in ADS constituents level as a result of oxidative stress condition in the rats, CP supplementation diet restored the CCl_4-induced ADS constituents towards to control (Table 2).

Microscopic Findings

With regard to liver histopathological changes, the livers of the rats from control and 10% dried CP group had no noticeable histological changes (Figure 1,3). Evident histopathological changes were consistently observed in livers of all the rats from CCl_4 and CCl_4+the functional food treated group. In the second group of rats with CCl_4 group, severe hydropic degeneration in the liver in the portal area, a small number of necrotic cells and intra-hepatic cholestasis was found. In addition, the portal area region toward setral fibrosis in the group II was observed (Figure 2). These findings in the group IV are similar to group II findings (Figure 4).

Table 1: Effect of CCl_4 and carob pods on serum liver and kidney damage biomarker and body weight of rats

Parameters	Control		CCl_4	CP	CP+CCl_4	
Body weight (g)	Beginning	Finally	Beginning Finally	Beginning Finally	Finally	Beginning
Food intake (g/week)	424.5±50.2 459.0±58.0		401.5±26.2 278.5±2.1	439.0±86.3 327.0±9.9*	440.0±84.9	473.0±66.5
AST (U/L)	86.0±11,7		347.3±22.5[a] 206.2±29.0[a,b]		80.0±7.6	
ALT (U/L)	33.5±1.9		398.5±22.3[a] 249.7±29.2[a,b]		34.5±2.3	
GGT (U/L)	0.9±0.1		2.5±0.6[a] 1.2±0.2[a,b]		1.0±0.1	
LDH (U/L)	1092.5±77.6		1851,5±165.5[a] 1483.3±118.3[a,b]		1120.3±91.3	
TC (mg/dl)	48.5±6.6		37.5±6.5[a] 42.3±1.5[a]		59.0±6.2[a]	
TP(mg/dl)	5.9±0.4		5.5±0.5 5.6±0.4		6.3±0.3	
Creatinine (mg/dl)	0.40±0.10		0.52±0.12[a] 0.42±0.10[a]		0.40±0.11	
Urea (mg/dl)	36.7±2.5		44.3±5.3[a] 28.8±6.7[a,b]		29.3±2.8[a]	

Each value is the mean ± SD of 6 animals per group (One way ANOVA).
*: Significantly different from the beginning.
[a]: Significantly different from control group.
[b]: Significantly different from CCl_4 group.

Table 2: Determination antioxidant capacity of carob pods against CCl_4-induced oxidative stress

