ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Protective and Antioxidant Effects of *Cynara Scolymus* Leaves against Carbon Tetrachloride Toxicity in Rats.

Madeha MAbdel-Kader[1], Eman M El-Sayed[*1], Seham S Kassem[1], Mohamed H. A. Shams El-Din[2], Mohammed MHaggag[3], and Zeinab El-Hawary[1]

[1]Department of Nutrition and Food Science .National Research Center, Cairo, Egypt.
[2]Department of Food Technology, National Research Centre Dokki, Cairo, Egypt.
[3]Department of Nutrition and Food Science, Faculty of Home Economics, Helwan University, Cairo, Egypt.

ABSTRACT

Cynarascolymus (artichoke) plant has many natural antioxidants. Artichokeleaves extract have multiple pharmacological actions. This study was designed to explore the potential of dietary artichoke leaves in the management of hepatic and renal dysfunctions induced by carbon tetrachloride (CCl_4) intoxication. Diets containing 20% or 40% artichoke leaves were provided to rats treated with CCl_4 (1ml/kgI.P.). Our data showed that artichoke treatments significantly restored the elevated activities hepatic enzymes; alanine aminotransferase (ALT), aspartate aminotranseferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH), ina dose dependent manner. Besides, plasma creatinine, urea and uric acid levels were markedly reduced by artichoke treatments as compared to CCl_4 group. Oxidative stress induced by CCl_4 was extremely managed by artichoke supplementation as the activities of both erythrocytes superoxide dismutase (SOD) and plasma catalase were noticeably increased by artichoke in a dose dependent manner as compared to those of CCl_4 group. These results demonstrate that artichoke leaves have a marked antioxidative and protective potential against CCl_4 intoxication.

Keywords: Artichoke leaves, Carbon tetrachloride, Hepatoprotection, Renal protection, Oxidative stress.

Corresponding author
Eman M. El-Sayed
Email: emanalsayed2000@yahoo.com

ISSN: 0975-8585

INTRODUCTION

Reactive oxygen free radicals have been known to produce tissue injury through covalent binding and lipid peroxidation and have been shown to augment fibrosis [1].Theymay also be a contributory factor in a progressive decline in the function of immune system[2].Cooperative defense systems that protect the body from free radical damage included the antioxidant nutrients and enzymes. Carbon tetrachloride (CCl_4) intoxication in animals is an experimental model that mimics oxidative stress in many physiological situation [3].The initial step in the tissue injury induced by CCl_4 is its cytochrome P450-mediated transfer of a single electron to the C-Cl bond, giving a radical anion as a transient intermediate that eliminates chlorine to form a carbon-centered radical, the trichloromethyl radical ($^{\bullet}CCl_3$) andchloride. $^{\bullet}CCl_3$can bind to macromolecules or attack fatty acidsin cellular membranes. The tricholoromethyl radical can also react with oxygen to form the peroxytrichloromethyl free radical ($^{\bullet}CCl_3O_2$), which is more reactive than ($^{\bullet}CCl_3$) and produces similar kinds of damage. $CCl_4$is considered a hepatotoxic[4]and n**e**phrotoxic chemical[5].Many natural antioxidants have been shown counteract the damage induced by CCl_4 such as dietary polyphenolic compounds[6], vitamin C[7]and vanillin[5].

Artichoke (*CynaraScolymus L*.) is one of the world`s oldest medicinal plants. It is belong to the family (*Asteraceae*).It has many medicinal properties and used as remedy. It has a hypoglycemic effect [8],and health promoting properties in preventing cardiovascular disease(CVD) by its hypolipidemic action[9]orby up regulation of endothelial nitric-oxide synthase or (eNOS)gene expression and eNOS protein expression [10]. Also, artichoke was reported to have anticancer effects by increasing apoptosis either in hepatocellular carcinoma in rats [11]or human hepatoma cells [12].Artichokeleaves have a protective actions against dyspepsia [13], chronic hepatitis-C [14] and other liver complaints[15].

