Karabulut et al., J Plant Physiol Pathol 2014, 2:3
http://dx.doi.org/10.4172/2329-955X.1000130

Journal of Plant Physiology & Pathology

Research Article

A SCITECHNOL JOURNAL

Nutri-Protection and Mediterranean Diet: Bitter Apricot Kernel and Amygdalin Treatment Effects on a Battery of Oxidative Stress and Apoptosis Biomarkers

Aysun Bay Karabulut[1*], Yunus Önal[2], Mehmet Gül[3], Onder Otlu[1], Mehmet Tuzcu[4], Semir Gül[3] and Kazım Sahin[4]

Abstract

The role of nutrition against oxidative stress is increasingly debated in the literature. Much of the traditional food in Mediterranean diet are claimed to possess antioxidant properties but are in need of greater empirical support. This study was designed to test the putative protective effect of 3% and 5% bitter apricot kernel containing food (frequently consumed in Mediterranean diet) treated with amygdalin on apoptosis and oxidative stress in a preclinical model: carbon tetrachloride (CCl_4)-induced hepatic damage in Sprage Dawley rats. The animals (n = 64) were divided into eight groups as follows: (i) control, (ii) CCl_4; (iii) Amygdalin, (iv) Amygdalin and CCl_4, (v) Bitter Apricot Kernel (3%), (vi) Bitter apricot kernel (5%), (vii) CCl_4 +Bitter apricot kernel (3%), (viii) CCl_4 and bitter apricot kernel (5%). Chronic liver injury was induced by intraperitoneally administering carbon tetrachloride (CCl_4) (1 mg/kg body weight for 3 d at the end of 28 days) to rats. The area of liver injury was found significantly decreased with 5% bitter apricot kernel feeding. Serum AST, ALT, TOS activities and hepatic Bcl 2 and NFκB levels were elevated following CCl_4 administration. However, their activities were markedly reduced by supplementation with the bitter apricot kernel ($P < 0.05$). Serum TAS and hepatic Bax, Caspase 3, Nrf2 levels were decreased by CCl_4 admistration. However, they increased in the bitter apricot kernel group versus CCl_4 groups. Histopathological examination revealed massive necrosis in the centrilobular area and degenerative changes caused by CCl_4 were ameliorated by dietary supplementation with bitter apricot kernel concentrates. These results suggest that bitter apricot kernel concentrates have hepatoprotective effects and may improve the symptoms of liver injuries.

Keywords

Oxidative stress; Apoptosis biomarkers; Mediterranean diet; Nutrition

Introduction

Carbon tetrachloride (CCl_4), a well-known hepatotoxicant, has been widely used in investigation of chemical toxin-induced hepatic injury in a range of experimental models as they show lesions similar to human of liver diseases [1-3].These effects of CCl_4's toxic byproducts binding to cellular macromolecules such as DNA, lipids, proteins and carbohydrates initiating free radical-mediated lipid peroxidation and consequently leading to liver damage [4]. It has been indicated that natural compounds from various species of medicinal plants, herbs and spices exhibited strong antioxidant activity that could prevent from diseases, because they contain lots of bioactive compounds such as phenolic and flavonoid compounds [5,6]. Amygdalin is compenent of *Prunus armeniaca*, and these are abundant in the seeds of bitter almond and apricots [7]. It has been reviewed as the evidence for effect of amygdalin as prevention and treatment of cancers. Amygdalin belongs to a family of compounds called cyanogenic glycosides [8].

Transcription factors play an important role in cancer tumorigenesis and progression. Bcl 2, Bax, Caspase 3, NF-ƙB and Nrf 2; as apoptosis-related cysteine peptidase, cell survival genes and induce cell proliferation, and tumor metastasis are very important apoptosis related parameters [9,10]. It regulates gene expression in response to a variety of stimuli, including cytokines, growth factors, stress, and cancer [11,12].

Bcl2 acts as an anti-apoptotic gene to promote cell survival, whereas *Bax*acts as a pro-apoptotic gene to promote cell death [13] by regulating the expression of inflammatory response genes and inhibiting apoptosis of HSCs [14]. Similarly, the ubiquitous transcription factor NF-kB also plays a crucial role in the onset of hepatic fibrosis [15].

Apricot kernels are generally produced in Malatya as eastern region of Turkey exported to European countries and used especially in medicine, cosmetic and oil production [16]. Apricot kernels, particularly rich in lipid and protein, are potentially useful in human nutrition along with significant amounts of oil and fiber [17]. Amygdalin is a major constituent of apricot kernels, bitter almonds and peach, plum, pear and apple seeds [18]. It is reported apricot seeds and bitter almonds contain approximately 20-80 μmol/g and 100 μmol/g of amygdalin, respectively [19].

However, research on the ability of bitter apricot kernel to help protect the liver and improve liver function is insufficient. In the present study, we tested the ability of bitter apricot kernel to protect against liver injury and enhance hepatic function against CCl_4-induced hepatic damage in Sprague-Dawley rats. The activity of aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), Bcl 2, Bax, Caspase-3 NF-ƙ B and Nrf2 in liver tissue were measured in order to analyze the hepatoprotective effects of bitter apricot kernel.

