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Assessment of hepatoprotective and apoptotic efficacy of *Cynara scolymus* leaf extract

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Abstract

Cynara scolymus is an important medicinal plant containing phenolic acids and flavonoids. One of the most active ingredients of cynara is cynarin, which is present in high concentrations, especially in the leaves. Experimental studies demonstrated a significant liver protecting and regenerating effects of cynara but only few of them discussed its anti-apoptotic effects on liver diseases. Fourty adult male albino rats were allocated into five groups. Group I: animals served as normal control, Group II: animals received olive oil, Group III: animals received cynara extract, Group IV: animals constituted the hepatotoxic group, which received CCl_4 in olive oil, Group V: CCl_4-prophylactic group administered cynara extract. Blood and liver tissues were collected for biochemical, molecular and histopathological examinations. The CCl_4 group showed significant increases in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin, albumin, malondialdehyde (MDA), interleukin-6 (IL-6), and decreases in albumin with reduced glutathione (GSH) content and superoxide dismutase (SOD) activity. The co-administration of cynara extract in prophylactic group significantly reduced the elevated levels of biochemical markers mentioned above as well as increased the level of tissue SOD, GSH and serum albumin. Histopathology of liver also confirmed the protective effective of cynara against CCl_4-induced hepatic damage. Gene expression measurements revealed that cynara administration ameliorated the effect of CCl_4 that induced alterations in genes expression of apoptotic related genes Bax, Bcl-2 and Caspase-3. The results confirm the strong protective and anti-apoptotic potential of cynara extract against CCl_4-induced hepatic toxicity in rats at both biochemical and molecular levels.

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Introduction

Hepatocellular carcinoma (HCC) is the most common primary hepatic malignancy of adults. It is the sixth most common cancer worldwide and the third most common cause of cancer death (Dai *et al*., 2014). In Egypt, liver cancer forms 11.75% of the malignancies of all digestive organs and 1.68% of the total malignancies. HCC constitutes 70.48% of all liver tumors among Egyptians (Mokhtar *et al*., 2007). It is the second most common cancer in men and the sixth most common cancer in women (GLOBOCAN database, 2008). HCC represents the main complication of cirrhosis, and shows a growing incidence in Egypt (Gomaa *et al*., 2014). Pre-existing cirrhosis is an important prerequisite for hepato-carcinogenesis, although some HCCs do arise in the absence of cirrhosis (Gao *et al*., 2012).

The liver is the largest and most metabolically active and fundamental organ of biotransformation and metabolism (Xu *et al*., 2011). It is a pivotal organ that removes and inactivates toxic substances and drugs to be excreted in urine. Hepatic toxicity is attributed primarily to the changes in oxidative stress and alteration in acute phase proteins (Flora *et al*., 2013). This stress induces damage to several biomolecules including lipids, proteins, DNA and, over time, contributes to liver injury. This injury is often differentiated as acute and chronic. Chronic liver disease has continuous formation from steatosis to chronic hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma (Loguercio and Federico, 2003 & Vitaglione *et al*., 2005 & Coballase-Urrutia *et al*., 2011). The condition of liver is important for our safety and health and its damage or disease is associated with DNA, protein, and lipid damage (Vera-Ramirez *et al*., 2013).

Hepatotoxicity is defined as injury to the liver that is associated with impaired liver function caused by exposure to a drug or another non-infectious agent (Bahirwani and Reddy, 2014). Chemical toxins including acetaminophen, carbon tetrachloride, galactosamine and thioacetamide are often used as the model substances causing experimental hepatocyte injury in both *in vivo* and *in vitro* conditions (Domenicali *et al*., 2009 & Remien *et al*., 2014).

Carbon tetrachloride (CCl_4) is a well-known and widely used synthesized chemical to establish animal model to study hepatic injury which is characterized by typical centrilobular necrosis and is similar to the hepatotoxicity in human (Li *et al*., 2017). It induces toxicity in rat liver, which closely resembles human cirrhosis. CCl_4 induces liver cell injury via the generation of trichloromethyl free radical by the mixed function oxidase system of the endoplasmic reticulum (Jain *et al*., 2012 & Hu *et al*., 2014). In addition to trichloromethyl free radical, other toxic products arising from peroxidative degeneration of membrane such as tricholoromethylperoxy ($^{\cdot}OOCCl_3$) and chlorine (Cl^{-}) free radicals, are responsible for the wide spread hepatocyte damage induced by CCl_4 (Khan *et al*., 2012). Compared to other body organs, liver is exposed to higher concentration of toxicants, such as CCl_4, and their metabolites because of the dual blood supply from the hepatic artery and the portal vein and high liver content of microsomal cytochrome P_{450} metabolizing enzymes (Canet *et al*., 2012).

Globe artichoke *(Cynara scolymus)*, belonging to the family of *Asteraceae (Compositae)*, is an herbaceous perennial crop, widely cultivated in the Mediterranean area. Cynara is one of the world's oldest medicinal plants. It has been known by the ancient Egyptians, while the ancient Greeks and Romans used it as a digestive aid (Magielse *et al*., 2014). Cynara leaf extract possesses antioxidant properties. It protected cultured rat hepatocytes against hydroperoxide-induced oxidative stress (Ben Salem *et al*., 2017). Cynara leaf extract also inhibited LDL oxidation (Mileo *et al*., 2012) and reduced the production of intracellular reactive oxygen species by oxidized LDL in cultured endothelial cells and monocytes (Zapolska-Downar *et al*., 2002).

Cynara leaves contain phenolic acids, sesquiterpene lactones, flavonoids, phytosterols (taraxasterol), sugars, inulin, enzymes, and essential oils (Simsek and Uysal, 2013). The phenolic acid derivatives include caffeoylquinic acids such as 3-caffeoylquinic acid, cynarin, and caffeic acids. Such properties are consistent with the well-known double role of

phenolic compounds as antioxidants and as substrates for oxidative browning reactions, primarily in the presence of high iron concentrations (Lattanzio *et al.*, 2009). Chemical activity of polyphenols in terms of their reducing properties, as hydrogen or electron-donating agents, predicts their potential effect as free-radical scavengers (Fratianni *et al.*, 2014).The flavonoid compounds of the leaf are luteolin-7-β-D-glucoside, luteolin-4-β-D-glucoside and luteolin-7-β-rutinoside (ESCOP, 2003 & Lattanzio *et al.*, 2009).

The leaf of the Cynara has been used for centuries as an antimicrobial, anti-inflammatory, choleretic, hepatoprotective, cholesterol-lowering, lipid-lowering, and glucose-lowering substance in Turkey, Southern Europe and Mediterranean countries (Jimenez-Escrig *et al.*, 2003).

In view of the above-mentioned beneficial effects of cynara, the aim of this study, therefore, was designed to evaluate the possible protective effect of the cynara leaf extract against CCl_4-induced hepatic injury, as a natural product able to reduce the induced oxidative and apoptotic damage in hepatocytes of male albino rats.

Materials and methods

Cynara preparation

Five % cynarin containing, commercially available cynara leaf extract powder extracted with 75 % ethanol was purchased from Kale Naturel Herbal Products Company, Ltd., Balikesir, Turkey.