Artichoke leaves (AL) extracts are known to have antioxidant potential which mainly results from their constituents, as cynarin, chlorogenic acid and flavonoids [16].In addition to their prebioticssuch as inulin which affects many physiological processes[17]. Pure compounds of ALhave been shown to produce less antioxidant activity than the extract itself [15].

In Egypt, the artichoke canning industry generates large amount of agricultural waste consisting mainly of the leaves ,stem and the bracts which are not suitable for human consumption and theycould be used as a source of food addative and nutratraceuticals .As far as we know ,there is no study concerning the effect of dietary artichoke leaves powder against CCl_4toxicity. Therefore, the object of this study was to evaluate the protection of artichoke byproduct (leaves) against CCl_4-induced damage in rat kidney and liver. Also, we tried to establish the mechanism by which such protection was achieved. Phenolic compounds in the leaves were also evaluated.

MATERIAL AND METHODS

Plant material

Artichoke leaves were collected after fresh handling processing of artichoke hearts. They authenticated in phytochemistry and plant systematic department at National Research Center (NRC), Egypt. Avoucher specimen has been deposited at the Herbarium units of NRC. Leaves were air dried at room temperature and powdered. The powdered samples were placed in air tight container for evaluation of phenolic compounds, and preparing the diets.

Determination of total phenolic compound

100mg of dried leaves powder were strongly shaked with 10ml of hydrochloric acid (0.1N). The mixture was heated at 100 ^{0}Cfor 1 hour, and filtered. One ml of the filtrate was mixed with 0.5ml folinCiocalteu reagent (diluted 1:1 with distilled water) and1ml of sodium carbonate and the volume was completed to 10 ml with distilled water. After incubation for30 min.at room temperature, the absorbance was determined at 750nm using Spectrophotometer against prepared reagent as blank. Total phenplic contents in samples were expressed as mg tannic acid equivalents (mg/g dry weight) .All samples were analyzed in triplicates[18].

Determination of total flavonoid content (TFC)

TFC of artichoke leaves was spectrophotometrically estimated by vanillin reagent using catechin as a standard [19].Briefly, 0.2gm dried leave powder was extracted for 1 hour at room temperature with10 ml methanol / HcL (1%). 0.5 ml of the filtrate was vigourslyshacked with 2.5 ml vanillin reagent and then incubated at 30 ^{0}C for20 minutes. The absorbance was measured against prepared blank at 500nm. TFC was expressed as mg catechin equivalent /g dry weight. Samples were analyzed in triplicates.

Identification of phenolics by HPLC

Phenolic compounds were extracted from powdered leaves by methanol according to Duke et al. [20]**.** Themethanolic extract was injected in high performance liquid chromatography (HPLC). Phenolic compounds were identified and evaluated by comparing their relative retention times with those of standards.

Animals

Male Sprague-Dawely rats weighing 120-150gm were purchased from Animal House of NRC, Dokki, Giza, Egypt. They were housed individually at roomtemperature (25±2^0)under 12 hr dark-light cycle. They were fed ad labitumwith standard diet and tap water for 1 week prior to experiment. Procedures and animal comfort were controlled by the guidelines of Animal Care and Ethics Committee of NRC.

Experimental procedure

Animals were divided into four groups each of six rats. Group 1 served as normal control, receiving normal saline (1ml /kg) and fed with balanced diet (Table 1). GroupII served as CCl_4 control that received 1ml CCl_4/kg body weight intraperitoneally[21]**,** twice per week for two weeks and fed with balanced diet. Group III injected with CCl_4in the same manner as group II but fed 20% artichoke diet. Meanwhile, group IV fed 40% artichoke diet and administered CCl_4 as previous two groups. The experiment was continued for 28 days. At the end of the experiment, rats were fasted over night, anaesthetized by light ether and blood samples were obtained from retro-orbital vein on heparin. One part of the blood was used for the determination of SOD in the erythrocytes. While plasma was separated from the other part and stored at -8o^{0}Cfor further analysis.

Table 1:Composition of the tested diets(g / kg).

