Hepatic fibrosis in biliary-obstructed rats is prevented by *Ginkgo biloba* treatment

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**Abstract**

**AIM:** To assess the antioxidant and antifibrotic effects of long-term *Ginkgo biloba* administration on liver fibrosis induced by biliary obstruction in rats.

**METHODS:** Liver fibrosis was induced in male Wistar albino rats by bile duct ligation and scission (BDL). *Ginkgo biloba* extract (EGB 761, 50 mg/kg per d) or saline was administered for 28 d. At the end of the treatment period, all rats were killed. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) levels were determined to assess liver functions and tissue damage, respectively. Tumor necrosis factor-α (TNF-α) was also assayed in serum samples. Liver tissues were taken for determination of the hepatic malondialdehyde (MDA) and glutathione (GSH) levels, myeloperoxidase (MPO) activity and collagen content. Production of reactive oxidants was monitored by chemiluminescence (CL) assay. Serum AST, ALT, LDH, and TNF-α levels were elevated in the BDL group as compared to control group and were significantly decreased by EGB treatment.

**RESULTS:** Hepatic GSH level, depressed by BDL, was elevated back to control level in EGB-treated BDL group. Increase in tissue MDA level, MPO activity and collagen content due to BDL were also attenuated by EGB treatment. Furthermore, luminol and lucigenin CL values in BDL group increased dramatically compared to control and reduced by EGB treatment.

**CONCLUSION:** Our results suggest that *Ginkgo biloba* protects the liver from oxidative damage following BDL in rats. This effect possibly involves the inhibition of neutrophil infiltration and lipid peroxidation; thus, restoration of oxidant and antioxidant status in the tissue.
**MATERIALS AND METHODS**

**Animals**
Male Wistar albino rats (200-250 g) were housed in a room at a mean constant temperature of 22±2 °C with a 12-h light-dark cycle, and free access to standard pellet chow and water. The study was approved by the Marmara University School of Medicine Animal Care and Use Committee.

**Induction of liver fibrosis**
Rats were anesthetized (100 mg/kg ketamine and 0.75 mg/kg chlorpromazine; ip) and the common bile duct was exposed and ligated by double ligatures with suture silk. The first ligature was made below the junction of the hepatic ducts and the second ligature was made above the entrance of the pancreatic ducts. Finally, the common bile duct was resected between the double ligatures[18]. In sham-operated rats, abdominal incision was made without a ligation.

**Experimental groups**
Ginkgo biloba extract was administered to control (EGb group) and bile duct ligated (BDL+EGb group) rats for 28 d at a daily dose of 50 mg/kg, ip. The sham-operated control (C) and BDL groups received equal amounts of saline for 28 d.

After 28 d of treatment, the rats were killed and trunk blood was collected. Serum samples were used for the measurement of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), bilirubin and tumor necrosis factor (TNF-α) levels. Liver samples were taken and stored at -70 °C for the measurement of malondialdehyde (MDA) and glutathione (GSH) levels, myeloperoxidase (MPO) activity and collagen contents. Formation of reactive oxygen species in liver samples was monitored by using chemiluminescence (CL) method.

**Determination of serum AST, ALT, LDH, bilirubin, and TNF-α levels**
Serum AST and ALT levels - as indicators of liver function - and LDH activity - as a marker of tissue injury - were assessed by using commercial kits (Roche Diagnostics, GmbH, D-68298, Mannheim, Germany) in Roche-Hitachi Modular Autoanalyzer (Roche Diagnostics, GmbH, D-68298, Mannheim, Germany)[23]. Serum total bilirubin level was evaluated by measuring the amount of the dyes Sirius red and fast green FCF to collagen and noncollagenous components, respectively. Both dyes were eluted readily and simultaneously by using 0.1 N NaOH-methanol (1:1, v/v). Finally, the absorbances at 540 and 605 nm were used to determine the amount of collagen and protein, respectively.

**Chemiluminescence (CL) assay**
Luminescence of the hepatic tissue samples was recorded at room temperature using Mini Lumat LB 9 506 luminometer (EG&G Berthold, Germany) in the presence of luminol or lucigenin, 0.2 mmol/L each. All counts were obtained at 15-s intervals for 5 min and the results were expressed as area under the curve of relative light unit (rlu) for 5 min/mg tissue[28].