Animals

Fourty adult male albino rats weighing 180-200 g obtained from the breeding unit of the Medical Research Center (Faculty of Medicine, Ain Shams University, Cairo, Egypt) were used throughout this study. The rats were housed in steel mesh cages on wood-chip bedding and maintained on a commercial pellet diet and tap water for one week before the start of the experiment as an acclimatization period. All animal experiments were performed according to the protocols approved by the local institutional animal ethics committee of Ain Shams University.

Experimental design

The rats were randomly divided into 5 groups of 8 rats each. Group I: animals served as normal control and received distilled water by gavage for 14 days. Group II: animals received olive oil (0.2 ml/kg b.wt.; *I.P.* twice daily) for 10 days. Group III: animals received cynara extract (1.5 g/kg b.wt.; daily; orally) for 14 days. Group IV: animals constituted the hepatotoxic group, which received CCl_4 in olive oil (0.2 ml/kg b.wt.; *I.P* twice daily) for 10 days. Group V: prophylactic group administered cynara extract (1.5 g/kg b.wt.; daily; orally) for 14 days and at the last 10 days received CCl_4 in olive oil (0.2 ml/kg b.wt., *I.P.* twice daily).

Blood and tissue collection

At the end of the experimental period, animals were sacrificed by cervical decapitation. Blood was allowed to clot and then centrifuged to prepare serum that was stored at -20°C. At necropsy, livers were excised immediately, rinsed from blood thoroughly in ice-cold isotonic saline and divided into three parts. The first part of liver (~100 mg) was immediately cut on ice, placed in 10 volumes RNA later (Qiagen, Hilden, Germany) and then stored at 4°C overnight. After the overnight incubation, the RNA later solution was replaced and the liver tissue was preserved at -30°C. The second part of the liver tissue was cut with a surgical blade and fixed in 10% phosphate-buffered formalin for histological examination. The remaining third part of liver was immediately stored in ice-cold sterile saline at -30°C for determination of caspase-3 oxidative stress and antioxidant activities.

Measurements of some biochemical parameters in serum

Serum alanine aminotransferase (ALT; EC 2.6.1.2), aspartate aminotransferase (AST; EC 2.6.1.1) and alkaline phosphatase (ALP; EC 3.1.3.1) activities, as well as total bilirubin level were assayed by the colorimetric methods of Reitman and Frankel (1957), Bessey *et al.* (1946) and Pearlman and Lee (1974), respectively, using kits purchased from Spectrum (Cairo, Egypt).

Total protein contents in liver homogenates (Lowry *et al*., 1951) and serum albumin concentration (Doumas *et al*., 1971) were quantified using kits purchased from Spectrum (Cairo, Egypt).

Determination of antioxidants and oxidative stress parameters in hepatic tissue

The liver was weighed and homogenized (10%) in chilled 50 mmol phosphate buffered saline (pH 7.4), centrifuged at 1200g, at 4°C for 15 min, using universal centrifuge (16R, Germany), then the supernatants were used for the determination of the following parameters: Total (Cu–Zn and Mn) Superoxide dismutase (SOD; EC 1.15.1.1) activity was assayed according to the previous described method (Minami and Yoshikawa, 1979), Reduced glutathione (GSH) concentration was measured according to Beutler *et al.* (1963), Hepatic lipid peroxide (LPO), in terms of malondialdehyde (MDA) was estimated according to the method of Esterbauer and Cheeseman (1990).

Determination of serum interleukin-6

Level of interleukin-6 (IL-6) was determined by using the corresponding commercially available enzyme-linked immunosorbent assay (ELISA) kit following protocols provided by the manufacturers (R&D Systems Kit, USA and DRG, USA).

Determination of Caspase-3 levels in the liver tissue

USCN Life Science Inc. Caspase-3 ELISA kit (Cat. No: SEA626Ra) was used to measure caspase-3 concentrations in the liver tissues.

Histological examination

Fixed liver samples were embedded in paraffin cubes, sliced into serial 4 µm-thick sections, stained with hematoxylin and eosin (H&E), examined and photographed under a light microscope.

Quantitative analysis of gene expression by real time PCR

Total RNA extraction

Total RNA was extracted from liver cells using SV Total RNA Isolation System (Promega, Madison, WI, USA) according to manufacturer's instruction.

The RNA concentrations and purity were measured with an ultraviolet spectrophotometer.

Complementary DNA (cDNA) synthesis

The cDNA was synthesized from 1µg RNA using SuperScript III First-Strand Synthesis System as described in the manufacturer's protocol (#K1621, Fermentas, Waltham, MA, USA). In brief, 1µg of total RNA was mixed with 50 µM oligo (dT) 20, 50 ng/µL random primers and 10 mM dNTP mix in a total volume of 10 µL. The mixture was incubated at 56°C for 5 min, and then placed on ice for 3 min. The reverse transcriptase master mix containing 2 µL of 10x RT buffer, 4µL of 25 mM $MgCl_2$, 2µL of 0.1 M dithiothreitol (DTT) and 1µL of SuperScript® III RT (200 U/µL) was added to the mixture and was incubated at 25°C for 10 min followed by 50 min at 50°C.

Real-time quantitative PCR

Real-time amplification was performed using an Applied Biosystem with software version 3.1 (Stepone™, USA). The reaction containing SYBR Green master mix (Applied Biosystem), gene-specific primer pairs which were shown in Table (1) and were designed with Gene Runner Software (Hasting Software), Inc., Hasting, NY) from RNA sequences from the gene bank. All primer sets had a calculated annealing temperature of 60°C.

Quantitative real-time PCR reactions were performed in a 25 µL reaction volume consisting of 2X SYBR Green PCR master mix (Applied Biosystem), 900 nM of each primer and 2 µL of cDNA. Amplification conditions were: 2 min at 50°C, 10 min at 95°C and 40 cycles of denaturation for 15 sec and annealing/extension at 60°C for 10 min. Data from real-time assays were calculated using the v1.7 sequence detection software from PF Biosystem (Foster City, CA). The relative difference in gene expression of studied gene mRNA between treated groups and the control was determined using the comparative Ct method.

The level of expression of each target gene were normalized to β-actin which was used as the control

housekeeping gene (endogenous reference gene) and reported as fold change over background levels detected in the diseased groups.

Statistical analyses

All data were expressed descriptively as the mean ± standard deviation (SD) of replicate determinations for quantitative parametric data. Statistical analysis was performed using Student t-test to test for differences in means of variables between groups. A probability of $P<0.05$ was considered significant and $P>0.05$ insignificant.

All data were analyzed by Statistical Package for Social Science (SPSS) version 21.0 for Windows (SPSS® Chicago, IL, USA) software program.

Results

Biochemical markers

No mortality was seen in animals during the study. The dose of cynara did not initiate any side effects for the animals, whereas many side effects were observed in animals treated with CCl_4 such as yellowish body hair, loosing of body weight, general weakness (completely loss of activity) and abdominal edema.

Table 1. Primer sequences of target genes.

