EVALUATION OF ANTIOXIDANT PROPERTIES OF SILYMARIN AND ITS POTENTIAL TO INHIBIT PEROXYL RADICALS *IN VITRO*

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ABSTRACT

Silymarin (milk thistle) has been used for centuries as a natural remedy for diseases of liver and biliary tract. The present study was carried out to evaluate the *in vitro* free radical scavenging activity and antioxidant properties of silymarin. Antioxidant properties of silymarin were evaluated by four methods: 1) FARP assay for total antioxidant capacity; 2) DPPH radical scavenging assay; 3) the inhibitory effect on RBC hemolysis induced by peroxyl radicals from APPH; 4) the inhibitory effect on plasma oxidation induced by Cu^{2+}. But total phenolic compound was evaluated by Folin-Ciocalteu method.

Total polyphenol compounds of silymarin was 0.484±0.017 mg Gallic acid equivalent per mg in comparison to that of green tea which is 0.313±0.095. High Antioxidant properties and significant protective effect of silymarin were in a concentration dependent manner in all methods. Therefore, silymarin is a powerful antioxidant herbal drug which can protect biological systems against the oxidative stress. It is suggested that silymarin may be used in preventing free radical-related diseases as a dietary natural antioxidant supplement.

Keywords: Silymarin, total antioxidant capacity, phenolic compounds.

INTRODUCTION

Reactive oxygen species (ROS) including free radicals such as superoxide anion radicals (O_2^-), hydroxyl radicals ($HO^{\cdot}$), hydrogen peroxide (H_2O_2) and peroxyl radicals ($R\text{-}COO^{\cdot}$) are active oxygen components that are often generated during aerobic conditions (Halliwell and Gutteridge, 1984). ROS are usually highly reactive and short-lived, known to cause damage to cellular components including lipid, DNA, protein, carbohydrate, and other biological molecules .They consequently lead to many pathological processes such as aging, cancer, cardiovascular diseases, diabetes, inflammation and neurodegenerative diseases (Casetta *et al.*, 2005; Haidara *et al*., 2006; Junqueira *et al*., 2004; Piconi *et al*., 2003; Singh *et al.*, 2005; Valko *et al*., 2006). Fortunately, biological systems can protect themselves against harmful effects of ROS and free radicals by designing and formation of antioxidants (Fang *et al.*, 2002). Antioxidant defense system includes enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and also nonenzymatic agents with endogenous and exogenous sources such as uric acid, bilirubin, Vitamin E, Vitamin C, carotenoids, polyphenols and etc. (Halliwell, 1995; Huang *et al.*, 2005). In recent years, antioxidants have been subjected to many epidemiological studies that have related their consumption to a reduction in the incidence of oxidative damage related diseases. Therefore, much attention has been focused on the use of antioxidants-specially natural antioxidants-for improvement of human health (Liu and Ng 2000; Zheng and Wang 2001).

Silymarin is a purified extract from the seeds of *Silybum marinum L*. (Asteraceae) also called "milk thistle". It is shown that silymarin consists of a large number of flavolignans, including silybin (or silybinin), which is the most active component, isosilybin, silydianin and silychristin (Khan *et al.*, 2006). Currently, silymarin is widely used as a hepatoprotectant and as a supportive therapy of liver disorders such as cirrhosis, hepatitis and fatty acid infiltration due to alcohol and toxic chemicals (Ball and Kowdley, 2005; Kren and Walterova, 2005). Silymarin also showed the beneficial effects in the case of radiation injury to the membrane of liver cells (Ramadan *et al*., 2002). It seems silymarin is capable to protect liver cells directly by stabilizing the membrane permeability through inhibiting lipid peroxidation and preventing liver glutathione depletion (Skottova *et al.*, 2003). Recent studies suggested that silymarin and its polyphenolic fraction could have beneficial effects on some risk factors of atherosclerosis. The results have shown that silymarin has hypolipidemic effect (Sobolova *et al*., 2006) and preventive effect on low density lipoprotein (LDL) peroxidation in vitro (Locher *et al*., 1998). Silymarin has also protective effects against stress-induced gastric ulcers and induces recovery of pancreatic function after alloxan damage in rats (Soto *et al*., 2004). The results have shown anticarcinogenic and cancer chemopreventive effects of silymarin. The combination of silibin and mitoxantrone exhibits a pattern of synergy to inhibit cell growth and induce apoptosis in human prostate cancer (Flaig *et al*.,