Materials and Methods

Experimental animals

In this study, 64 female albino Sprage Dawley rats in Inonu

***Corresponding author:** Aysun Bay Karabulut, Department of Biochemistry, Faculty of Medicine, Inonu University, Malatya, Turkey, E-mail: aysun.karabulut@inonu.edu.tr

Received: June 26, 2014 **Accepted:** August 22, 2014 **Published:** August 26, 2014

University Animal Research Center, Malatya, Turkey, Rats were weighting between 200-250 g, housed in individual cages for 28 days in a well ventilated room with a 12: 12-hour light/dark cycle at 21°C. Animals were fed with standart rat chow and tap water ad libitum. The experiments were performed in accordance with the guidelines for animal research from the National Institute of Health and were approved by the Committee on animal Research at Inonu University, Malatya.

Experimental design

The rats were divided according to body weights (BW), which were similar, into eight equal groups, 8 each. The groups were as follows: Group (i), normal control rats, did receive 0.8 ml/kg/day olive oil and fed with standard rat diet. Group (ii), rats fed with standard rat diet and was administered with Amigdal in 50 mg/kg dose in drinking water. Group (iii), rats fed with%3 ratio Bitter Apricot Kernel, Group (iv), rat fed with% 5 ratio Bitter Apricot Kernel, Group (v), rats were induced with 2 ml/kg/s.c . doses of CCl4 doses was resolved 1:3 ratio with in olive oil; Group (vi) rats were induced 2 ml/kg/s.c. doses of CCl4 ; 1:3 ratio with in olive oil with 50 mg/kg dose in drinking water Amigdalin; Group (vii) 2 ml/kg/s.c. doses of CCl4 and feding with 3% ratio Bitter Apricot Kernel ; Group (viii) 2 ml/kg/s.c. doses of CCl4 and feeding with 5% ratio Bitter Apricot Kernel. Treatment was continued for 6 weeks. The apricot was supplied by Fruit of Research Institute Malatya, Turkey. After bitter apricot kernel was harvested, apricot kernels collected grinding rats were fed for 8 weeks. Inside of apricot core for amygdalin was subjected to extraction using ethyl alcohol. Soxhlet extraction apparatus specifically in the known extraction apparatus. Get here a mixture of ethyl alcohol in the environment using the heater ethyl alcohol was recovered by distillation. Rest on the addition of diethyl ether extracts of ice was amygdalin was allowed to precipitate in the form of white crystals.

Histological examination

Liver tissue samples were separated for histopathologic and biochemical examinations. Each liver sample was processed for light microscopic examination. The samples were placed in 10% neutral formalin for 24 h and prepared for routine paraffin embedding. Tissue samples were cut into 5 µm thick sections, mounted on slides, and stained with hematoxylin–eosin (H&E). Sections were examined by blind histologist in a light microscope (Leica DFC280, UK) and analyzed by the Leica Q Win Plus V3 Image Analysis system (Leica Micros Imaging Solutions, Cambridge, UK). The degree of inflammation was scored (0: absent; 1: mild; 2: moderate; 3: severe) and histopathologic damage (hepatic necrosis, intracellular vacuolization and vascular congestion) was expressed within each slide of liver sections, classified on a scale of 0–3 (0, normal; 1, mild; 2, moderate; 3, severe) with a maximum score of 12.

Laboratory analyses

For analyses, the frozen liver was thawed and homogenized gently for about 45 s in 1/10 volume of ice-cold 10 mM phosphate-buffered saline (PBS, pH 7.4) containing 1.15% KCl, and centrifuged at 800*g* to remove cell debris and nuclei. Supernatant was further centrifuged at 10.000*g* for 10 min and kept for analysis.

TAS, TOS and OSI index analysis

Liver tissue TAS and TOS were measured using a colorimetric method with commercially available kits (Rel Assay). The results were expressed as millimolar Trolox equivalent per liter (mmol Trolox equivalent/gr protein) for TAS and micromolar hydrogen peroxide equivalent per liter (µmol H_2O_2 equivalent/gr protein) for TOS. The ratio of TOS to TAS was accepted as the OSI. For the calculation, the resulting unit of TAS was converted to µmol/gr protein, and the OSI value was calculated according to the following formula: OSI (arbitrary unit) = TOS (mmol H_2O_2 equivalent/L)/TAS (mmol Trolox equivalent/L) [20].

Serum AST, ALT levels were measured with Abbott kits (Abbott Diagnostics, Abbott Park, Ill, United States) on the Abbott clinical autoanalyser (Architect c16000).