Gene	Primer sequence	Accession numbers
Bax	Forward: 5'-TAGCAAACTGGTGCTCAAGG-3' Reverse: 5'-TCTTGGATCCAGACAAGCAG-3'	NM_017059.2
Bcl-2	Forward: 5'- CCAGGCCTTCAACCATTATC-3' Reverse: 5'- CTCATTGAACTCGTCTCCGA-3'	NM_022612.1
Caspase-3	Forward: 5'- CAAGTCGATGGACTCTGGAA-3' Reverse: 5'- GTACCATTGCGAGCTGACAT-3'	NM_012922.2
β-actin	Forward: 5'-CCTGTATGCCTCTGGTCGTA-3' Reverse: 5'-CCATCTCTTGCTCGAAGTCT-3'	NM_031144.3

Liver function tests

The data summarized in Table 2 indicates that, AST, ALT, ALP activities and total bilirubin were significantly increased ($P<0.05$) in rats receiving CCl_4 either alone or in combination with cynara extract when compared with the control group. However, the same parameters were significantly lower ($P<0.05$) in prophylactic group when compared to the intoxicated CCl_4 group.

In addition, albumin level was significantly decreased in rats received CCl_4 when compared with the control group, however, all the other groups showed no significance. For almost all parameters, cynara extract showed normalization of liver function when compared to control group.

Hepatic oxidative stress

The hepatic GSH content and SOD activity were significantly decreased ($P<0.05$) in CCl_4 and prophylactic groups compared to control group. However, they were significantly increased ($P<0.05$) when cynara extract was co-administered in prophylactic group, compared to CCl_4 group. Animals treated with CCl_4 had a significant increase ($P<0.05$) in the level of MDA compared to control group. Simultaneous treatment with cynara extract significantly abolished the enhancing effect of CCl_4 on hepatic lipid peroxidation that was expressed as a lower level of MDA in hepatocytes ($P<0.05$) (Table 2).

IL-6 level

IL-6 level was significantly increased *($P<0.05$)* when CCl_4 was administered either alone or combined with cynara compared with control group (Table 2).

However, administration of cynara extract alone showed anti-inflammatory effect by decreasing the level of IL-6 significantly *($P<0.05$)* when compared to control group. On the other hand, the prophylactic group showed a significant decreased level of IL-6 *($P<0.05$)* when compared to the CCl_4 group.

Caspase-3 activity

Caspase-3 activity was markedly increased in CCl_4 group (*P<0.05)*, meanwhile co-administration of cynara extract in prophylactic group showed an ameliorating effect by decreasing the level of caspase-3 (*P<0.05)* in liver tissue when compared to CCl_4 group (Table 2).

Table 2. Biochemical parameters among the studied groups.

Parameters \ Groups	Control	Olive oil	Cynara	CCl_4	Prophylactic
AST (U/L)	89.4 ± 7.1	91.3 ± 3.2	108.6 ± 8.6*	316.8 ± 7.7*	93.7 ± 3.7#
ALT (U/L)	39.2 ± 3.1	42.1 ± 2.5	43.2 ± 3.6	187.6 ± 4.6*	59.4 ± 2.4*#
ALP (U/L)	97.5 ± 3.1	101.3 ± 4.4	100.6 ± 3.7	274.1 ± 5.6*	112.3 ± 7.7*#
Total Bilirubin (mg/dL)	0.64 ± 0.06	0.59 ± 0.08	0.63 ± 0.03	3.43 ± 0.05*	1.09 ± 0.16*#
Albumin (mg/dL)	4.1 ± 0.39	3.9 ± 0.4	4.2 ± 0.2	2.4 ± 0.6*	3.8 ± 0.9
MDA (mmol/g tissue)	56.8 ± 4.1	58.1 ± 3.3	55.4 ± 2.9	116.0 ± 3.6*	47.2 ± 4.1*#
GSH (µmol/g tissue)	33.4 ± 1.7	32.6 ± 2.1	33.9 ± 1.8	14.6 ± 0.9*	29.8 ± 1.0*#
SOD (U/mg protein)	2.81 ± 0.5	2.63 ± 0.1	2.70 ± 0.2	1.35 ± 0.4*	2.5 ± 0.3#
IL-6 (pg/ml)	38.2 ± 1.3	37.9 ± 1.1	29.4 ± 1.4*	125.1 ± 6.7*	74.6 ± 3.1*#
Caspase 3 (ng/mg protein)	1.34 ± 0.1	1.29 ± 0.09	1.31 ± 0.1	2.9 ± 0.1*	1.62 ± 0.2#

Data are expressed as mean ± SD. *P* values was statistically measured by independent t-test, **P* value < 0.05 when compared to control group; # *P* value < 0.05 when compared to CCl_4 group.

AST, aspartate transaminase; ALT, alanine transaminase, ALP, alkaline phosphatase; MDA; malondialdehyde; GSH, reduced glutathione; SOD, superoxide dismutase; IL-6, interleukin-6.

Molecular studies

Gene expression of pro-apoptotic (Bax), anti-apoptotic (Bcl-2) and Caspase-3 genes

Bax, Bcl-2 and caspase-3 gene expression were normalized to the housekeeping beta actin gene. As evident from Fig. 1 & 2, real time PCR evaluation of liver cells among the different studied groups showed a significant increase in Bax and caspase-3 mRNA expression in CCl_4 group.

On the contrary, Bcl-2 mRNA expression was relatively decreased in CCl_4 and prophylactic groups, meanwhile cynara extract showed normal pattern of expression. However, the Bax/Bcl-2 ratio appeared to have a tendency to markedly increase in CCl_4 group and to lesser extent in prophylactic group with almost normal level in cynara group compared to that in control group.

Histopathological studies

Histology of the liver sections of control animals showed normal hepatic architecture and liver lobular with well-preserved cytoplasm, prominent nucleus with normal histological structure of the central vein and surrounding hepatocytes in the parenchyma (Fig. 3A). Liver of animals treated with olive oil and cynara extract showed no histopathological alteration and almost normal architecture of hepatic lobules as recorded in Fig. 3B & 3C, respectively. The liver sections of CCl_4-treated animals showed accumulation of adipoblasts with fibrosis while the underlying hepatocytes had degenerative change in Fig. 3D. Dilatation was observed in the central vein also associated with fatty change in the surrounding few hepatocytes in Fig. 3E. Finally, mild thickening was detected in the capsule associated with degeneration in the underlying hepatocytes in group of cynara intoxicated with CCl_4 in Fig. 3F.

Discussion

In absence of a reliable and effective agent for prevention and treatment of liver diseases, many researchers are focusing on introducing hepatoprotective compounds from natural products (Kumar *et al.*, 2011). Many epidemiological studies suggest that diet rich in fruits and vegetables has healthy properties (Mileo *et al.*, 2016). The phenolic and flavonoid contents of these natural products have many advantages either through scavenging the free radicals (Kalaiselvan *et al.*, 2016) or exhibiting some apoptotic events (Supic *et al.*, 2013).