Tissue	Parameters	Control	CCl_4	CP	CP+CCl_4
Erythrocyte	GSH (mg/ ml)	75.21±0.13	72.88[a]±0.42	75.43±0.35	75.34[b]±0.34
	MDA (nmol/ml)	1.41±0.20	2.92[a]±0.52	1.40±0.29	1.96[a,b]±0.24
	GST U/ml	14.92±2.64	24.58[a]±4.67	16.00±2.14	16.91[b]±2.24
	GPx (U/ml)	176.25±30.29	160.88±17.05	177.03±20.06	167.61±22.19
	GR (U/ml)	2.41±0.56	2.13±0.48	2.24±0.55	2.35±0.51
	SOD (U/ml)	2105.46±90.08	2079.21±82.97	2089.11±54.13	2051.66±78.02
	CAT (U/ml)	232.91±32.91	244.98±45.54	238.70±37.74	267.60±53.83
Liver	GSH (mg/g)	71.20±2.93	67.63[a]±2.08	70.34±1.45	68.22±6.30
	MDA (nmol/g)	41.01±5.78	59.36[a]±5.56	41.34±6.89	48.05[a,b]±6.68
	GST (U/g)	84.47±8.37	108.14[a]±8.45	76.17±7.33	70.65[a,b]±5.22
	GPx(U/g)	141.55±15.80	114.45[a]±5.43	127.87±16.73	126.71[b]±18.85
	GR (U/g)	35.42±2.02	31.75[a]±2.25	35.55±3.19	33.09±3.29
	SOD (U/g)	2019.87±73.67	1983.59±761.36	1888.81[a]±50.45	2006.37±43.14
	CAT (U/g)	336.51±34.70	397.56[a]±57.60	338.74±33.35	322.36[b]±42.58
Brain	GSH (mg/g)	18.28±1.63	19.66±3.67	18.14±4.29	19.94±3.03
	MDA (nmol/g)	39.28±4.17	70.91[a]±8.51	39.50±4.75	37.69[b]±6.09
	GST (U/g)	57.23±10.24	72.95[a]±11.35	56.10±5.95	57.79[b]±5.66
	GPx(U/g)	163.57±30.46	154.79±21.86	165.37±36.19	160.81±17.51
	GR (U/g)	32.83±0.33	29.09[a]±3.17	33.07±2.22	31.47±2.83
	SOD (U/g)	2165.24±47.09	2161.02±32.72	2114.92±69.27	2117.61[a,b]±22.54
	CAT (U/g)	2.16±0.61	6.21[a]±1.36	2.65±0.50	2.58[b]±0.41
Kidney	GSH (mg/g)	70.52±1.77	59.10[a]±2.89	65.40[a]±4.87	63.45[a]±6.97
	MDA (nmol/g)	115.06±10.86	151.76[a]±12.32	118.94±15.29	158.28[a]±23.00
	GST (U/g)	38.53±4.64	61.40[a]±7.17	40.27±6.27	52.61[a,b]±4.58
	GPx(U/g)	157.70±12.05	145.07±18.72	152.99±32.95	149.70±24.63
	GR (U/g)	53.21±4.33	46.38[a]±3.30	52.46±1.49	51.76[b]±2.27
	SOD (U/g)	2147.03±44.03	2100.49±40.82	2145.76±28.33	2146.31±54.73
	CAT (U/g)	269.69±48.16	308.78±50.29	285.32±21.75	296.49±57.39
Spleen	GSH (mg/g)	72.75±2.54	73.98±1.55	69.63±3.59	73.44±0.69
	MDA (nmol/g)	158.67±25.60	199.90[a]±14.15	159.96±17.88	153.87[b]±19.73
	GST (U/g)	34.68±3.99	47.20[a]±6.38	37.43±4.55	36.67[b]±5.64
	GPx(U/g)	151.20±16.11	143.42±11.04	150.00±33.60	145.67±26.61
	GR (U/g)	12.10±0.60	12.94±2.37	11.96±1.63	12.06±0.68
	SOD (U/g)	2258.75±63.55	2179.14[a]±56.44	2218.86±43.06	2239.09[b]±26.95
	CAT (U/g)	20.28±1.39	34.43[a]±8.95	21.86±1.67	22.05[b]±1.32

Each value is the mean ± SD of 6 animals per group (One way ANOVA).
a: Significantly different from control group.
b: Significantly different from CCl_4 group.

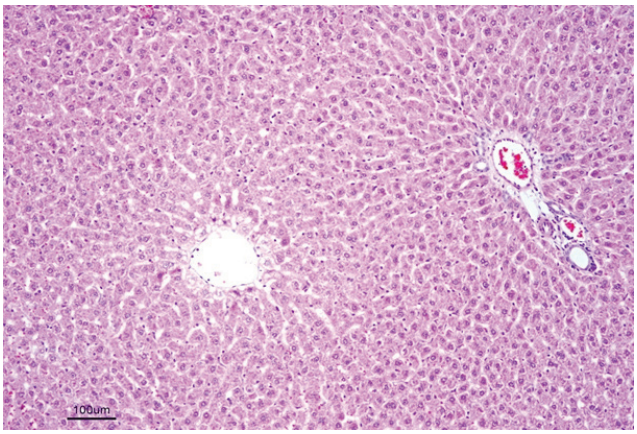

Figure 1: (control group): The liver of control group rats shows normal architecture of lobules (haematoxylin-eosin, Bar= 100μ)