Western blot analysis

For Bax, Bcl-2, Caspase-3, NFkB and Nrf-2 expressing levels western blot analysis, liver was homogenized in PBS with protease inhibitor cocktail (Calbiochem, San Diego, CA, USA) and the protein concentration was quantitated. The sample (20 mcg of protein per lane) was mixed with sample buffer, boiled for 5 min, separated by SDS-polyacrylamide (12%) gel electrophoresis under denaturing conditions, and electroblotted onto nitrocellulose membrane. Nitrocellulose blots were washed in PBS and blocked with 1% bovine serum albumin in PBS for 1 h prior to application of the primary antibody. Primary antibody was diluted (1:1000) in the same buffer containing 0.05% Tween-20. The nitrocellulose membrane was incubated overnight at 4°C with protein antibody. Antibodies against Bax, Bcl-2, Caspase-3(19-kDa), NFkB and Nrf-2 expressing levels and β-actin were purchased from Santa Cruz Biotechnology Inc, CA, USA. The next day, the immunoreactions were continued with the secondary goat antirabbit horseradish-peroxidase-conjugated antibody after washing for 2 h at room temperature. Specific binding was detected using diaminobenzidine and H_2O_2 as substrates. Protein levels were analyzed densitometrically using an image analysis system (Image J; National Institute of Health, Bethesda, USA).

Statistical Analysis

Statistical analysis was done with SPSS 17.00.11.5 software. Normality test was done with Shapiro-Wilk method. One-way ANOVA test was used to determine the significance of biochemical parameters among the study groups. Tukey' test was used for multiple comparisons to estimate the difference between the two study groups. Multiple linear regression analysis was performed to evaluate the associations between serum levels of oxidative stress and liver markers. Histopathologically data are described median as minimum and maximum values, Kruskal-Wallis test was used for group comparisons. Kruskal-Wallis test after multiple comparisons were made by the method of Conover. 0.001 and 0.05 level of significance for all tests was considered.

Results

Biochemical parameters (ALT, AST, TAS, TOS and OSI) of CCl_4 and% 3 and% 5 Bitter Apricot kernel and control groups are presented in Table 1. TAS levels decreased in CCL_4 group. Statistically significant increase in blood plasma TOS, ALT and AST levels in CCl_4, Amydaline, 3% BAK, 5% BAK, CCl_4+Amyg, CCl_4+3%BAK, CCl_4+5% BAK in comparison to the values from control group.

The effects of Amyg and Bitter Apricot Kernel ratio with%3 and% 5 on the expression of Bcl-2, Bax, caspase 3, and NFκβ and Nrf-2 protein expression levels in the liver tissue are shown. As the studies results; CCl_4 induced group was the lower levels of caspase 3 and Nrf-2 protein

Table 1: TAS, TOS, ALT, AST and OSI levels are expressed as Mean±SD in all the groups.

	TAS (mmol Trolox equivalent/gr protein)	TOS (μmol H_2O_2 equivalent/gr protein)	OSI (Arbitrary unit)	ALT (IU/l)	AST (IU/l)
Control	0.69±0.006	0.39±0.16	0.40±0.06	*38.23±2.5*	50.14±5.5
Amyg	0.58±0.06	2.3±0.15	0.40±0.03	44.22±2.11	55.26±2.2
%3 BAK	0.57±0.09	2.13±0.18	0.41±0.05	$52.36±3.98^{a\,b}$	$68.96±3.78^{b}$
%5 BAK	0.60±0.06	2.33±0.28	0.38±0.05	53.21±2.84	69.32±4.12
CCl_4	$0.37±0.15^{b,d,g}$	$2.88±0.1^{b,d,e,g}$	$0.68±0.06^{a,b,c,d,e,f,g}$	$115.23±8.56^{a,b,c,d,e,f,g}$	$145.63±9.45^{a}$
CCl_4+Amyg	0.66±0.01	0.65±0.01	0.27±0.02	$95.36±4.35^{a}$	$125.28±7.48^{a}$
CCl_4+%3 BAK	0.63±0.09	0.64±0.09	0.30±0.05	85.32±5.63	98.63±7.25
CCl_4+%5 BAK	2.02±0.18	0.50±0.08	0.40±0.04	$80.12±2.36^{a,b}$	$92.32±5.36^{a\,b}$

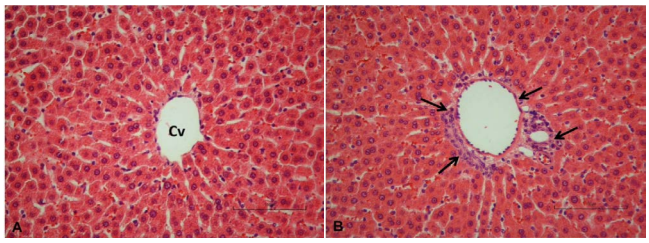

Figure 1: Normal histological appearance (A) Central vein (Cv). (B) Portal area (arrows). H&E. Scale bar = 100 µm.

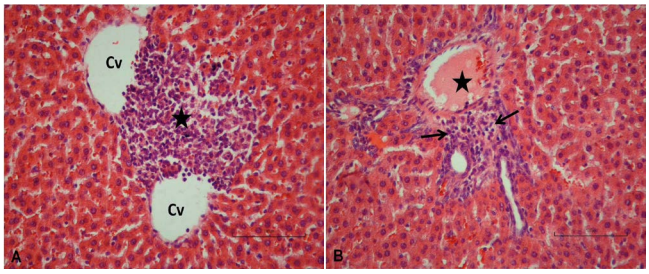

Figure 2: Central vein (Cv). Hepatic necrosis (asters) (A). Inflammatory cell infiltration in periportal area (arrrows). Vascular conges¬tion (aster) (B). H&E. Scale bar = 100 µm.