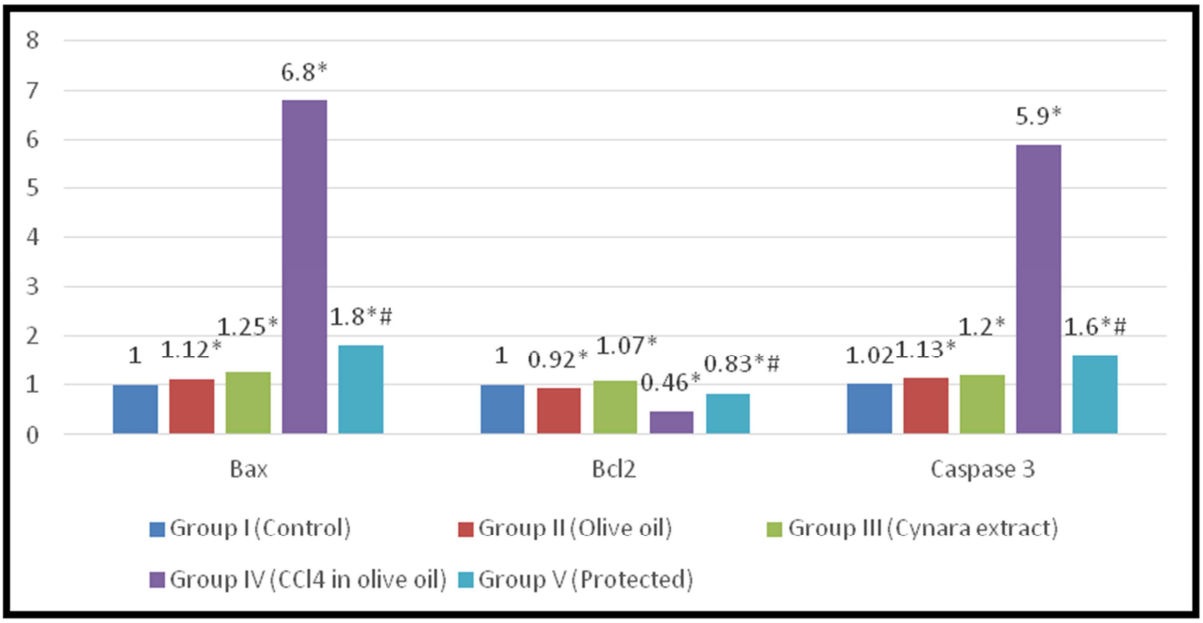

Fig. 1. Quantitative RT-PCR analysis of Bax, Bcl-2, and Caspase-3 mRNA expressions in liver of the studied groups. Data are expressed as mean ± SD. *P* values was statistically measured by independent t-test, **P* value < 0.05 when compared to control group; # *P* value < 0.05 when compared to CCl_4 group.

Artichoke *(Cynara scolymus)* is a healthy food and folk medicine with antioxidative and anti-inflammatory properties. Although the most important active ingredient of cynara, cynarin, is present in the whole plant, the highest concentrations are found in the leaves. For this reason, most of the natural medicines obtained from this plant are prepared from leaves. Like silymarin, cynarin has demonstrated significant liver protecting and regenerating effects (Moglia *et al.*, 2008).

Liver function tests

One of the ways for estimating of the extent of hepatic damage is through the determination of the serum level of cytoplasmic enzymes that leak from damaged liver cells into the blood stream. The results of the present work indicated that the administration of CCl_4 caused severe liver injury which was evidenced by the increase in the activities of serum hepatic enzymes AST, ALT, ALP and total bilirubin accompanied with decrease in the albumin content. This may be due to the fact that hepatic cells posses a variety of metabolic activities and in case of liver injury the transport function of hepatocytes is disturbed, resulting in the leakage of these metabolites (Biswas *et al.*, 2010). Also, these results are in agreement to previous studies, showing that increased bilirubin levels indicated diffused harm to the liver (El-Kott and Owayss, 2008).

At the same time, the current results showed a pronounced decrease in the activities of serum ALT, AST, ALP and total bilirubin after oral treatment with prophylactic dose of cynara extract when compared to the CCl_4 group. This effect of cynara is in agreement with previous study (Heidarian and Rafieian-Kopaei, 2013) which stated that cynara significantly improved the liver status and showed more profound therapeutic effects. This protective effect may be due to the antioxidant effect of cynara which was previously confirmed (Desai *et al.*, 2012).

Another study (Header *et al.*, 2017) reported that the treatment with cynara extract significantly prevented the release of transaminases and significantly enhanced protein content towards control, suggesting its hepatoprotective potential. The pharmacological activities of cynara extract as hepatoprotective, antioxidative, anticarcinogenic, hypocholesterolemic and antibacterial effects, which may be attributed to the presence of phenolic and flavonoid compounds can be useful in the treatment of liver damage (Gouveia and Castilho, 2012).

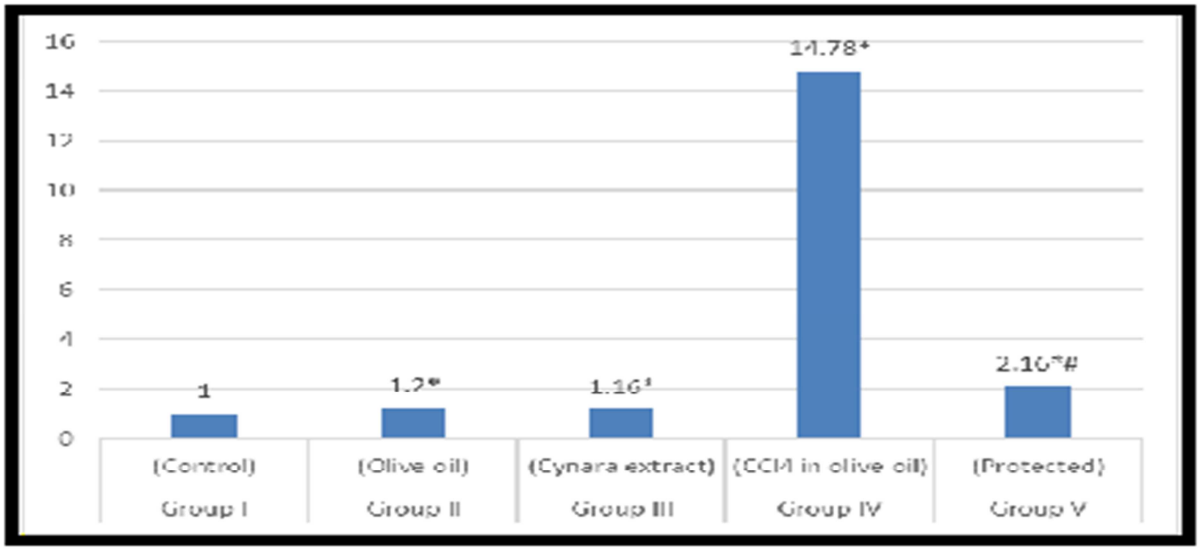

Fig. 2. Quantitative RT-PCR analysis of Bax/Bcl-2 ratio mRNA expressions in liver of the studied groups. Data are expressed as mean ± SD. *P* values was statistically measured by independent t-test, **P* value < 0.05 when compared to control group; # *P* value < 0.05 when compared to CCl_4 group.