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2007). It is suggested that many of these properties may be related to the antioxidant and free radical scavenging activity of silymarin. For example, silymarin increases antioxidant enzymes and reduced glutathione in alloxan-induced diabetes in rat pancreas (Soto *et al*., 2003). Silymarin and its related compounds are also found to be scavengers of active oxygen such as the superoxide anion radicals, hydroxyl radicals and hydrogen peroxide (Kiruthiga *et al*., 2007a; Pascual *et al*., 1993). In addition to these active oxygen species, peroxyl radicals ($ROO^{\cdot}$) and alkoxyl radicals ($RO^{\cdot}$) are soluble in membranes, considered to be the major generators of lipid peroxides, which cause many kinds of diseases (Zennaro *et al*., 2007).

However, as far as we know there are no reports on the estimation of scavenging activity of silymarin against peroxyl radicals in biological system like red blood cell (RBC) and also its inhibitory effect on plasma oxidation *in vitro*. Therefore, the present study evaluates the antioxidant activities of silymarin by using several bioassays: total antioxidant capacity, DPPH radical scavenging activity, anti-hemolysing effect through the peroxyl radical scavenging activity, inhibitory effects on plasma oxidation induced by copper ions and total phenolic compounds. In the present study, we also compare the antioxidant activity of silymarin with green tea and some standard antioxidants.

MATERIALS AND METHODS

Chemicals

Chemicals and reagents including 1, 1-diphenyl-2-picrylhydrazyl (DPPH), 2, 4, 6- tripyridyl-s-triazine (TPTZ), and Butylated Hydroxy Toluene (BHT) were purchased from Fluka Company (Buchs, Switzerland). Vitamin C was obtained from Merck (Darmstadt, Germany) and Vitamin E was from Sigma Chemical Co. (St. Louis, USA). 2, 2′- azobis 2-amidinopropane hydrochloride (AAPH) was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). All other chemicals and solvents used in this study were of the analytical grade. The Green tea extract was prepared by freeze-drier apparatuses. The extract of silymarin was from Darou-Pakhs pharmaceutical Company (Tehran, Iran).

Total phenol evaluation by Folin-Ciocalteu method

Total polyphenols were determined by Folin-Ciocalteu (FC) procedure (Singleton *et al*., 1999). Aliquots (0.1 ml) of extracts were transferred into the test tubes and their volumes made up to 0.5 ml with distilled water. After addition of 0.25 ml Folin-Ciocalteu reagent (FCR) and 1.25 ml 20% aqueous sodium carbonate solution, tubes were vortexed and absorbance of blue colored mixtures recorded after 120 min at 765 nm. The amount of total polyphenols was calculated as a Gallic acid equivalent from the calibration curve of Gallic acid standard solutions (covering the concentration range between 0.1 and 1.0 mg/ml), and expressed as mg Gallic acid per mg dry material. All measurements were done in triplicate and values were expressed as the mean ± SD.

Ferric Reducing/Antioxidant Power (FRAP) assay

For this investigation, the FRAP assay reported by Benzie and Strain (1996), a simple and reliable test that depends upon the reduction of ferric [Fe(III)-TPTZ] to [Fe(II)-TPTZ] complex by a reductant at low pH, was adopted. This complex has an intense blue color that can be monitored at 593 nm. Assays were performed in 1.5 ml reaction mixtures containing 1.45 ml of FRAP solution and 0.05 ml of 0.1 mg/ml silymarin and other antioxidants and samples. Absorbance was measured at 593 nm. Total antioxidant capacity of silymarin was compared with Vitamin C, Vitamin E, BHT, and green tea. All experiments were done in triplicate and values were expressed as the mean ± SD.

DPPH radical scavenging activity

Scavenging activity of DPPH radicals of silymarin was measured according to the method described by Brand-Williams *et al* (1995). Assays were performed in 2 ml reaction mixtures containing 1.95 ml of 0.1 mM DPPH-ethanol solution and 0.05 ml of the samples. The inhibitory effect of different concentrations of silymarin and green tea extracts (0.05-2 mg/ml) on DPPH were measured by spectrophotometeric method. Absorbance of the reaction mixtures at 517nm was continuously monitored for 90 min. IC_{50} represents the level where 50% of the radicals were scavenged by test samples.