Ingredients	Control[a]	Artichoke	
		20%	40%
Casein	120	120	120
Sucrose	100	100	100
Hydrogenated fat	100	100	100
Oil	50	50	50
Salt mix.	35	35	35
Vitamin mix.	10	10	10
L-Cystin	1.8	1.8	1.8
Cholin chloride	2.5	2.5	2.5
Coriander powder	-	200	400
Starch	580.7	380.7	180.7

[a:]Control diet contents [22]

Analytical methods

Measurement of liver function markers

Plasma aspartate and alanine aminotransferases activities were assayed colorimetricallyusing BioDiagnosticskit [23].Alkaline phosphatase activity was determined according to the method of Belfied and Goldberg [24]using commercial kit. Finally, lactate dehydrogenase activity was evaluated by kinetic procedure [25] using Biosystem S.S.A kit.

ISSN: 0975-8585

Measurement of kidney function markers

Plasma urea, uric and creatinine were evaluated colorimetrically using commercial kits provided from BioDiagnostics [26-28].

Measurement of antioxidant parameters

Erythrocyte SOD was evaluated according to the method of Nishikimi et al. [29]. Catalase activity was assessed by the decrease in the absorbance that corresponds to H_2O_2 decomposition [30].

Statistical analysis

Data were expressed as mean ±standarderror.Student's T-Test (2-tailed) was applied to compare between groups. Differences were considered to be significant at $P<0.05$.

RESULTS

Phytochemical determinations revealed that total polyphenolic compounds in artichoke leaves were 855mg/gm dry weight as tannic acid equivalents. Also the evaluation of total flavonoids was determined as catechin equivalents. one gram of artichoke leaves powder had 11.09 mg catechinequivalent.HPLC analysis demonstrated that coumarin 1,1.30,protocatechol 0.82,p-hydroxybenzoic acid 0.9% and salicylic acid 5.64%were the major phenolic constituents in the methanolic extract of artichoke leaves according to the available standards in our laboratories.

Table 2 shows the effects of feeding artichoke diets on plasma levels of hepatic enzymes. Where the hepatotoxicity induced by CCl_4 was evidenced by significant ($P<0.001$) increase in AST, ALT, ALP and LDH activities.The percentage increases were 29.3, 123, 153 and 49, respectively as compared to normal group. Treatment by artichoke considerably reduced such elevation in hepatic enzymes activities as compared with CCl_4 group.The restoring effect of artichoke was dose dependent as a diet containing 40% artichoke exhibited more pronounced effects than that had 20% artichoke.

Table 2: Effects of artichoke on CCL_4 induced hepatotoxicity.

Groups / Parameters	Control	CCL_4	CCL_4 + 20% Artichoke	CCL_4 + 40% Artichoke
AST (U/ml)	51.8±o.448	67^{a}*±1.49	45.9^{b}*±1.25	37.3^{b}*±1.27
ALT (U/ml)	16.3±0.514	36.4^{a}*±1.85	18.7^{b}*±0.228	16.9^{b}*±0.153
ALP (U/L)	105±0.547	266^{a}*±10.6	197^{b}*±1.48	155^{b}*±3.52
LDH (U/L)	116±0.968	173^{a}*±5.23	141^{b}*±0.723	117^{b}*±0.958

Values are expressed as mean ± S.E.
[a]: values significantly differ from normal control.
[b]: values significantly differ from CCL_4 group
*: $P< 0.01$

Nephrotoxicity induced by CCl_4 was manifested by a significant alteration ($P<0.001$) in plasma levels of urea, uric acid and creatinine.The increase was 30.4%, 73% and 51%, respectively as compared to control. Feeding artichoke diets significantly ($P<0.001$) ameliorated the concentrations of urea, uric acid and creatinine in the plasma as compared with CCl_4-treated rats. Both the 20%and 40% artichoke diets approximately exerted the same ameliorating effects (Table3).

ISSN: 0975-8585

Table3: Effects of artichoke on CCL_4 induced nephrotoxicity.