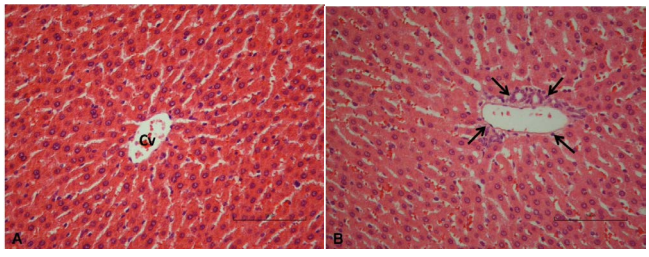

Figure 3: Normal histological appearance (A) Central vein (Cv). (B) Portal area (arrows). H&E. Scale bar = 100 µm.

expression levels compared to other groups. (p< 0,005) Amygdalin,% 3 and% 5 BAK feeding group was found higher levels of caspase 3 compared to other groups. Administration of CCl_4 significantly elevated Bcl-2 expression and decreased Bax and caspase 3expressions as compared with control animals. However, amygdalin and% 3 and 5 Bitter apricot Kernel feding combination to CCl_4 -treated animals significantly decreased Bcl-2 expression and significantly increased Bcl2 and NFκβ expressions compared to only CCl_4 induced rats.

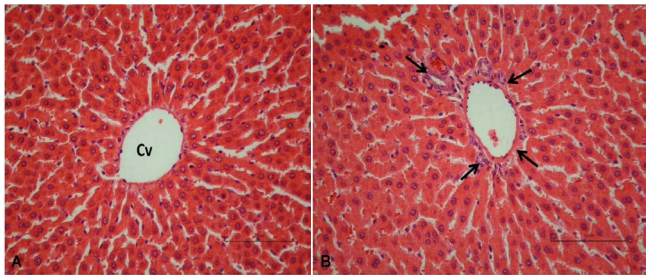

Figure 4: Normal histological appearance (A) Central vein (Cv). (B) Portal area (arrows). H&E. Scale bar = 100 µm.

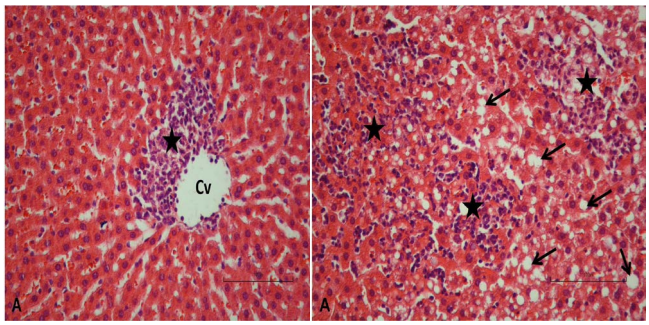

Figure 5: Hepatic necrosis (asters). intracellular vacuolization (arrows) (A). Hepatic necrosis (asters). intracellular vacuolization (arrows). Inflammatory cell infiltration in the periportal area (B). H&E. Scale bar = 100 µm.

As to; the Liver sections from the control groups were normal in histological appearance (Figures 1A and 1B).

The liver sections from the A treated group demonstrated some histopathological changes. Such as hepatic necrosis especially in the zone 3 location in the center of hepatic lobule and moderately common inflammatory cell infiltration especially in the periportal area and vascular congestion were observed (Figures 2A and 2B).

Administration of A%3 (Figures 3A and 3B) and B%5 (Figures 4A and 4B) groups rare minimal inflammation was evaluated otherwise normal histological structure.

Administration of CCl induced histopathological changes in liver. Such as inflammatory cell infiltration especially in the periportal area. Widespread hepatic necrosis. Intracellular vacuolization and vascular congestion (Figures 5A and 5B).

Administration of CCl+A induced histopathological changes in liver. moderately inflammatory cell infiltration in the periportal area

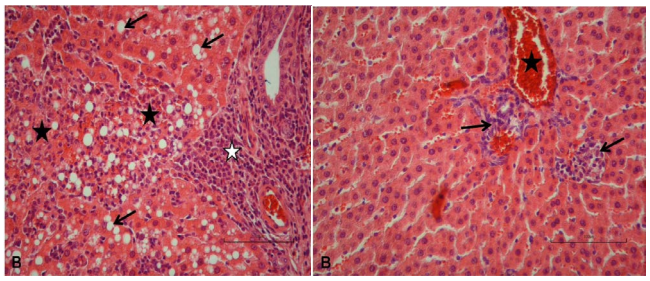

Figure 6: Central vein (Cv). Hepatic necrosis (aster) (A). inflammatory cell infiltration in the periportal area (arrows). Vascular conges¬tion (aster) (B). H&E. Scale bar = 100 µm.