Anti-hemolysing effect

The hemolysis in RBC suspensions were induced by peroxyl radicals generated by AAPH (50mM) and the effect of different concentrations of silymarin (0.05-0.5 mg/mL) were evaluated according to Zou *et al* (2001). Scavenging of peroxyl radical was measured by spectrophotometeric method at 37°C. Absorbance of the reaction mixtures at 540nm (absorbance of hemoglobin) was continuously monitored for 4 h and percent of hemolysis was calculated. Anti-hemolysing effect silymarin was compared with green tea and BHT

Inhibition of copper – catalyzed human plasma oxidation

Measurement of oxidizability of blood plasma has been reported by Kontush and Beisiegel (1999). The Inhibitory effect of different concentrations of silymarin (0.01-1.0 mg/ml) on Cu^{2+} (50 μM) - induced conjugated diene (CD) formation in plasma (diluted 1:150) were evaluated. Plasma oxidation was initiated by adding $CuSO_4$ at 37°C and absorbance was continuously monitored at 240nm for 8 h.

STATISTICAL ANALYSIS

The experimental results are expressed as mean ± standard deviation of triplicate measurements. The statistical analysis was performed by one-way ANOVA, followed Ducan's multiple range test. Differences are considered significant when $p<0.05$.

RESULTS

Total phenol content of silymarin was 0.484±0.017 mg Gallic acid equivalent per mg in comparison to that of Green tea which is 0.313±0.095. The results of total antioxidant capacity of silymarin, Green tea and standard antioxidants were shown in fig. 1. Total antioxidant capacity of silymarin (0.1 mg/ml) was compared with 56.7µM Vitamin C, 23.2µM Vitamin E, 45.3µM BHT, and 0.1 mg/ml green tea. It was 131±10.5, 622.7±13.6, 242.3±10.8, 535±24.3, and 169.3±21.9 µM/L in silymarin, Vitamin C, Vitamin E, BHT, and green tea respectively. The different concentrations of silymarin (0.1-1.0 mg/ml) showed reducing power as total antioxidant capacity in a dose dependent manner. The observed total antioxidant capacity significantly correlated ($r = 0.999$) with the total phenolic content present in the extract ($p<0.05$).

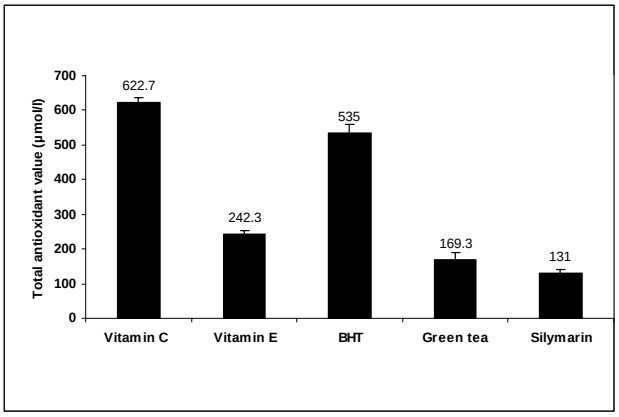

Fig. 1: Total antioxidant capacity of 0.1 mg/ml of the silymarin extract compared with 56.7µM Vitamin C, 23.2µM Vitamin E, 45.3µM BHT, and 0.1 mg/ml of the green tea extract measured by FRAP assay. Results are presented as the mean ± S.D based on three experiments.

Evaluation of antioxidant activity of silymarin was also performed with a DPPH radical scavenging system. As the data shows in fig. 2, silymarin had significant scavenging effects on the DPPH radical by reduction in absorbance at 517 nm and the effects increasing with increasing concentrations in the range of 0.05-2.0 mg/ml. Compared with that of BHT and the green tea extract, the scavenging effect of silymarin was lower. The IC_{50} value of silymarin on DPPH radical scavenging assay was found to be 1.34 mg/ml compared with 0.83 and 0.3 mg/ml those of BHT and green tea respectively. The results found to be statistically significant ($p<0.05$).