Groups / Parameters	Normal Control	CCL_4	CCL_4 + 20% Artichoke	CCL_4 + 40% Artichoke
Urea (mg/dl)	35.2±o.702	45.9[a]*±o.411	35.2[b]*±1.32	35.5[b]*±1.47
Uric acid (mg/dl)	2.51±0.154	4.35[a]*±0.15	2.83[b]*±0.255	2.78[b]*±0.08
Creatinine (mg/dl)	0.77±0.056	1.16[a]*±0.012	1.04[b]*±0.020	0.925[b]*±0.029

Values are expressed as mean ± S.E.
[a]: values significantly differ from normal control.
[b]: values significantly differ from CCL_4 group
*: P< 0.01

The status of enzymatic antioxidant is presented in Table (4).CCl_4 treatment significantly (P<0.001) reduced the activities of SOD and catalase. The percentage of decrease was 48.9 and 82.5,respectively as comparedwith control group. Administration of artichoke diets brought marked improvement (p<0.001) of antioxidant enzymes activities as compared to CCl_4- treated group. The restoring effect onenzymes activities by artichoke was dose dependent;40% artichoke diet showed more marked effect than 20% artichoke diet.

Table4: Effects of artichoke on antioxidant enzymes

Groups / Parameters	Normal Control	CCL_4	CCL_4 + 20% Artichoke	CCL_4 + 40% Artichoke
SOD (U/ml)	399±o.86	204[a]*±2.50	234[b]*±3.66	299[b]*±0.827
Catalase (U/ml)	600±18.50	105[a]*±9.17	250[b]*±20.10	457[b]*±43.300

Values are expressed as mean ± S.E.
[a]: values significantly differ from normal control.
[b]: values significantly differ from CCL_4 group
*: P< 0.01

DISCUSSION

Liver is the largest and most complex internal organ in the body. It plays an important role in the maintenance of internal environment through its multiple and diverse functions[31]. Liver is the first organ to metabolize all foreign compounds and hence it is susceptible to many diseases. Also, the kidney removes the metabolic wastes, the xenobiotics and their metabolites which expose the kidney to numerous disorders. Synthetic or conventional drugs used in treatment of those vital organs are either inadequate or can have serious adverse effects.So, there is a worldwide trend togo back to traditional medicinal plants and functional food [32].Many studies have reported that antioxidants that present in natural products can diminish nephropathy and hepatic damage caused by various toxins [7].Artichoke was reported as one of the oldest medicinal plants used as a remedy[33]. Therefore, thepresent study was designed to evaluate the efficacy of artichoke by products in the management of hepatic and renal toxicity induced by CCl_4.

CCl_4injection induced liver injury mediated by reactive oxygen species as normal metabolites in the hepatocytes.Where,the active metabolite of CCl_4, CCl_3^* binds to the macro-molecules and inducesperoxidativedegradation of membrane lipids.Also, CCl_3^* and other toxic intermediates initiate the chain reaction of lipid peroxidation which attacks polyunsaturated fatty acids. This affects the permeability of mitochondrial, endoplasmic reticulum and plasma membranes. Resulting hepatocelluar necrosis and increase in plasma levels of hepatic enzymes[34].Besides, the increased cholesterol/phospholipids ratio in serum and liver by CCl_4 administration indicates that the membrane integrityand fluidity have been compromised [35].In addition,loss of phospholipid asymmetry triggers apoptosis [36].In this context, Rahmat et al.,also reported that CCl_4 significantly inactivated calcium ATPase activity, consequently caused cytosolic calcium overload that may contribute to mitochondrial dysfunction which is considered from pro-apoptotic signals.