Hepatic oxidative stress

Lipid peroxidation produces a progressive loss of cell membrane integrity, impairment in membrane transport function and disruption of cellular ion homeostasis (Bano and Bhatt, 2007). The current study revealed that the administration of CCl_4 resulted in a significant increase in the liver MDA content which is the most important oxidative stress parameters in the liver. These results are in agreement with the previous study which confirmed the liver damage of CCl_4 through the higher level of MDA which directly stimulated the activation of hepatic stellate cells and accelerated liver fibrosis (Guo *et al.*, 2017). On the contrary, it has been suggested that therapeutic activities of cynara extract depend mainly on the presence of phenolics and flavonoids. These compounds are known for their strong scavenging effect on free radicals and may also be able to suppress the formation of free radicals by binding to heavy metal ions which are known to catalyze many processes leading to the generation of free radicals.

In the present study, cynara extract attenuated the CCl_4-induced MDA formation possibly due to its intrinsic antioxidant properties, thus cynara extract may prevent peroxidative changes in liver tissue. These results are in agreement with previous study (Colak *et al.*, 2016) that also documented the effect of cynara through the renewal mechanism of the liver by the re-stabilization of the cell membranes and repair mechanisms in the hepatic tissue as a result of recovery against the oxidative stress.

Furthermore, the accumulation of free radicals may cause decrease in antioxidant capacity such as a decline in SOD and GSH levels and hence diminished capacity of scavenging free radicals (Wang *et al.*, 2003). In the present study, the decrease of the GSH and SOD content of the liver tissues after the application of CCl_4 have also been described previously (El-Sayed *et al.* 2015). On the other hand, the present study showed significant improvement in GSH content

nd SOD activity in rats protected by cynara extract. This improvement may be due to its rich content of essential elements such as K, Ca, Na, Mg, I, Mn, Zn, Cu and Cr and some vitamins such as A, B1, B2, B3 and C, that might be responsible for reactivating antioxidant enzymes by providing optimum trace elements and so enhancing the SOD and GSH levels (Monti *et al.*, 2008).

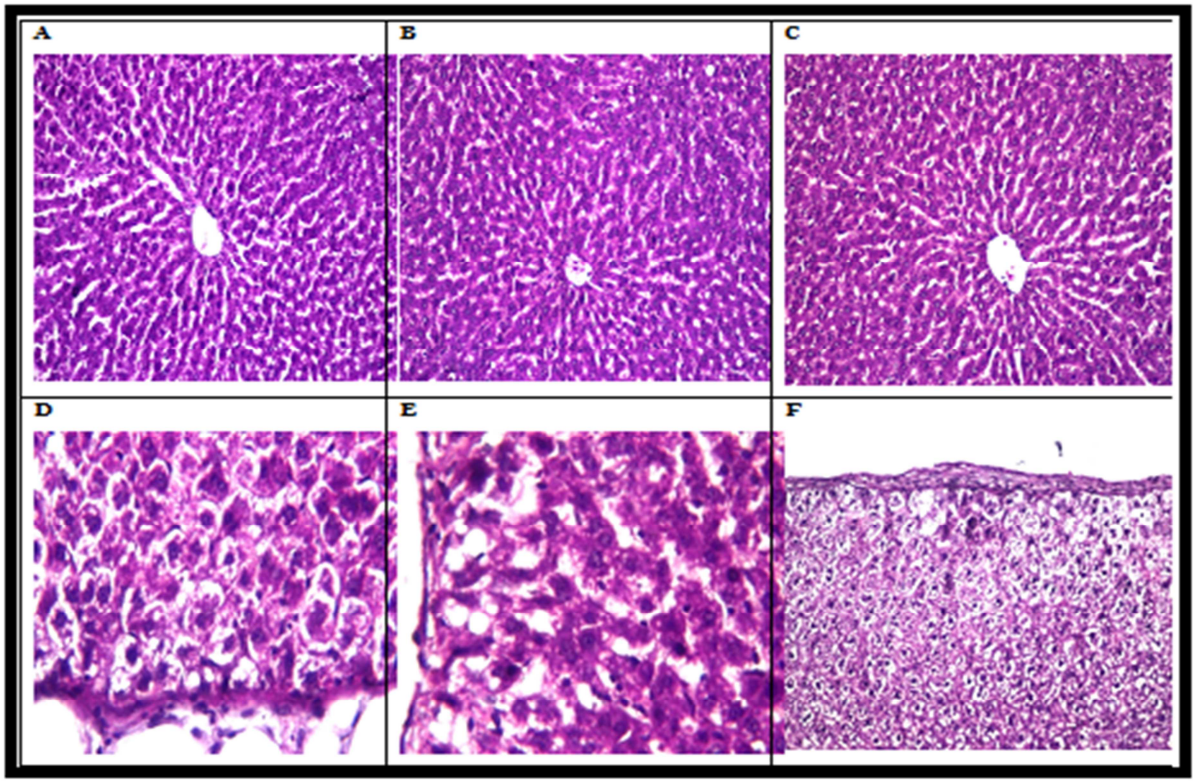

Fig. 3. Histological investigations of hematoxylin and eosin-stained liver tissue sections. A; Control group (10X), B; Olive oil group (10X), C; Cynara leaf extract group (10X), D, E; CCl_4-induced group (20X), F; Prophylactic group (10X).

IL-6 level

The production of some pro-inflammatory cytokines by hepatic Kupffer cells is increased in acute inflammatory responses. These cytokines can trigger hepatic stellate cells and promote their production; therefore, enhancing extracellular matrix (ECM) deposition and exacerbating the fibrogenesis process (Friedman, 2008). In the present study, CCl_4 administration caused an increase in the levels of IL-6 compared to control group. These results are in agreement with previous studies that reported the capability of CCl_4 to induce some inflammatory responses by elevation of some cytokines such as IL-6 (Hassan *et al.*, 2012) and activation of caspase cascade of apoptosis as indicated by the increase in activity of active caspase-3 (Yao *et al.*, 2017).

Meantime, cynara extract exhibited an ameliorating effect which may be due to the unique active constituents of cynara that have been shown to exert a variety of medical properties including anti-inflammatory activity (Mocelin *et al.*, 2016).

Caspase-3 activity

Cell apoptosis can be induced by the activation of death receptors, mitochondrial dysfunction, DNA damage, excessive autophagy, and endoplasmic reticulum stress.

In liver cells, mitochondrial dysfunction can amplify the apoptotic signal and integrate various pathways to promote apoptotic response (Fernandez *et al.*, 2015). Caspase-3 is a 32 kDa caspase protein that interacts with caspase-8 and caspase-9 and has a central role in the execution-phase of cell apoptosis. Data of the current study demonstrated that CCl_4 induced high levels of caspase-3 in the rat liver.

This result suggested the capability of CCl_4 to induce the apoptosis as previously proved by El-Halawany *et al.* (2014). Meanwhile, the pretreatment of cynara leaf extract inhibited caspase-3 activity in prophylactic group which was elevated after the administration of CCl_4 and protect against hepatic damage via an anti-apoptotic function. These results are in agreement with the previous study of Colak *et al.* (2016).

Molecular studies

Furthermore, the dramatic effects of CCl_4 and the valuable effect of cynara extract on the activity of liver enzymes, antioxidant, inflammatory and apoptotic markers are supported by the molecular studies on gene expression of anti-apoptotic (Bcl-2), pro-apoptotic (Bax) and caspase-3 genes. Bax and Bcl-2 have been reported to play a major role in determining cellular fate under injured conditions. Increased expression of Bax can induce apoptosis, while upregulation of Bcl-2 protein protects cells (Tallman *et al.*, 2005).