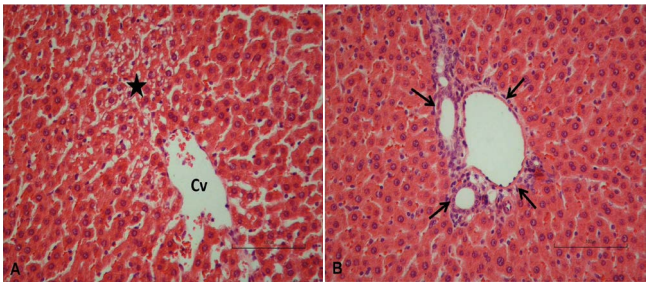

Figure 7: Central vein (Cv). local intracellular vacuolization in the liver parenchyma (aster) (A). Portal area (arrows). H&E. Scale bar = 100 µm.

and mild hepatic necrosis and rarely intracellular vacuolization and vascular congestion (Figures 6A and 6B).

In the CCl_4+%3 A (Figures 7A and 7B) and CCl_4+%5 A (Figures 8A and 8B) groups. Histopathological evidence of hepatic damage was markedly reduced. In this groups liver sections showed rare focal hepatic necrosis and mild inflammatory cell infiltration. Intracellular vacuolization and vascular congestion was limited in localized areas Figures 9A – 9F. Histopathological damage scores of the groups are summarized in Table 2.

Discussion

Leatril (amygdaline) is an alternative for cancer treatment and it relates to cyanogenic glycosides received from kernels of various fruits (almonds, apricots, peaches, etc.) The basis of suggestion of leatril as an antitumor agent is known about selective fermentative processing of amygdaline in tumor cells. In clinical trials there was reported efficiency of leatril with a very high ratio to develop cyanide intoxication. Cyanide can cause to apoptosis as a result of aerobic glycolysis suppression. However some scientific data and absence of permission from the supervising institutions (FDA) leatril is still advertised, produced and distributed as anti-tumor drug [21].

Our results increased activity of ALT, AST and TOS and histpathologically changes show that CCl_4 damaged liver changes with Bcl-2 expression and decreased Bax and caspase 3expressions as compared with control animals. Only Amygdalin given rat group's has an increased inflammation in histopathologically value show that it has some side effects but 3-5% BAK was not. Amigdalin and Bitter apricot kernel treated that damaged liver decreased Bcl-2 expression and significantly increased Bcl2 and NFκβ caspase 3. This changes

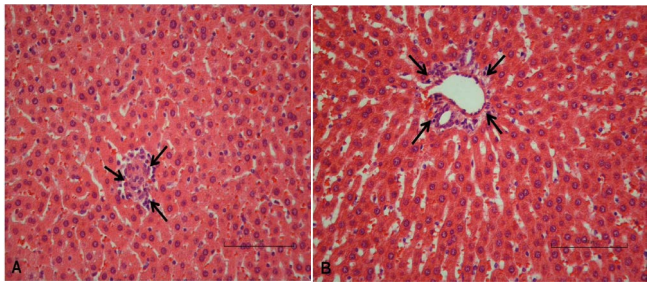

Figure 8: Focal necrosis in the liver paranchyma (arrows) (A). Portal area (arrows). H&E. Scale bar = 100 µm.

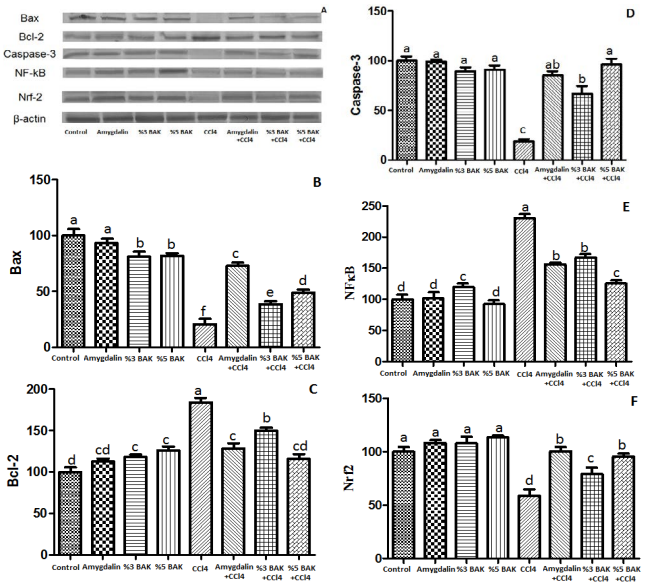

Figure 9: Liver tissue, Bax, Bcl-2, Caspase-3, NFkB and Nrf-2 expressing levels western blot strips (A), The Effect of CCl_4 and %3 and % 5 BAK on Bax (B), Bcl-2 (C), Caspase-3 (D), NFkB (E) and Nrf-2 (F) Protein Expression levels of liver tissue in CCl_4 Induced Rats. The intensity of the bands was quantified by densitometric analysis. Data are expressed as a ratio of normal control value (set to 100 %). The bar represents the standard error of the mean. Blots were repeated at least 3 times (n=3) and a representative blot is shown. β-actin was included to ensure equal protein loading. (a–f) Means on the bars with no common superscript differ significantly at the level of P < .01 by Fisher's multiple comparison test.

confirms that positively effect of Amygdalin and Bitter Apricot Kernel ratio of 3% the more effective for CCL_4 damaged liver of rat.