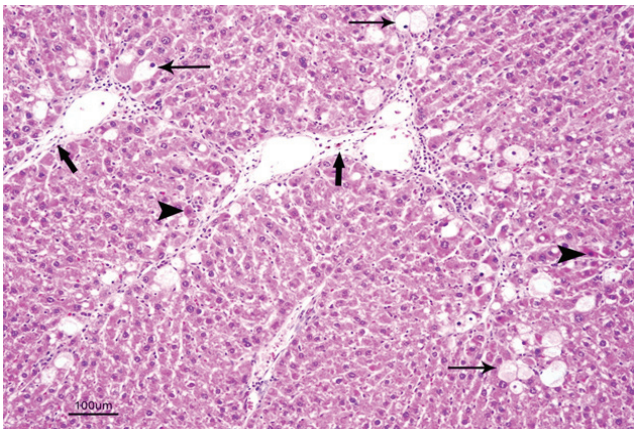

Figure 2: (CCl_4-treated group): Hydropic degeneration (fine arrows) and coagulation necrosis (arrow heads) in the hepatocytes, and fibrous bands extending from periacinar regions to parenchyma. (Haematoxylin-eosin, Bar= 100μ)

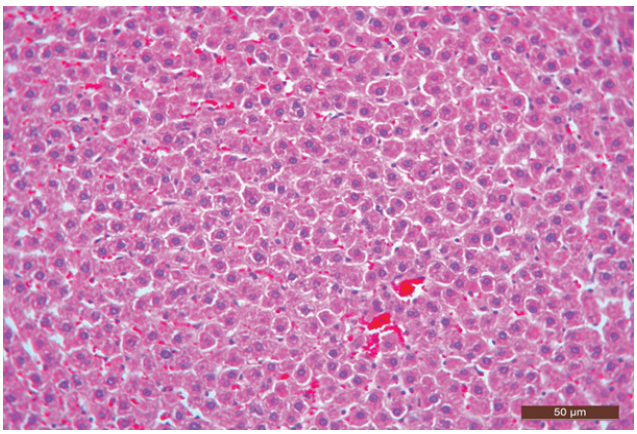

Figure 3: (CP-treated group): The liver of 10%dried fig group rats shows normal architecture of lobules (haematoxylin-eosin, Bar = 100μ)

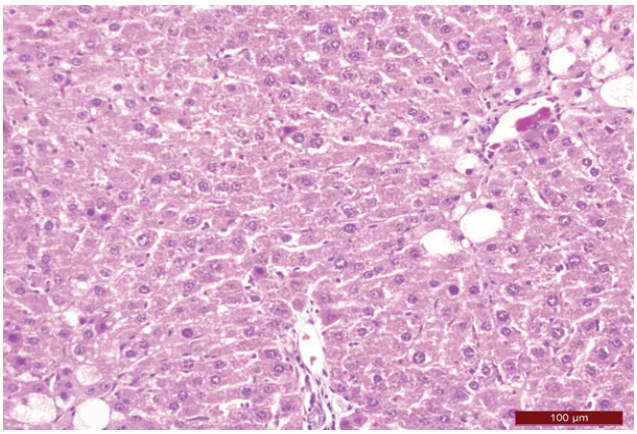

Figure 4: (CP + CCl_4-treated group): Note that there is no hydropic degeneration and coagulation necrosis in the hepatocytes, and fibrosis. (Haematoxylin-eosin, Bar = 100μ)

DISCUSSIONS

Today, the world of natural products rather than synthetic drugs continues to seek to use the power of treatment. Replace synthetic food additives with natural antioxidants are also increasing their efforts to change. Functional foods through their specific components or prophylactic or therapeutic effect are. Numerous studies have demonstrated that a great number of medicinal and aromatic herbs, as well as fruits and leaves of some berry plants, biosynthesis phytochemicals possessing antioxidant property and may be used as a natural source of free radical scavenging compounds.[24,25] Therefore, studies are needed to provide effective protection from the damaging agents and experimental studies have implicated the influence of a functional plant, CP in this regard. The first aim of this study was to investigate whether the CP supplementation could prevent CCl_4 toxic damages on the liver and kidney, decrease content of the MDA and efficacy on the antioxidant defense system and histopatholgical changes of liver in rats.