In the current study, the expression levels of Bcl-2, Bax, and caspase-3 were investigated by quantitative real time PCR. As expected, CCl_4 treatment increased the Bax expression and downregulated the Bcl-2 expression (Hamid *et al.*, 2017), which could be reversed by cynara extract effect. Meanwhile, the expression of caspase-3 was increased by the treatment with CCl_4 and marvelous decreased by cynara.

These results support that cynara protects the liver against hepatocyte apoptosis during CCl_4-induced liver injury as previously reported by Mileo *et al.* (2012). Also, as stated previously, cynara polyphenols can act over different stages of apoptosis by modifying Bcl-2 and Bax expression levels (Jahanafrooz *et al.*, 2016). The ultimate vulnerability of cells to diverse apoptotic stimuli is determined by the relative ratio of various pro-apoptotic and anti-apoptotic members.

The balance between Bax and Bcl-2 expression levels help to determine the susceptibility of a cell to apoptotic stimuli.

The present work confirmed that co-administration of cynara extract with CCl_4 significantly decreased the expression of Bax/Bcl-2 mRNA ratio compared with CCl_4 group. This result agreed with the earlier work of Kakkar and Singh (2007) that showed that cells' survival is frequently regulated by pro-apoptotic and anti-apoptotic family proteins. A similar result was also reported by Ding *et al.* (2010).

Histopathological studies

In terms of histopathological changes, several previous experimental studies have reported that administration of CCl_4 led to a variety of structural changes in the liver tissue such as inflammation, apoptosis, fibrosis, fatty degeneration, mononuclear cell infiltration, steatosis, hemorrhage, formation of regenerative nodules in liver tissue, sinusoid dilation, vacuolisation and centrilobular necrosis (Kim *et al.*, 2014). The present study support these documented findings in CCl_4 group. Following the CCl_4-induced hepatic injury, the administration of cynara leaf extract led to reduction of these histopathological changes where mild thickening were detected in prophylactic group, meanwhile there was no histopathological alterations were observed in cynara group. These findings are in agreement with earlier study that confirmed the ameliorating effect of cynara in rat liver which showed a mild degree of lymphocyte infiltration compared to the control (Heidarian and Rafieian-Kopaei, 2013).

Conclusion

In conclusion, according to all the achieved biochemical, molecular and histopathological findings in the present study, cynara extract can prevent or alleviate the liver injury induced by CCl_4 in rats towards the normal state with the reversal of altered antioxidant enzymes, apoptotic status and peroxidation damage in tissues.

These confirmed its hepatoprotective effect and potential role in defense against free radicals. The protective effects may be, at least in part, related to the antioxidant and anti-apoptotic properties of the extract that may be due to its content of the phenolic acids and flavonoids.

These results suggested the pharmaceutical phytomedicine role of cynara leaf extract in the treatment of liver diseases.

References

Bahirwani R, Reddy KR. 2014. Drug-induced liver injury due to cancer chemotherapeutic agents. Seminars in Liver Disease **34(2)**, 162-171. http://dx.doi.org/10.1055/s-0034-1375957.

Bano M, Bhatt DK. 2007. Neuroprotective role of a novel combination of certain antioxidants on lindane (g-HCH) induced toxicity in cerebrum of mice. Research Journal of Agriculture and Biological Sciences **3(6),** 664-669.

Ben Salem M, Hanen Affes H, Athmouni K, Ksouda K, Dhouibi R, Sahnoun Z, Hammami S, Zegha KM. 2017. Chemicals Compositions, Antioxidant and Anti-Inflammatory Activity of *Cynara scolymus* Leaves Extracts, and Analysis of Major Bioactive Polyphenols by HPLC. Evidence-Based Complementary and Alternative Medicine. https://doi.org/10.1155/2017/4951937

Bessey OA, Lowry OH, Brock MJ. 1946. A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. Journal of Biological Chemistry **164**, 321-329. PMID: 20989492 [Indexed for MEDLINE].

Beutler E, Duron O, Kelly BM. 1963. Improved method for the determination of blood glutathione. Journal of Laboratory and Clinical Medicine **61**, 882–890. PMID: 13967893 [Indexed for MEDLINE].

Biswas K, Kumar A, Babaria BA, Prabhu K, Setty RS. 2010. Hepatoprotective effect of leaves of *Peltophorum pterocarpum* against paracetamol Induced acute liver damage in rats. Journal of Basic and Clinical Pharmacy **1(1)**, 10–15. PMCID: PMC4158896.

Canet MJ, Hardwick RN, Lake AD. 2012. Altered arsenic disposition in experimental nonalcoholic fatty liver disease. Drug Metabolism and Disposition **40(9)**, 1817-1824. http://dx.doi.org/10.1124/dmd.112.046177

Coballase-Urrutia E, Pedraza-Chaverri J, Cárdenas-Rodríguez N, Huerta-Gertrudis B, García-Cruz ME, Ramírez-Morales A, Sanchez-Gonzalez DJ, Martínez-Martínez CM, Camacho-Carranza R, Espinosa-Aguirre JJ. 2011. Hepatoprotective effect of acetonic and methanolic extracts of *Heterotheca inuloides* against CCl(4)-induced toxicity in rats. Experimental and Toxicologic Pathology **63(4)**, 363–370. http://dx.doi.org/10.1016/j.etp.2010.02.012.

Colak E, Ustuner MC, Tekin N, Colak E, Burukoglu D, Degirmenci I, Gunes HV. 2016. The hepatocurative effects of *Cynara scolymus* L. leaf extract on carbon tetrachloride-induced oxidative stress and hepatic injury in rats. Springer Plus **5**, 216-225. http://dx.doi.org/10.1186/s40064-016-1894-1.

Dai L, Ren P, Liu M, Imai H, Tan EM, Zhang JY. 2014. Using immunomic approach to enhance tumor-associated autoantibody detection in diagnosis of hepatocellular carcinoma. Journal of Clinical Immunology **152**, 127–139. https://doi.org/10.1016/j.clim.2014.03.007

Desai SN, Patel DK, Devkar RV, Patel PV, Ramachandran A. 2012. Hepatoprotective potential of polyphenol rich extract of *Murraya koenigii* L.: an in vivo study. Food and Chemical Toxicology **50**, 310–314. http://dx.doi.org/10.1016/j.fct.2011.10.063

Ding M, Potter JJ, Liu X, Torbenson MS, Mezey E. 2010. Selenium supplementation decreases hepatic fibrosis in mice after chronic carbon tetrachloride administration. Biological Trace Element Research **133**, 83–97. http://dx.doi.org/10.1007/s12011-009-8414-x

Domenicali M, Caraceni P, Giannone F. 2009. Cannabinoid type 1 receptor antagonism delays ascites formation in rats with cirrhosis. Gastroenterology **137(1)**, 341-349. http://dx.doi.org/10.1053/j.gastro.2009.01.004

Doumas BT, Watson WA, Biggs HG. 1971. Albumin standards and the measurement of serum albumin with bromocresol green. Clinica Chimica Acta **31**, 87-96. PMID: 9049440 [Indexed for MEDLINE].