Our emerged data revealed that liver enzymes; AST, ALT, andALP, were significantly elevated in the plasma of rats treated by CCl_4 confirming an extreme necrosis of hepatic cells. These results are in accordance with those of Murayama et al., Abdalla et al. andAldoba-Muruato et al. [37-39].It is clear from the increased enzymatic levels that ALP was the most affected enzyme meanwhile ASTwas the least one. Lin et al.[40], demonstrated that ALT is more sensitive test for hepatocellular damage than AST.However,the elevated activities of both are indicative of cellular leakage and loss of functional integrity of cell membranes,since these cytosolic enzymes are extremely released into circulation after hepatic damage[41].While, ALP isexcreted normally via bile by the liver.CCl_4impair the integrity, structure and function of the hepatocytes via its ROS,leading to defective secretion of the bile due to the damaged bile ducts consequently elevation of ALP level in the blood [6]. Also, our results show an increase in LDH activity which coincides with the finding reported by Motawi et al.and Breikka et al.[42,43].$LDH_4$and LDH_5 are two isomers of LDH that are predominantly present in the liver .Rodrigue et al.[44],reported that increased LDH activity in plasma is indicative to major necrosis in the liver during intoxication.

Nephrotoxicity induced by CCl_4 in our animals was manifested by a marked increase in urea, uric acid and creatininelevels. This result was previously reported [45,46,35].In our work uric acid level was the most affected renal marker ,followed by creatinine level and then urea concentration .However, there have been controversial reports concerning the levels of biomarkers of kidney function in CCl_4-induced toxicity depending on its dose, route of administration and duration of treatment.Manna et al.[47],found nochange in serum urea or creatinine.Meanwhile,Bashandy and Al-Wasel[7],demonstrated that urea level was decreased and creatinine level was elevated by CCl_4 intoxication.

Increased levels of renal function biomarkers could be attributed to a marked damage in the glomeruli and tubules resulted from ROS generated by CCl_4.Morever, CCl_4 produced hydroperoxide accumulated in the kidney and caused cytotoxicity.Besides, CCl_4 produced a local ischemic environment that aggravates cellular damage[48].Also, DNA fragmentation wasa hall mark feature produces renal necrosis by CCl_4[46].

Oxidative stress is an imbalance between ROS production and antioxidative mechanisms[49].The results of the present study revealed that CCl_4-induced oxidative stress as evidenced by the significant depletion of antioxidant enzymes, SOD and catalase.Similar results were reported [40,42,50].

The coordinate actions of various cellular antioxidants are critical for the effective detoxification of free radicals [51]. SOD, catalase and glutathione peroxidase (GPx) work in concert to detoxify superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2). O_2^- generation was increased from the free radical chain reactions initiated by CCl_4**.** SOD catalyzes the dismutation of superoxide radical to H_2O_2 which is consequently reduced by catalase and GPx. Overproduction of O_2^- extremely consumed SOD and catalase leading to their depletion . Generally, such antioxidant enzymes are easily inactivated by lipid peroxides or ROS.

Where the end product of lipid peroxidation by $CCl_4$is malonaldhyde that compromised the conformation and biological activity of antioxidant enzymes by reacting with their amino, sulfhydryl, and imidazole groups [52].Besides,CCl_4 down regulates the expression of SOD and catalase genes [53].

Treatment with artichoke leaves counteracted the deleterious effects of CCl_4 on liver and kidney and protected both from injury. Where, our emerged data revealed that artichoke diets significantly ameliorated the increase of hepatic AST, ALT and ALP which in accordance with the results of Mehmetick et al. [54], Kücükgerin et al. [55],Abdalla et al. [38]and Heidarian andRafieian-Kopaei[56].Also, artichoke treatment significantly reduced the elevated LDH activity by CCl_4 which in agreement with the result of Gebhardt[57].Besides, renal function biomarkers showed a significant improvement by artichoke where plasmalevels of urea, uric acid and creatinine were markedly reduced. This result was previously reported [58,59].Also, oxidative stress induced by CCl_4 was opposed by artichoke treatment, as appeared from the significant induction of both SOD and catalase activites. Such antioxidant effect of artichoke was previously reported [38,60-62].