Parellely to our study; changes in serum AST and ALT activities as well as MDA, TAS, and TOS levels showed that CCl_4 caused lipid peroxidation and liver damage. However, lipid peroxidation and liver damage were reduced in the N-acetyl cysteine group. Increased levels in 8-hydroxy-2-deoxy guanosine and histone acetyltransferase activities, decreased histone deacetylase activities, and DNA breakage detected in nuclear extracts showed that CCl_4 intoxication induces oxidative stress and apoptosis in rat liver. The results of their study indicate that N-acetyl cysteine has a protective effect on CCl_4-induced DNA damage [20].In addition of these TAS levels of 5% BAK and CCl_4 group higher than control as very important situation for

Table 2: Histopathologically evaluation of the all groups.

	Inflammation	Necrosis	Vacuolization	Congestion	Total score
Control	0	0	0	0	0
Amyg	1.25±0.2^{c,d}	1	0	0.5±0	2.75±0.4^{b,e}
%3 BAK	0.2±0	0	0	0	0.2
%5 BAK	0.1±0	0	0	0	0.1
CCl_4	1.4±0.3	1.5±0.4^{b,c,d}	2.3 ±0.4^{a,b,g}	1.2±0.3^{b,c,d,g}	6.4 ±1.2^{a,b,g}
CCl_4+Amyg	1.4±0.3	1.2±0.2	0.4	0.4	3.4±0.5
CCl_4+%3 BAK	0.5±0	0	0.4	0.5	1.4±0.4
CCl_4+%5 BAK	0.5±0	0.1	0	0.4	1.01±0.2

a= Compared to Amygdaline; b= compared to control; c= compared to 3% BAK; d= compared to 5% BAK; e= compared to CCl_4+% 3 BAK; f= compared to CCL_4 + 3% BAK; g= compared to CCl_4+A.

protection of damage. The BAK has some other antioxidant phenolic content is an effective for damage.

Kuzu et al. showed that genistein reduced liver malondialdehyde, increased GSH levels, and had positive effects on inflammation, necrosis but did not change the fibrosis score. In their studies, treated with *L*-carnitine and genistein together with CCl_4 significantly reduced the ALT and AST levels compared to control groups that were only given CCl_4 [22]. BCL2 acts as an anti-apoptotic gene to promote cell survival, whereas BAX acts as a pro-apoptotic gene to promote cell death.

Smilary to our study, Pan PH et all showed NF-κB and its associated inflammatory cascade play an important role in cholangiocyte survival/damage, hepatocyte survival/damage, stellate cell and inflammatory cell activation, proinflammatory cytokine production, fibrosis, and liver injury [18] and [19]. Their results showed that rutin supplementation was accompanied by the suppression of inflammatory cell recruitment/accumulation, chemotactic cytokine expression, proinflammatory cytokine production, and NF-κB activation. Therefore, it was demonstrated that rutin attenuated BDL-induced hepatic inflammation [23]. Liver fibrosis was induced in a mouse model using CCL_4 (intraperitoneal injection, three times a week for 8 weeks), and astaxanthin was administered everyday at three doses (20, 40, and 80 mg/kg). Their pathologically results showed that astaxanthin significantly improved the pathological lesions of liver fibrosis. The levels of ALT and AST and hydroxyproline were also significantly decreased by astaxanthin. The same results were confirmed in bile duct liagtion, (BDL) model. In addition, astaxanthin inhibited hepatic stellate cells (HSCs) activation and formation of extracellular matrix (ECM) by decreasing the expression of NF-kappaB and TGF-beta1 [24] This findings is consistent with our results.

CCl4-induced depletion of cytosolic nuclear factor E2-related factor 2 (Nrf2) and suppression of nuclear translocation of Nrf2, which, in turn, up-regulated phase II/antioxidant enzyme activities. Taken together, these results demonstrate that DADS increases the expression of phase II/antioxidant enzymes and simultaneously decreases the expression of inflammatory mediators in CCl4-induced liver injury. These findings indicate that DADS induces antioxidant defense mechanism by activating Nrf2 pathway and reduces inflammatory response by inhibiting NF-kappaB activation [3].