El-Halawany AM, Salah-El Dine R, El Sayed NS Hattori M. 2014. Protective Effect of *Aframomum melegueta* phenolics Against CCl4-Induced Rat Hepatocytes Damage; Role of Apoptosis and Pro-inflammatory Cytokines inhibition. Scientific reports **4,** 5880-5889.
http://dx.doi.org/10.1038/srep05880.

El-Kott AF, Owayss AA. 2008. Protective effects of propolis against the amitraz hepatotoxicity in mice. American Journal of Pharmacology and Toxicology **3(5)**, 402-408.
http://dx.doi.org/10.3923/jpt.2008.402.408

El-Sayed YS, Lebda MA, Hassinin M, Neoman SA. 2015. Chicory (*Cichorium intybus* L.) root extract regulates the oxidative status and antioxidant gene transcripts in ccl_4-induced hepatotoxicity. PLoS One **10(3)**, e0121549.
https://doi.org/10.1371/journal.pone.0121549

ESCOP; European Scientific Cooperative on Phytotherapy 2003. Cynarea folium artichoke leaf. ESCOP monographs: the scientific foundation for herbal medicinal products, 2nd Edn. Thieme, New York, 118–125.

Esterbauer H, Cheeseman KH. 1990. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Methods Enzymology **186**, 407-421.
https://doi.org/10.1016/0076-6879(90)86134-H

Fernandez A, Ordonez R, Reiter RJ, Gonzalez-Gallego J, Mauriz JL. 2015. Melatonin and endoplasmic reticulum stress: relation to autophagy and apoptosis. Journal of Pineal Research **59**, 292–307.
http://dx.doi.org/10.1111/jpi.12264.

Flora G, Gupta D, Tiwari A. 2013. Preventive efficacy of bulk and nanocurcumin against lead-induced oxidative stress in mice. Biological Trace Element Research **152**, 31-40.
http://dx.doi.org/10.1007/s12011-012-9586-3.

Fratianni F, Pepe R, Nazzaro F. 2014. Polyphenol composition, antioxidant, antimicrobial and quorum quenching activity of the “Carciofo di Montoro” (*Cardunculus* var. *scolymus*) global artichoke of the campania region, Southern Italy. Food and Nutrition Sciences **5(21),** 2053-2062.
http://dx.doi.org/10.4236/fns.2014.521217

Friedman SL. 2008. Mechanisms of hepatic fibrogenesis. Gastroenterology **134**, 1655–1669.
http://dx.doi.org/10.1053/j.gastro.2008.03.003.

Gao J, Xie L, Yang WS. 2012. Risk factors of hepatocellular carcinoma--current status and perspectives. Asian Pacific Journal of Cancer Prevention **13**, 743-52. PMID: 22631642 [Indexed for MEDLINE].

GLOBOCAN. 2008 database (version 1.2). Available online: 2008.
http://globocan.iarc.fr

Gomaa AI, Hashim MS, Waked I. 2014. Comparing staging systems for predicting prognosis and survival in patients with hepatocellular carcinoma in Egypt. PLoS One **9,** e90929.
https://doi.org/10.1371/journal.pone.0090929.

Gouveia SC, Castilho PC. 2012. Phenolic composition and antioxidant capacity of cultivated artichoke, Madeira cardoon and artichoke based dietary supplements. Food Research International **12**, 712–724.
https://doi.org/10.1016/j.foodres.2012.05.029

Guo Y, Liang X, Meng M, Chen H, Wei X, Li M, Li J, Huang R, Wei J. 2017. Hepatoprotective effects of Yulangsan flavone against carbon tetrachloride (CCl_4)-induced hepatic fibrosis in rats. Phytomedicine **33**, 28-35.
https://doi.org/10.1016/j.phymed.2017.07.005.

Hamid M, Liu D, Abdulrahim Y, Liu Y, Qian G, Khan A, Gan F, Huang K. 2017. Amelioration of CCl4-induced liver injury in rats by selenizing *Astragalus* polysaccharides: Role of proinflammatory cytokines, oxidative stress and hepatic stellate cells. Research in Veterinary Science **114**, 202–211. https://doi.org/10.1016/j.rvsc.2017.05.002.

Hassan MH, Edfawy M, Mansour A, Hamed AA. 2012. Antioxidant and antiapoptotic effects of capsaicin against carbon tetrachloride-induced hepatotoxicity in rats. Toxicology and Industrial Health **28(5)**, 428-438. https://doi.org/10.1177/0748233711413801.

Header EA, ElSawy NA, Alkushi AG. 2017. Biological effect of *Cynara cardunculus* on liver and heart status for hypercholesterolemic rats. Australian Journal of Basic and Applied Sciences **11(2)**, 41-49.

Heidarian E, Rafieian-Kopaei M. 2013. Protective effect of artichoke (*Cynara scolymus*) leaf extract against lead toxicity in rat. Pharmaceutical Biology **51(9)**, 1104–1109. https://doi.org/10.3109/13880209.2013.777931.

Hu L, Li L, Xu D, Xia X, Pi R, Xu D, Wang W, Du H, Song E, Song Y. 2014. Protective effects of neohesperidin dihydrochalcone against carbon tetrachloride-induced oxidative damage in vivo and in vitro. Chemico-Biological Interactions **213**, 51-59. https://doi.org/10.1016/j.cbi.2014.02.003.

Jahanafrooz Z, Motameh N, Bakhshandeh B. 2016. Comparative evaluation of silibinin effects on cell cycling and apoptosis in human breast cancer MCF-7 and T47D cell lines. Asian Pacific Journal of Cancer Prevention **17**, 2661–2665. https://doi.org/10.7314/APJCP.2016.17.5.2661.

Jain M, Kapadia R, Jadeja RN. 2012. Protective role of standarderized *Feronia limonia* stem bark methanolic extract against carbon tetrachloride-induced hepatotoxicity. Annals of Hepatology **11(6)**, 935-943. PMID: 23109458 [Indexed for MEDLINE].

Jimenez-Escrig A, Dragsted LO, Daneshvar B, Pulido R, Saura-Calixto F. 2003. *In vitro* antioxidant activities of edible artichoke (*Cynara scolymus* L.) and effect on biomarkers of antioxidants in rats. Journal of Agricultural and Food Chemistry **51(18)**, 5540–5545. https://doi.org/10.1021/jf030047e

Kakkar P, Singh B. 2007. Mitochondria: a hub of redox activities and cellular distress control. Molecular and Cellular Biochemistry **305**, 235–253. https://doi.org/10.1007/s11010-007-9520-8

Kalaiselvan I, Samuthirapandi M, Govindaraju A, Sheeja Malar D, Kasi PD. 2016. Olive oil and its phenolic compounds (hydroxytyrosol and tyrosol) ameliorated TCDD-induced hepatotoxicity in rats via inhibition of oxidative stress and apoptosis. Pharmaceutical Biology **54**, 338-346. https://doi.org/10.3109/13880209.2015.1042980.

Khan RA, Khan MR, Sahreen S. 2012. CCl4-induced hepatotoxicity: protective effect of rutin on p53, CYP2E1 and the antioxidative status in rat. BMC Complementary and Alternative Medicine **12(1)**, 178. https://doi.org/10.1186/1472-6882-12-178.