The results showed that CCl_4 caused a significant increase in the AST, ALT, GGT, and LDH levels in comparison to those of control rats whereas CP supplementation caused a significant decrease in these serum marker enzymes in comparison to those of CCl_4 treated rats. The reasons for such effect of CCl_4 and the CP supplementation were not certainly understood at present. However, it is known that several soluble enzymes in blood serum have been considered as indicators of the hepatic dysfunction and damage. Further, ALT and AST levels are also of value indicating the existence of liver diseases, as this enzyme is present in large quantities in the liver. ALT increases in serum

when cellular degeneration or destruction occurs in this organ.[26] The increase in plasma LDH activity may be due to the hepatocellular necrosis leading to leakage of the enzyme to the blood stream.[27] Thus, when CCl_4 may lead to the release of these enzymes into plasma because of autolytic breakdown or cellular necrosis, the CP supplement imparts protection against CCl_4 induced oxidative injury that may result in development of liver damage.

As shown in the Table 2, the present study demonstrated that the CP could have antioxidative role in rats. This was obvious from our observation that, by the consequence of additional CP treatment *in vivo* as the concentration of MDA in the tissues differed from that of CCl_4-exposed group. According to the obtained results, while MDA concentrations increased in the erythrocytes, liver, brain, kidney and spleen of rats treated with CCl_4, the MDA contents of the tissue significantly decreases in the CP supplementation group compared to that of CCl_4 group. The reasons for such effect of CCl_4 and the CP additions are not understood at the present. Nevertheless, the increased MDA content might have resulted from an increase of ROS as a result of stress condition in the rats with CCl_4 intoxication. Studies have shown that CCl_4 is potent liver toxicant and cause severe damage in vital organs like liver.[28,29] The excessive generation of free radicals in CCl_4 induced liver damage will provokes a massive increase of lipid peroxidation in liver.[30] On the other hand, it is known that the elevation of lipid peroxidation after the consumption of some xenobiotics and following superoxide over production that produce dismutation singlet oxygen and H_2O_2, can be easily converted later into the reactive ·OH. Both single oxygen and OH radicals have a high potential to initiate free radicals chain reactions of lipid peroxidation. Further, it is known that ·OH can initiate lipid peroxidation in tissues and MDA is a major oxidating product of peroxidized polyunsaturated fatty acids.[31] Increased MDA content is an important indicator of lipid peroxidation.[32] Meanwhile, SOD, GR, GPX GST and CAT activities and GSH levels were fluctuated at significant levels in the CCl_4-treated, generally as an increase whereas the administration of CP supplementation restored the CCl_4 induced imbalance between the fluctuated antioxidant system to near normal levels, particularly in erythrocytes, liver, brain, kidney and spleen. The reasons for such effect of functions of plant supplementation are not understood at the present. However, oxidative stress can affect the activities of protective enzymatic antioxidants in organisms exposed to CCl_4. The fluctuated ADS activities may reflect an adaptive change against CCl_4-induced ROS toxicity.[30] However, the increased activities of ASD are known to serve as protective responses to eliminate xenobiotics.[33] Thus, the existence of an inducible antioxidant system may reflect an adaptation of organisms. Also, the reasons for such effect of the CP addition may be due to antioxidant activity. CP supplementation treatment protected against lipid peroxidation and depletion of antioxidant enzyme activities. Previous studies have shown the riches of polyphenols in carob fruit.[34,35]