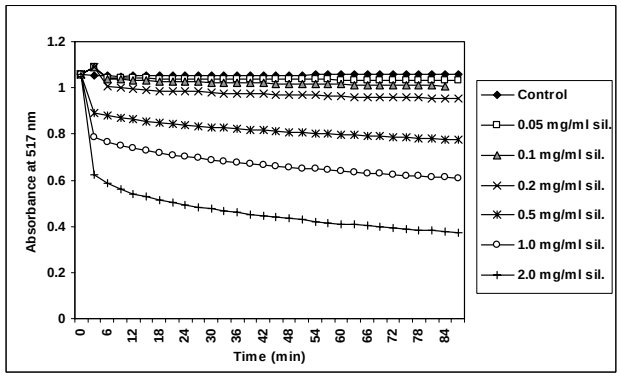

Fig. 2: DPPH radical scavenging activity of silymarin. The concentration of DPPH was 100µM and concentrations of silymarin (sil.) extract were 0.05, 0.1, 0.2, 0.5, 1.0, and 2.0 mg/ml. Absorbance was continuously taken for 90 min at 517 nm.

Scavenging of peroxyl radicals by different concentrations of silymarin were evaluated by its inhibitory effect on RBC hemolysis induced by peroxyl radicals. Percent of hemolysis decreased in a concentration dependent manner (fig. 3). Silymarin at concentrations from 0.05-0.5 mg/ml inhibited the hemolysis by 32.5-92.5%. In comparison, the silymarin extract shown a significantly higher peroxyl radical scavenging activity (92.5%) than the green tea extract and BHT (17.7 and 45.5%) at the same concentration of 0.5 mg/ml and 240 min time interval ($p<0.05$).

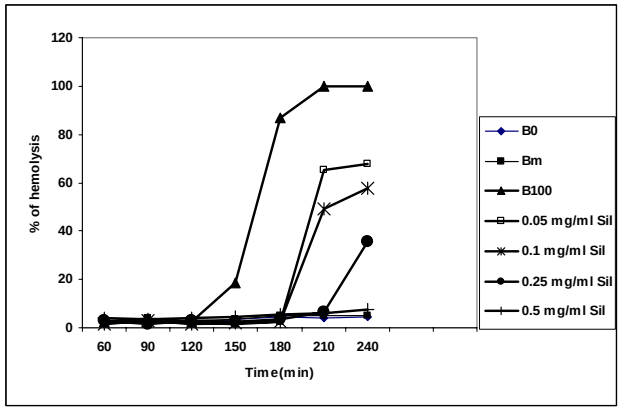

Fig. 3: The inhibitory effect of different concentrations of silymarin on RBC hemolysis induced by peroxyl radical generated by AAPH. The concentration of AAPH was 50mM. Concentrations of silymarin extract (Sil.) were 0.05, 0.1, 0.25 and 0.5 mg/ml. Absorbance was continuously taken for 4 h at 37°C.
B_0: RBC suspension without silymarin and AAPH; B_{100}: RBC suspension with AAPH, without silymarin; B_M: RBC suspension with silymarin, without AAPH.

The effect of silymarin on inhibition of plasma oxidation was also shown a concentration dependent manner. CD formation was retarded by silymarin extract mixture. CD formation was initiated about 2.5 hr after adding copper ions and this period was increased in presence of different concentrations of silymarin (fig. 4).

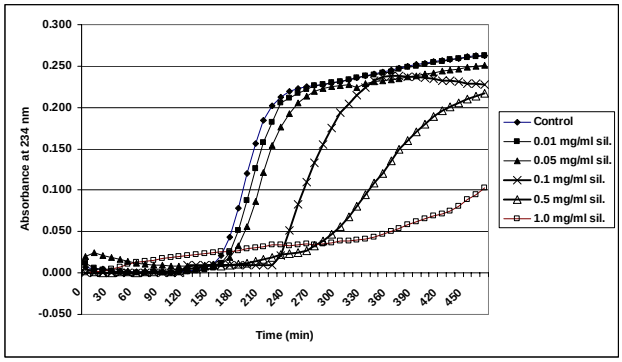

Fig. 4: The inhibitory effect of different concentrations of silymarin on conjugated diene formation induced by copper ions in human plasma. The concentration of Cu^{2+} was 50μM and plasma was diluted 1:150. Concentrations of silymarin extract (Sil.) were 0.01, 0.05, 0.1, 0.5 and 1.0 mg/ml. Absorbance was continuously taken for 8 h at 37°C.

DISCUSSION

The higher levels of polyphenol compounds recorded in the silymarin and green tea (as a known and powerful herbal antioxidant) can explain its antioxidative properties. However, some studies strongly suggest that amount of polyphenol content should be considered as an important feature of herbal drugs. Some of its pharmacological effects like antioxidant, anti-inflammatory, and diuretic activity can be attributed to the presence of these valuable constituents of many medicinal plants (Maksimovic *et al*., 2005; Zheng and Wang, 2001).