Kim Y, You Y, Yoon HG, Lee YH, Kim K, Lee J, Kim MS, Kim JC, Jun W. 2014. Hepatoprotective effects of fermented Curcuma longa L. on carbon tetrachloride induced oxidative stress in rats. Food chemistry **151**, 148-153. https://doi.org/10.1016/j.foodchem.2013.11.058.

Kumar CH, Ramesh A, Kumar JS, Ishaq BM. 2011. A review on hepatoprotective activity of medicinal plants. International Journal of Pharmaceutical Sciences and Research **2**, 501-515. http://dx.doi.org/10.13040/IJPSR.09758232.2(3).501-15.

Lattanzio V, Kroon PA, Linsalata V, Cardinali A. 2009. Globe Artichoke: A Functional Food and Source of Nutraceutical Ingredients. Journal of Functional Foods **1**, 131-144. https://doi.org/10.1016/j.jff.2009.01.002

Li X, Wang L, Chen C. 2017. Effects of exogenous thymosin β4 on carbon tetrachloride-induced liver injury and fibrosis. Scientific Reports **7,** 5872. https://doi.org/10.1038/s41598-017-06318-5.

Loguercio C, Federico A. 2003. Oxidative stress in viral and alcoholic hepatitis. Free Radical Biology and Medicine **34(1)**, 1–10. https://doi.org/10.1016/S0891-5849(02)01167-X

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the folin phenol reagent. Journal of Biological Chemistry **193**, 265-272. PMID: 14907713 [Indexed for MEDLINE].

Magielse J, Verlaet A, Breynaert A, Keenoy BM, Apers S, Pieters L, Hermans N. 2014. Investigation of the *in vivo* antioxidative activity of *Cynara scolymus* (artichoke) leaf extract in the streptozotocin-induced diabetic rat. Molecular Nutrition and Food Research **58(1),** 211-215. https://doi.org/10.1002/mnfr.201300282.

Mileo AM, Di Venere D, Linsalata V, Fraioli R, Miccadei S. 2012. Artichoke Polyphenols Induce Apoptosis and Decrease the Invasive Potential of the Human Breast Cancer Cell Line MDA-MB231. Journal of Cellular Physiology **227**, 3301–3309. https://doi.org/10.1002/jcp.24029

Mileo AM, Di Venere D, Miccadei S. 2016. Antitumour effects of artichoke polyphenols: cell death and ROS-mediated epigenetic growth arrest. Stem Cell Epigenetics **3**, e1242. https://doi.org/10.14800/sce.1242.

Minami M, Yoshikawa H. 1979. A simplified assay method of superoxide dismutase. Clinica Chimica Acta **92**, 337-342. https://doi.org/10.1016/0009-8981(79)90211-0

Mocelin R, Marcon M, Santo GD, Zanatta L, Sachett A, Schönell AP, Bevilaqua F, Giachini M, Chitolina R, Wildner SM, Duarte MM, Conterato GM, Piato AL, Gomes DB, Roman Junior WA. 2016. Hypolipidemic and antiatherogenic effects of *Cynara scolymus* in cholesterol-fed rats. Revista Brasileira de Farmacognosia **26**, 233–239. http://dx.doi.org/10.1016/j.bjp.2015.11.004

Moglia A, Lanteri S, Comino C, Acquadro A, Vos R, Beekwilder J. 2008. Stress-Induced Biosynthesis of Dicaffeoylquinic Acids in Globe Artichoke. Journal of Agricultural and Food Chemistry **56(18)**, 8641–8649. https://doi.org/10.1021/jf801653w.

Mokhtar N, Gouda I, Adel I. 2007. Cancer pathology registry 2003-2004 and time trend analysis. In: Mokhtar N, Gouda I, Adel I, eds. Malignant digestive system tumors. Cairo: Elsheraa Press, 55–67.

Monti A, Virgilio N, Venturia G. 2008. Mineral composition and ash content of six major energy crops. Biomass and Bioenergy **32(3)**, 216-223. https://doi.org/10.1016/j.biombioe.2007.09.012

Pearlman FC, Lee RT. 1974. Detection and measurement of total bilirubin in serum, with use of surfactants as solubilizing agents. Clinical Chemistry **20,** 447-453. PMID: 4818198 [Indexed for MEDLINE].

Reitman S, Frankel S. 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology **28,** 56-63. PMID: 13458125 [Indexed for MEDLINE].

Remien CH, Sussman NL, Adler FR. 2014. Mathematical modeling of chronic acetaminophen metabolism and liver injury. Mathematical Medicine and Biology **31(3)**, 302-317. https://doi.org/10.1093/imammb/dqt010.

Simsek EN, Uysal T. 2013. In vitro investigation of cytotoxic and apoptotic effects of *Cynara* L. species in colorectal cancer cells. Asian Pacific Journal of Cancer Prevention **14(11)**, 6791-6795. https://doi.org/10.7314/APJCP.2013.14.11.6791.

Supic G, Jagodic M, Magic Z. 2013. Epigenetic: a new link between nutrition and cancer. Nutrition and Cancer **65,** 781-792. https://doi.org/10.1080/01635581.2013.805794.

Tallman MS, Gilliland DG, Rowe JM. 2005. Drug therapy for acute myeloid leukemia. Blood **106**, 1154–1163.
https://doi.org/10.1182/blood-2005-01-0178

Vera-Ramirez L, Perez-Lopez P, Varela-Lopez A, Ramirez-Tortosa M, Battino M, Quiles JL. 2013. *Curcumin* and liver disease. Bio Factors **39**, 88–100.
https://doi.org/10.1002/biof.1057.

Vitaglione P, Morisco F, Caporaso N, Fogliano V. 2005. Dietary antioxidant compounds and liver health. Critical Reviews in Food Science and Nutrition **44(7–8),** 575–586.
https://doi.org/10.1080/10408690490911701

Wang M, Simon JE, Aviles IF, He K, Zheng QY, Tadmor Y. 2003. Analysis of antioxidative phenolic compounds in artichoke (*Cynara scolymus* L.). Journal of Agricultural and Food Chemistry **51(3),** 601–608.
https://doi.org/10.1021/jf020792b

Xu L, Gao J, Wang Y. 2011. *Myrica rubra* extracts protect the liver from CCl4-induced damage. Evidence-Based Complementary and Alternative Medicine.
https://doi.org/10.1093/ecam/nep196.

Yao Y, Wang L, Jin P, Li N, Meng Y, Wang C, Xu M, Zhang Y, Bian J, Xiaoming Deng X. 2017. Methane alleviates carbon tetrachloride induced liver injury in mice: anti-inflammatory action demonstrated by increased PI3K/Akt/GSK-3β-mediated IL-10 expression. Journal of Molecular Histology **48(4),** 301-310.
https://doi.org/10.1007/s10735-017-9728-1.

Zapolska-Downar D, Zapolski-Downar A, Naruszewicz M, Siennicka A, rasnodebska B, Koldziej B. 2002. Protective properties of artichoke (*Cynara scolymus*) against oxidative stress induced in cultured endothelial cells and monocytes. Life Sciences **71,** 2897–2908.
https://doi.org/10.1016/S0024-3205(02)02136-7