CCl_4 induced rats showed severe and common degenerative and necrotic changes. The pathological findings, observed in cases of CCl_4 toxicity are similar to previous reports.[28,29,30] These results of our investigations are in accordance with those of[28,29,30] who have reported that the CCl_4-induced hepatotoxicity and the potent effect of CCl_4 on the excessive generation of free radicals in CCl_4 induced liver damage will provoke a massive increase of lipid peroxidation in liver. The pathological our findings, observed in cases of CCl_4, are similar to the previous reports. CP treated rats did not showed significantly less histological abnormalities including hydropic degeneration, bile-duct proliferation and periportal fibrosis as compared with CCl_4-treated rats. Thereby, CP protective role in countering the hepatotoxicity induced by CCl_4 is arises. So far, no study examining the preventive role of CP supplemented food *in vivo* has been made on rat histological abnormalities, serum biomarkers, antioxidant defense systems and MDA content as a containing diet supplementation. Therefore, we had no chance to compare our results with the previous ones.

CONCLUSION

In conclusion, this study demonstrated that CCl_4 exposure gave rise to lipid peroxidation via inducing the ROS and had different effects on various tissues of rats on their antioxidant defense enzymes systems. This can result from adaptation of different qualities of cell physiology for different tissues. According to the data obtained by the survey of CP tissues of the liver, it can be concluded that there is a protective feature, and has antioxidant activity. CP has protective effects on liver, but to understand the molecular mechanism and nature of this process better and to reach a conclusion more studies are needed. The protective and anti-oxidant effect of CP on liver need to be studied more on the rats *in vivo*.

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AVAILABILITY OF DATA AND MATERIALS

Data are all contained within the paper.

AUTHORS' CONTRIBUTIONS

HS: designed the study and performed all the experiments. AD: designed the study and analyzed data. IC: analyzed data wrote the manuscript and supervised the study. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The ethic regulations have been followed in accordance with The National and Institutional guidelines for the protection of animal welfare during experiments. Ethical approval was obtained from the Animal Experiments Local Ethics Committee of Yuzuncu Yil University.

ABBREVIATIONS USED

CCl_4: Carbon tetrachloride; **CP:** Carob pods; **AST:** Aspartate aminotransferase; **ALT:** Alanin aminotransferase; **GGT:** Gamma glutamyl transpeptidase; **LDH:** Lactate dehydrogenase; **TP:** Total proteine; **TC:** Total cholesterol; **ADS:** Antioxidant defense systems; **GSH:** Reduced glutathione; **GR:** Glutathione reductase; **SOD:** Superoxide dismutase; **GST:** Glutathione *S*-transferase; **CAT:** catalase; **GPx:** glutathione peroxidase; **MDA:** Malondialdehyde.

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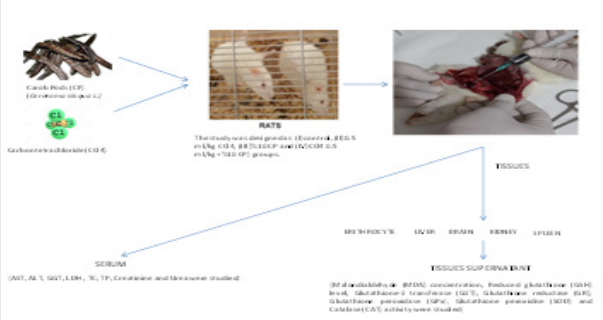
PICTORIAL ABSTRACT



SUMMARY

- Biochemical analysis showed a considerable increase in the serum hepatic and renal damage biomarkers in II group as compared to control group whereas decrease in the group IV with compared to II group.
- Carob pods has had a antioxidant role according to carob pods supplementation diet restored the CCl_4 induced malondialdehyde and antioxidant defense system constituents towards to control.
- The results indicated that carob pods could be as an important as diet-derived antioxidants in preventing oxidative damage. by reducing the production of CCl_4-induced free radicals.

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