Hepatic and renal protection by artichoke leaves involves many mechanisms that must be taken into consideration. Artichoke leaves contained many pharmacologically active ingredients including phenolic compounds, minerals and dietary fiber. The principlephenolicsare phenolic acids as cynarin, chlorogenic acid

and caffeic acid and flavonoids such as luteolin and apigenin[63].Also, Ibrahim et al.[64],reported that artichoke leaveshave significant levels of silymarin a flavonoid that with other phenolics exhibited powerful antioxidant activity via radical scavenging and their iron chelating activity. Their lipophilic properties, high affinity to and permeability through plasma membranes facilitate their actions.Besides, these active principles are structurally related and exert their actions synergistically. Therefore, phenolics prevent ROS from reaching biomolecules; polyunsaturated fatty acids, DNA, and proteins.Moreover,silymarin promotes ribosomal RNA synthesis,which stimulates liver regeneration[65].Additionally, artichoke extracts may have an anti-apoptotic effects as they protect the mitochondria and restore mitochondrial function [66].It has been demonstrated that phenolics protect ca-ATPase activity and membrane fluidity and integrity which were critical in preventing cytosolic calcium overload ,alteration of calcium homeostasis mitochondrial membrane collapse,cytochrome C release and caspase-3 activation and ultimately mitochondrial mediated cell death by CCl_4[35].

Also CCl_4upregulates the gene expression of many inflammatorycytokines and activating nuclear factor kappB [67] that participate in tissue injury. Artichoke exhibited an anti-inflammatory potential [68], and enhanced the immunity [59].In addition,many phenolic compounds induce xenobiotic detoxification enzymes of phase I and II[69, 54]subsequently increase CCl_4detoxification with concomitant tissue protection and antioxidants restoring.

In conclusion, our findings revealed that feeding artichoke leaves protected against CCl_4induced hepatic and renal toxicity as manifested by ameliorating hepatic and renal biomarkers. Moreover, artichoke leaves treatment improved the levels of antioxidant enzymes by their phenolic bioactive constituents. Our results clearly demonstrated that artichoke byproducts are rich source of nutraceuticals that have health promoting properties with no deleterious effects.

REFRENCES

[1] Geesin, J.C.; Gordon, J.S. and Bergand, R.A. 1990. Arch. Biochem. Biophys. 278: 350-355.
[2] Pike, J. and Chandra, R.K.1995. Int. J. Vit. Nutr. 65: 117-120.
[3] Ivor, J.B and Schneider, M.D. 2005. J. Clin. Invest. 115: 495-499.
[4] Weber, L.W.; Boll, M.; and Stampfl, A. 2003. Crit. Rev. Toxicol. 23: 105-113.
[5] Makni,M.,Chtourou, Y. and Garoui, E.M. 2012.Human and Experimental Toxicology. 31:844-852.
[6] Sreelatha, S., Padma , P.R. and Umdevi , M.2009. Food and Chemical Toxicology. 47: 702-708.
[7] Bashandy, S.A. and Al-Wasel, S.H. 2011. J. Pharmacol. Toxicol. 6: 283-292.
[8] Kim, O.O.; Jeong, S.W. and Lee, C.Y. 2003. Foodchem. 81: 321-326.
[9] Rondanelli, M.; Monteferrario, F.; Perna, S.; Faliva, M.A. and Opizzi, A. 2013. Monaldi Arch. Chest Dis. 80: 17-26.
[10] Li, H.; Xia, N.; Brausch, I.; Yao, Y. and Forster-mann, U. 2004. J. Pharmacol. Experimental Therapeutics. 310: 926-932.
[11] Metwally, N.S.; Kholeif, T.E.; Ghanem, K.Z.; Farrag, A.R.; Ammar, N.M. and Abdel-Hamid, A.H. 2012. Eur. Rev. Med. Pharmacol. Sci 15:1429-1444.
[12] Miccadei, S.; Di Venere, D.; Cardinali, A. et al. 2008.Nutr. Cancer 60: 276-283.
[13] Sannia, A. 2010. MinervaGastroenterology Dietol.56:93-9.
[14] Huber, R.;Müller, M.; Naumann, J.; Schenk, T. and Ludtke, R. 2009. Phytomedicine, 16:801-4.
[15] Speroni, E.; Cervellati, R.; Govoni, P.; Guizzardi, S.; Renzulli, C. andGuerra, M.C.2003. J. Ethnopharmacol 66:203-211.
[16] Perez- Garcia, F.; Adzet, T. and Canigueral, S. 2000. Free Rad. Res. 33: 661-665.
[17] Kaur, N. and Gupta, A. K. 2002. J. Biosci. 27: 703-14.
[18] A.O.A.C. 1990. Association of Official Analytical Chemists. (15thed) Washaington,D.C.
[19] Price,M.L.Van-Scoyo,C.S.and Butter,L.O.1978.J.Agri.Food Chem.26:5-6.
[20] Duck, O. Rimanob,A.M.,Pace,P.F.Reddy,K.N.andSemeda,K.J.2003.J.Agri.Food Chem.51:344-350.
[21] Wong,C.K.,Ooi,V.E.C.andWong,C.K. 2003.Enviro.Toxicol. Pharm. 14:109-116.
[22] A.O.A.C. 2000.Association of Official Analytical Chemists (Official Methods of Analysis International), 17th Ed., Gaithersburg, MD, U.S.A.
[23] Reitman,S.andFrankel,S.1957. Am.J.Clin.Path.28:56-72.
[24] Belfield,A.andGoldberg,M.D.(1971.Enzym.12:561—573.
[25] Young, D.S. 1995. Effects of Drugs on Clinical Laboratory Tests, 4th Edition, AACC Press, Washington, D.C.