In this study, FRAP assay shows high antioxidant capacity of silymarin compared with other standards and green tea. Although FRAP was initially elaborated for estimation of the total antioxidant activity in biological samples, this method was successfully modified for routine analysis of the antioxidative activity of pure chemical substances and plant extracts (Guo *et al*., 2003). FRAP assay offers a rapid and convenient procedure for evaluation of reducing power as antioxidant capacity in plant extracts; we could not find any similar study for measuring of total antioxidant capacity by this method. DPPH scavenging activity of silymarin also indicated high antioxidant power of this compound. Other studies on DPPH radical scavenging of silymarin have shown similar results (Yang *et al*., 2006). Because of their high reactivity, most free radicals react rapidly with oxidizable substrates. Therefore, stable model free radicals such as DPPH which can be used as indicators for radical-scavenging abilities of biological samples are widely used (Wang and Zhang, 2003). Silymarin also delayed AAPH induced hemolysis. This is due to suppression of lipid peroxidation and protection of membrane proteins against degradation induced by peroxyl radicals. AAPH as an azo compound can decompose to form carbon-centered radicals that can react with O_2 to yield peroxyl radicals capable of removing hydrogen from membrane lipids and to stimulate lipid peroxidation (Thomas *et al*., 1997). Many studies have focused on the free-radical-initiated peroxidation of membrane lipid, associated with a variety of pathological events. Both natural and synthetic antioxidants have been used to trap peroxyl radicals and other radicals to protect the membrane lipids against free radical chain reactions (Adom and Liu, 2005). The erythrocyte membrane contains abundant polyunsaturated fatty acids which are very susceptible to free radical induced peroxidation. Since the generation rate of free radical from the decomposition of AAPH at physiological temperature can be easily controlled, this water-soluble azo compound can be used as free radical resource to attack the erythrocyte membrane to induce hemolysis. Hence, the AAPH-induced hemolysis provides a good approach to research the free-radical-induced membrane damage (Zou *et al*., 2001). There is no report about the effect of silymarin against peroxyl radicals in RBC models, but some studies have indicated that silymarin can increase the activity of both SOD and GPx, which may explain its protective effect against free radicals and also the stabilizing effect on the red blood cell membrane (Altorjay *et al*., 1992; Lang *et al*., 1993). Anti-hemolitic effects of silymarin indicates higher activity of peroxyl radical scavenging in silymarin than green tea. From the results of our study and some other researches, it is suggested that silymarin possesses a substantial protective effect and free radical scavenging mechanism against oxidative stress damage inducers (Kiruthiga *et al*., 2007a).

The inhibitory effect of silymarin on plasma oxidation was shown by delay of CD formation induced by copper ions in human plasma. The results can indicate the protective effect of silymarin on lipid peroxidation in plasma (Skottova *et al*., 2004). The silymarin compositions can protect plasma lipids or lipoproteins against oxidation via chelating redox-active transition metal ions and/or scavenging hydrophilic free radicals (Thomas *et al*., 1997). In the literature, absorbance increase of oxidizing plasma or serum has been shown to reflect the oxidation of plasma lipoproteins such as LDL and this suggests that the plasma oxidizability measured as an increase in absorbance at 234 nm may be proposed as a practical measure of the oxidizability of plasma lipoproteins (Kontush and Beisiegel, 1999). The oxidative hypothesis of atherosclerosis implies that the oxidation of LDL plays a central role in the early development of

atherosclerosis (Berliner *et al*., 1995). Several antioxidant and phenolic compounds have been found to be effective on protection of plasma lipoproteins against oxidation (Fuhrman and Aviram, 2001).

In conclusion, the silymarin extract can be very effective antioxidant and can protect biological systems against the oxidative stress that is found to be an important pathophysiological event in a variety of diseases including aging, cancer, diabetes, cardiovascular disorders and rheumatoid arthritis. As far as we know, this is the first report to describe an antioxidant effect of silymarin toward peroxyl radical-mediated RBC hemolysis and copper induced plasma oxidation. Therefore, as it is stated above silymarin shows high antioxidant capacity mainly due to its phenolic compounds and inhibits lipid peroxidation in plasma and RBC models, the present study suggests that silymarin may be used in preventing free radical-related diseases as a dietary natural anti-oxidant supplement.