CCl_4 is one of the most excessively studied hepatotoxicants to date and is commonly used as a model for screening the anti-hepatotoxic and/or hepatoprotective properties. Increased serum AST and ALT levels have been confirmed to the damaged structural integrity of hepatocytes. This changes parellely to our studies, the altered permeability of the membrane causes enzymes from the cells to be released into the circulation after cellular damage. In this study, the single oral dose of CCl_4 caused a significant elevation in serum AST and ALT levels, indicating the acute hepatotoxicity induced by CCl_4. The hepatotoxic effects observed in the CCl_4 treated rats were confirmed histopathologically, characterized by massive hepatocellular degeneration/necrosis, fatty changes, and inflammatory cell infiltration. However, pretreatment with DADS effectively improved the CCl_4-induced elevation in serum AST and ALT levels, indicating the hepatoprotective effect of DADS against the acute intoxication of CCl_4. This phenomenon was also confirmed by histopathological examination. Nrf2 has emerged as an indispensable regulator of both constitutive and inducible expression of detoxifying phase II and antioxidant enzyme genes in various tissues and cell types. The induction of cytoprotective enzymes is an important event in the cellular stress response during which a variety of oxidative toxicants can be eliminated or inactivated before they damage critical cellular macromolecules. Nrf2-null mice are particularly susceptible to oxidative stress, contributing to increased hepatotoxicity by ethanol and acetaminophen. In this study, rats treated with CCl_4 showed depletion of cytoplasmic Nrf2 and suppression of Nrf2 nuclear translocation. Consistent with this observation, a dramatic down-regulation of Nrf2 target genes, NQO1, HO-1, and GSTα levels was observed in the CCl_4-treated rat liver. These findings were in accordance with those of previous studies [3,25].

Parelely to our study Zhang S et al., investigated for rotective effect of the total flavonoids (TFs) from Rosa laevigata Michx fruit against carbon tetrachloride (CCl_4)-induced hepatotoxicity in mice. Pretreatment with TFs significantly decreased CCl_4-induced elevation of AST and ALT levels as well as the relative liver weight. Histopathological observation also revealed that TFs reduced the incidence of liver lesions and improved hepatocyte abnormality. Moreover, oral administration of TFs significantly enhanced antioxidant enzyme activities (superoxide dismutase, catalase and glutathione peroxidase), increased the content of glutathione and decreased the content of malondialdehyde. Further research indicated that TFs prevented the DNA fragmentation and mitochondrial ultrastructural alterations caused by CCl_4 based on TUNEL and transmission electron microscopy (TEM) assays. Moreover, pretreatment with TFs down-regulated the protein expressions of CYP2E1, iNOS, NF-kappaB, Bak and Caspase-3. Quantitative Real-time PCR assay suggested that TFs markedly decreased the levels of TNF-alpha, Fas/FasL and Bax gene expressions, and increased the level of Bcl-2. This is the first time to report the significant hepatoprotective effect of TFs from R. laevigata Michx fruit against CCl_4-induced liver

injury in mice and the action should be through reducing oxidative stress and suppressing inflammation and apoptosis [26].

We concluded that bitter apricot kernel feeding had beneficial effects on CCl_4-induced liver injury and damage probably due to its amygdaline contents and high radical-scavenging capacity. Dietary intake of amygdaline and bitter apricot kernel ratio of 5% can reduce the risk of liver steatosis and damage caused by free radicals.