[26] Fawcett,J.W.andScatt,J.E.1960.J.Clin.Path.13:156.
[27] Watts, R.W.F. 1974. Ann. Clin. Biochem. 11:103-111.
[28] Hauot, O. 1985. interpretation of clinical laboratory tests,Edited by SiestG.,Henny,J.Schiele,F.andYoung,D.S.(Biomedical Publication) pp: 220.
[29] Nishikimi.M.,Rea,N.A.andYagi.K.X.1972.Biochem.Biophs.Res.Commun 46:849-853.
[30] Aebi, H. 1984.Methods Enzymol.105:121-126.
[31] Wang, N.; Wang, L.P. and Peng, W. 2008. J. Ethanopharmacol 116:1-6.
[32] Block, G.; Peterson, B. and Subar, A. 1992. A review of epidemiological evidence. Nutra Cancer, 18:1-29.
[33] Englisch, W.; Beckers, C.; Unkauf, M. and Zinserling, V. (2000. Arzneim-Forsch 50: 260-265.
[34] Koneri, R.; Balaraman, R. and Firdous, K. M. V. 2008. Pharmacology 1: 365-374.
[35] Rahmat, A.A.;Dar, F.A. and Choudhary, I.M. 2014.Phrmacognosy Res. 6:19-28.
[36] Fadok, V. A.; De Cathelineau, A.; Daleke, D. L.; Henson, P.M. and Bratton, D.L. 2001. J. Biol. Chem. 276: 1071-1077.
[37] Murayama, H.; Ikemoto, M.; Fukuda, Y. and Nagata, A.2008. Clin. Chim. Acta. 391:31-35.
[38] Abdalla**,** O.A.; Risha, E.F. and El-Shopakey G. E.2013. Life Science J. 10:1436-1444.
[39] Aldoba-Muruato, L.R.Moreno,M.G,Shibayanna,M.,Tsutsumi,V.andMuriel, P.2013.Pharmacology. 92: 138-149.
[40] Lin, C.C.; Shich,D.C. and Yen, M.H.1997. J.Ethnopharm. 56: 193-200.
[41] Rajesh, M.G. and latha , M.S. 2004. Ind. J. pharmacy 36: 254 –256.
[42] Motawi, T.K., Hamed, M.A., Shabana, M.H., Hashem, R.H. and Aboul Nasr, A.F. 2011.Nutri.Metab.8:40-54.
[43] Breikaa, R.M., Algandaby, M.M., El-Demerdash,E.,Abdel-Naim, A.B. 2013. Bioscoi. Biotechnol. Biochem. 77:909-916.
[44] Rodrigue,F.,Boyer,D.,Feillet,F.,lemonnier,A.1995.Transplant. Proc. 27:1871 – 1874.
[45] Ozturk,F., Ucar,M., Ozturk, I.C., Vardi,N. and Batcioglu,K.2003. Urology 62:353-356.
[46] Makni,M.,Chtourou,Y.,Garoui,E.M.2013. Human and Experimental Toxicol. 31:844-852.
[47] Manna, P.; Sinha, M. and Sil, P.C. 2006.BMC Complement Altern. Med.6:33.
[48] khan ,S.A,. Priyamvadca, S., andYusufi,A.N,.K.2009. Pharmacological Research 59: 254 – 262.