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REFERENCES

Adom KK and Liu RH (2005). Rapid peroxyl radical scavenging capacity (PSC) assay for assessing both hydrophilic and lipophilic antioxidants. *J. Agric. Food Chem.*, **53**(17): 6572-6580.

Altorjay I, Dalmi L, Sari B, Imre S and Balla G (1992). The effect of silibinin (Legalon) on the free radical scavenger mechanisms of human erythrocytes *in vitro*. *Acta Physiol Hung.*, **80**(1-4): 375-380.

Ball KR and Kowdley KV (2005). A review of Silybum marianum (milk thistle) as a treatment for alcoholic liver disease. *J. Clin.Gastroenterol.*, **39**(6): 520-528.

Benzie IF and Strain JJ (1996). The ferric reducing ability of plasma(FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem.*, **239**(1): 70-76.

Berliner JA, Navab M, Fogelman AM, Frank JS, Demer LL, Edwards PA, Watson AD and Lusis AJ (1995). Atherosclerosis: basic mechanisms. Oxidation, inflammation and genetics. *Circulation*, **91**(9): 2488-2496.

Brand-Williams W, Cuvelier ME and Berset C (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensm.-Wiss.u.-Technol.*, **28**: 25-30.

Casetta I, Govoni V and Granieri E (2005). Oxidative stress, antioxidants and neurodegenerative diseases. *Curr. Pharm. Des.*, **11**(16): 2033-2052.

Fang YZ, Yang S and Wu G (2002). Free radicals, antioxidants, and nutrition. *Nutrition*, **18**(10): 872-879.

Flaig TW, Su LJ, Harrison G, Agarwal R and Glode LM (2007). Silibinin synergizes with mitoxantrone to inhibit cell growth and induce apoptosis in human prostate cancer cells. *Int. J. Cancer*, **120**(9): 2028-2033.

Fuhrman B and Aviram M (2001). Flavonoids protect LDL from oxidation and attenuate atherosclerosis. *Curr. Opin. Lipidol.*, **12**(1): 41-48.

Guo C, Yang J, Wei J, Li Y, Xu J and Jiang Y (2003). Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. *Nutrition Research*, **23**(12): 1719-1726.

Haidara MA, Yassin HZ, Rateb M, Ammar H and Zorkani MA (2006). Role of oxidative stress in development of cardiovascular complications in diabetes mellitus, *Curr. Vasc. Pharmacol.*, **4**(3): 215-227.

Halliwell B (1995). Antioxidant characterization. Methodology and mechanism. *Biochem. Pharmacol.*, **49**(10): 1341-1348.

Halliwell B and Gutteridge JM (1984). Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem. J.*, **219**(1): 1-14.

Huang D, Ou B and Prior RL (2005). The chemistry behind antioxidant capacity assays. *J. Agric. Food Chem.*, **53**(6): 1841-1856.

Junqueira VB, Barros SB, Chan SS, Rodrigues L, Giavarotti L, Abud RL and Deucher GP (2004). Aging and oxidative stress. *Mol. Aspects Med.*, **25**(1-2): 5-16.

Khan SA, Ahmad B and Alam T (2006). Synthesis and antihepatotoxic activity of some new chalcones containing 1, 4 - dioxane ring system. *Pak. J. Pharm. Sci.*, **19**(4): 290-294.

Kiruthiga PV, Shafreen RB, Pandian SK, Arun S, Govindu S and Devi KP (2007a). Protective effect of silymarin on erythrocyte haemolysate against benzo(a)pyrene and exogenous reactive oxygen species (H_2O_2) induced oxidative stress. *Chemosphere*, **68**(8): 1511-1518.

Kontush A and Beisiegel U (1999). Measurement of oxidizability of blood plasma. *Methods Enzymol*, **299**: 35-49.

Kren V and Walterova D (2005). Silybin and silymarin – new effects and applications. *Biomed. Pap. Med. Fac. Univ Palacky. Olomouc. Czech.Repub.*, **149**(1): 29-41.

Lang I, Deak G, Muzes G, Pronai L and Feher J (1993). Effect of the natural bioflavonoid antioxidant silymarin on superoxide dismutase (SOD) activity and expression *in vitro*. *Biotechnol. Ther.*, **4**(3-4): 263-270.