References

1. Subhapradha N, Saravanan R, Ramasamy P, Srinivasan A, Shanmugam V, et al. (2014) Hepatoprotective Effect of beta-Chitosan from Gladius of Sepioteuthis lessoniana Against Carbon Tetrachloride-Induced Oxidative Stress in Wistar Rats. Appl Biochem Biotechnol 172: 9-20.
2. Ramasamy P, Subhapradha N, Shanmugam V, Shanmugam A (2014) Protective effect of chitosan from Sepia kobiensis (Hoyle 1885) cuttlebone against CCl4 induced hepatic injury. Int J Biol Macromol 65: 559-563.
3. In-Chul Lee, Sung-Hwan Kim, Hyung-Seon Baek, Changjong Moon, Seong-Soo Kang, et al.(2014) The involvement of Nrf2 in the protective effects of diallyl disulfide on carbon tetrachloride-induced hepatic oxidative damage and inflammatory response in rats. Food Chem Toxicol 63: 174-185.
4. Peng W, Jiang X, Haiqin L, Zhang C, Zhu J, et al. (2009) Protective effects of transgene expressed human PON3 against CCl4-induced subacute liver injury in mice. Biomed Pharmacother 63: 592-598.
5. Mancini-Filho J, Novoa AV, González AE, de Andrade-Wartha ER, de O e Silva AM, et al. (2009) Free phenolic acids from the seaweed Halimeda monile with antioxidant effect protecting against liver injury. Z Naturforsch C 64: 657-663.
6. Khan TH, Sultana S (2009) Antioxidant and hepatoprotective potential of Aegle marmelos Correa. against CCl4-induced oxidative stress and early tumor events. J Enzyme Inhib Med Chem 24: 320-327.
7. Hwang HJ, Kim P, Kim CJ, Lee HJ, Shim I, et al. (2008) Antinociceptive effect of amygdalin isolated from Prunus armeniaca on formalin-induced pain in rats. Biol Pharm Bull 31: 1559-1564.
8. Santos Pimenta LP, Schilthuizen M, Verpoorte R, Choi YH (2014) Quantitative analysis of amygdalin and prunasin in Prunus serotina Ehrh. using (1) H-NMR spectroscopy. Phytochem Anal 25: 122-126.
9. Ashida H, Kihara K, Shiotani B, Hashimoto T, Kanazawa K, et al. (2000) Detection of biomarkers for apoptosis in rat liver after perfusion with 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1). Biosci Biotechnol Biochem 64: 2021-2024.
10. Xu T, Ren D, Sun X, Yang G (2012) Dual roles of sulforaphane in cancer treatment. Anticancer Agents Med Chem 12: 1132-1142.
11. Okoh V, Deoraj A, Roy D (2011) Estrogen-induced reactive oxygen species-mediated signalings contribute to breast cancer. Biochim Biophys Acta 1815: 115-133.
12. Azuma Y, Miura K, Higai K, Matsumoto K (2007) Protein O-N-acetylglucosaminylation modulates promoter activities of cyclic AMP response element and activator protein 1 and enhances E-selectin expression on HuH-7 human hepatoma cells. Biol Pharm Bull 30: 2284-2289.
13. Zhang GM, Gu CH, Zhang YL, Sun HY, Qian WP, et al. (2013) Age-associated changes in gene expression of goat oocytes. Theriogenology 80: 328-336.
14. Xiao J, Ho CT, Liong EC, Nanji AA, Leung TM, et al.(2014) Epigallocatechin gallate attenuates fibrosis, oxidative stress, and inflammation in non-alcoholic fatty liver disease rat model through TGF/SMAD, PI3 K/Akt/FoxO1, and NF-kappa B pathways. Eur J Nut 53: 187-199.
15. Krithika R, Jyothilakshmi V, Verma RJ (2014) Phyllanthin inhibits CCl4-mediated oxidative stress and hepatic fibrosis by down-regulating TNF-alpha/ NF-kappaB and pro-fibrotic factor TGF-beta1 mediated inflammatory signaling. Toxicol Ind Health.
16. Turan S, Topcu A, Karabulut I, Vural H, Hayaloglu AA (2007) Fatty acid, triacylglycerol, phytosterol, and tocopherol variations in kernel oil of Malatya apricots from Turkey. J Agric Food Chem 55: 10787-10794.
17. Abdel-Rahman MK (2011) Can apricot kernels fatty acids delay the atrophied hepatocytes from progression to fibrosis in dimethylnitrosamine (DMN)-induced liver injury in rats? Lipids Health Dis 10: 114.
18. Dicenta F, Martínez-Gómez P, Grané N, Martín ML, León A, et al. (2002) Relationship between cyanogenic compounds in kernels, leaves, and roots of sweet and bitter kernelled almonds. J Agric Food Chem 50: 2149-2152.
19. el-Adawy TA, Rahma EH, el-Badawey AA, Gomaa MA, Lásztity R, et al. (1994) Biochemical studies of some non-conventional sources of proteins. Part 7. Effect of detoxification treatments on the nutritional quality of apricot kernels. Nahrung 38: 12-20.
20. Aksit H, Bildik A (2014) Determination of DNA Damage in Experimental Liver Intoxication and Role of N-Acetyl Cysteine. Cell Biochem Biophys .
21. Korman DB (2012) [Alternative means of drug therapy in cancer: letril]. Vopr Onkol 58: 698-704.
22. Demiroren K, Dogan Y, Kocamaz H, Ozercan IH, Ilhan S, et al. (2014) Protective effects of L-carnitine, N-acetylcysteine and genistein in an experimental model of liver fibrosis. Clin Res Hepatol Gastroenterol 38: 63-72.
23. Pan PH, Lin SY, Wang YY, Chen WY, Chuang YH, et al. (2014) Protective effects of rutin on liver injury induced by biliary obstruction in rats. Free Radic Biol Med 73: 106-116.
24. Shen M, Chen K, Lu J, Cheng P, Xu L, et al. (2014) Protective effect of astaxanthin on liver fibrosis through modulation of TGF-Î²1 expression and autophagy. Mediators Inflamm 2014: 954502.
25. Dong D, Zhang S, Yin L, Tang X, Xu Y, et al.(2013) Protective effects of the total saponins from Rosa laevigata Michx fruit against carbon tetrachloride-induced acute liver injury in mice. Food Chem Toxicol 62: 120-130.
26. Zhang S, Lu B, Han X, Xu L, Qi Y, et al. (2013) Protection of the flavonoid fraction from Rosa laevigata Michx fruit against carbon tetrachloride-induced acute liver injury in mice. Food Chem Toxicol 55: 60-69.

Author Affiliations

Top

[1]*Department of Biochemistry, Faculty of Medicine, Inonu University, Malatya, Turkey*

[2]*Department of Chemistry, Faculty of Engineering, Inonu University, Malatya, Turkey*

[3]*Department of Histology and Embryology, Inonu University Faculty of Medicine, Malatya, Turkey*

[4]*Department of Biology, Faculty of Science, Firat University, Elazig, Turkey*

[5]*Department of Nutrition, Faculty of Fisheries, Inonu University, Malatya, Turkey*

[6]*Department of Animal Nutrition, Faculty of Veterinary, Firat University, Elazig, Turkey*