[49] Frei, B. 1994. Am. J. Med. 97: 13-22.
[50] Ottu, O.J., Atawodi, S.E.,and Onyike,E,2013.Asian Pac. J. Trop. Med. 6: 609 – 615.
[51] Yang, J.; Li, Y.; Wang, F. and Wu, C. 2010. J. Agric. Food Chem. 58: 6525-6531.
[52] Esterbauer, H.,Schaur, R.J., and Zollner, H. 1991. Free Radic. Boi. Met. 11: 81 – 128.
[53] de Oliveira e Silva, A.M.; Vidal- Novoa, A.; Batista- Gonzalez, A.E. et al. 2012. Redox Rep.17:47-53.
[54] Mehmetcik, G.; Ozdemirter, G.; Kocak-Toker, N.; Cerikbas, U. and Uysal, M. 2008. ExpToxicol. Pathol. 60: 475-480.
[55] Kücükgergin, C.; Aydin, A.F.; Ozdemirler-Erata,G.;Mehmetcik, G.;Kocak- Toker,N.; Uysal, M. 2010:. Biol. Trace Elem. Res. 135: 264-274.
[56] Heidarian, E. and Rafieian- Kopaei, M.2013.Pharm. Biol. 51:1104-9.
[57] Gebhardt, R. 1997. Toxicol. Appl. Pharmacol. 144: 279-286.
[58] Ghanem, K.Z.; Ramadan, M.M.; Farrag, A.H.; Ghanen, H.Z. and Farouk, A. 2009. Pol. J. Food Nutr. Sci. 59: 175-181.
[59] Stoey, S.D.; Djuvinov, D.; Mirtcheva, T.; Pavlov, D. and Mantie, P. 2002. Toxicol. Lett. 135:33-50.
[60] Aktay, G.; Deliorman, D.; E.; Ergun, F.; Yesilada, E. and Ceevik, C.2000. Ethnopharmacology 73:121-129.
[61] Jimenez- Escrig, A.; Dragsted, L.O.; Daneshvar, B.; Pulido, R. and Saura- Calixto, F. (2003). Agric. Food Chem. 51:5540-5545.
[62] Gaafar, A.A.; Salama, Z. A.; El-Buz, F. K.2013. Nat.Sc. Res. 3: 17-24.
[63] Lattanzio, V.; Cicco, N. and Linsalata, V. 2005. Acta. Hort. 681:421-428.
[64] Ibrahim, G.E.; El-Raey, M. A.; Eldahshan, O.A. and Souleman, A.M.A.2013. EuropScien. J. 9: 100-110.
[65] Fraschini, F.; Demartini, G. and Esposti, D. 2002. Clin. Drug Invest. 22: 51-65.
[66] Juzyszyn, Z.; Czerny,B.; Mysliwiec, Z.; Pawlik, A. and Drozdzik, M. 2010. Phytother Res. 24: 123-8.
[67] Yeh, Y.H.,Hsieh,Y.L., Lee, Y.T. (2013):. J.Agric Food chem. 61:7387-7393.
[68] Gurel, E.; Ustunova, S.; Erqin, B.; Tan, N. et al. 2013. Chin J. Physiol. 56: 253-62.
[69] Sotelo- Felix, J.I.; Martinez- Fong, D.; Muriel, P. et al. 2002:. J. Ethnopharmacology 81: 145-15