Liu F and Ng TB (2000). Antioxidative and free radical scavenging activities of selected medicinal herbs. *Life Sci.*, **66**(8): 725-735.

Locher R, Suter PM, Weyhenmeyer R and Vetter W (1998). Inhibitory action of silibinin on low density

lipoprotein oxidation. *Arzneimittelforschung.*, **48**(3): 236-239.

Maksimovic Z, Malencic D and Kovacevic N (2005). Polyphenol contents and antioxidant activity of *Maydis stigma* extracts. *Bioresour. Technol.*, **96**(8): 873-877.

Pascual C, Gonzalez R, Armesto J and Muriel P (1993). Effect of silymarin and silybinin on oxygen radicals. *Drug Development Research*, **29**(1): 73-77.

Piconi L, Quagliaro L and Ceriello A (2003). Oxidative stress in diabetes. *Clin. Chem. Lab Med.*, **41**(9): 1144-1149.

Ramadan LA, Roushdy HM, Bu Senna GM, Amin NE and El-Deshw OA (2002). Radio-protective effect of silymarin against radiation induced hepatotoxicity. *Pharmacol. Res.*, **45**(6): 447-454.

Singh U, Devaraj S and Jialal I (2005). Vitamin E, oxidative stress and inflammation. *Annu. Rev. Nutr*, **25**: 151-174.

Singleton VL, Orthofer R and Lamuela-Raventos RM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol*, **299**: 152-177.

Skottova N, Kazdova L, Oliyarnyk O, Vecera R, Sobolova L and Ulrichova J (2004). Phenolics-rich extracts from *Silybum marianum* and *Prunella vulgaris* reduce a high-sucrose diet induced oxidative stress in hereditary hypertriglyceridemic rats. *Pharmacol. Res.*, **50**(2): 123-130.

Skottova N, Vecera R, Urbanek K, Vana P, Walterova D and Cvak L (2003). Effects of polyphenolic fraction of silymarin on lipoprotein profile in rats fed cholesterol-rich diets. *Pharmacol. Res.*, **47**(1): 17-26.

Sobolova L, Skottova N, Vecera R and Urbanek K (2006). Effect of silymarin and its polyphenolic fraction on cholesterol absorption in rats. *Pharmacol. Res.*, **53**(2): 104-112.

Soto C, Mena R, Luna J, Cerbon M, Larrieta E, Vital P, Uria E, Sanchez M, Recoba R, Barron H, Favari L and Lara A (2004). Silymarin induces recovery of pancreatic function after alloxan damage in rats. *Life Sci.*, **75**(18): 2167-2180.

Soto C, Recoba R, Barron H, Alvarez C and Favari L (2003). Silymarin increases antioxidant enzymes in alloxan-induced diabetes in rat pancreas. *Comp. Biochem. Physiol. C. Toxicol. Pharmacol.*, **136**(3): 205-212.

Thomas MJ, Chen Q, Franklin C and Rudel LL (1997). A comparison of the kinetics of low-density lipoprotein oxidation initiated by copper or by azobis (2-amidinopropane). *Free Radic. Biol. Med.*, **23**(6): 927-935.

Valko M, Rhodes CJ, Moncol J, Izakovic M and Mazur M (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem. Biol. Interact.*, **160**(1): 1-40.

Wang LF and Zhang HY (2003). A theoretical investigation on DPPH radical-scavenging mechanism of edaravone. *Bioorg. Med. Chem. Lett.*, **13**(21): 3789-3792.

Yang L, Gong J, Wang F, Zhang Y, Wang Y, Hao X, Wu X, Bai H, Stockigt J and Zhao Y (2006). Synthesis and antioxidant evaluation of novel silybin analogues. *J. Enzyme Inhib. Med. Chem.*, **21**(4): 399-404.

Zennaro L, Rossetto M, Vanzani P, De M, Scarpa VM, Battistin L and Rigo A (2007). A method to evaluate capacity and efficiency of water soluble antioxidants as peroxyl radical scavengers. *Arch. Biochem. Biophys.*, **462**(1): 38-46.

Zheng W and Wang SY (2001). Antioxidant activity and phenolic compounds in selected herbs. *J. Agric. Food Chem.*, **49**(11): 5165-5170.

Zou CG, Agar NS and Jones GL (2001). Oxidative insult to human red blood cells induced by free radical initiator AAPH and its inhibition by a commercial antioxidant mixture. *Life Sci.*, **69**(1): 75-86.