**Measurement of liver collagen content**
Tissue samples were cut with a razor blade and immediately fixed in 40 g/L formaldehyde in 0.1 mol/L phosphate buffer (pH 7.2) in paraffin and sections of 15-μm thick were obtained. Collagen content was measured according to a method described by Lopez de Leon and Rojkind[27]. The method is based on selective binding of the dyes Sirius red and fast green FCF to collagen and noncollagenous components, respectively. Both dyes were eluted readily and simultaneously by using 0.1 N NaOH-methanol (1:1, v/v). Finally, the absorbances at 540 and 605 nm were used to determine the amount of collagen and protein, respectively.

**Measurement of liver MDA and GSH levels**
Tissue samples were homogenized with ice-cold trichloroacetic acid (1 g tissue +10 mL 10% TCA) in an Ultra Turrax tissue homogenizer. The MDA levels were assayed for products of lipid peroxidation by monitoring thiobarbituric acid reactive substance formation as described previously[24]. Lipid peroxidation was expressed in terms of MDA equivalents using an extinction coefficient of 1.56×10^5 mol/(L·cm) and results were expressed as nanomole MDA per gram tissue. GSH was determined by a spectrophotometric method, which was based on the use of Ellman’s reagent[25]. GSH levels were calculated using an extinction coefficient of 13 600 mol/(L·cm) and the results were expressed in micromole per gram tissue.

**Measurement of liver myeloperoxidase (MPO) activity**
Tissue samples (0.2-0.3 g) were homogenized in 10 volumes of ice-cold potassium phosphate buffer (50 mmol/L K2HPO4, pH 6.0) containing hexadecyltrimethylammonium bromide (HETAB, 5 g/L). The homogenate was centrifuged at 30 000 g for 10 min at 4 °C, and the supernatant was discarded. The pellet was then rehomogenized with an equivalent volume of 50 mmol/L K2HPO4, containing 5 g/L HETAB and 10 mmol/L EDTA (Sigma). MPO activity was assessed by measuring the H2O2-dependent oxidation of 4-dianizidine 2HCl. One unit of enzyme activity was defined as the amount of the MPO present per gram of tissue weight that caused a change in absorbance of 1.0 per min at 460 nm and 37 °C[26].

**Statistical analysis**
All data were expressed as mean±SE. Groups of data were compared with multiple comparison tests. Values of P<0.05 were regarded as significant.

**RESULTS**
As shown in Table 1, serum bilirubin, AST, ALT, and LDH levels were significantly higher in BDL rats compared to controls (P<0.001) and EGb administration to BDL group reduced these values (P<0.001). In untreated BDL group, serum TNF-α level showed a significant increase compared to that of the sham-operated control group (P<0.001). Similarly, BDL-induced rise in serum TNF-α level was significantly reduced by EGb treatment (P<0.001, Figure 1).
The liver MDA was found to be significantly higher in the BDL group (51.0±3.3 nmol/g; P<0.01) compared to that of the sham-operated control group (32.2±3.1 nmol/g). Treatment with EGb reversed the MDA level (33.8±2.6 nmol/g; P<0.01) back to control level (Figure 2).

The hepatic GSH level showed a marked reduction in the BDL group (0.88±0.07 μmol/g; P<0.001) compared to that of the sham-operated control group (2.1±0.15 μmol/g). Similarly, treatment with EGb reversed this effect (1.8±0.12 μmol/g; P<0.01) (Figure 3).

Liver MPO activity, which was observed to be higher in the BDL group (21.8±2.0 U/g; P<0.01) compared to sham-operated control group (13.8±1.3 U/g) was also attenuated by EGb treatment (13.3±0.7 U/g; P<0.01) (Figure 4).

Hepatic collagen content of the untreated BDL rats (21.3±2.1 μg/mg protein; P<0.01) was found to be significantly higher than that of the control group (12.8±1.4 μg/mg protein). EGb treatment significantly reduced the increase in hepatic collagen content (13.2±1.2 μg/mg protein; P<0.05) to the levels that were close to control values (Figure 5).

Luminol and lucigenin CL values of the liver samples of the BDL group (18.7±1.1 and 38.3±3.2 rlu/mg, respectively) were found to be significantly higher than those of the control group (11.1±1.0 and 14.8±1.1 rlu/mg; P<0.01 and P<0.001, respectively) and EGb treatment reversed these values (12.5±2.0 and 16.1±2.6 rlu/mg; P<0.05 and P<0.001, respectively) (Figures 6 and 7).

**DISCUSSION**

The present study demonstrates that EGb treatment improved BDL-induced impairment in liver functions and decreased BDL-induced elevations in serum LDH activity and TNF-α levels. Furthermore, increase in hepatic lipid peroxidation, MPO activity, oxidant production and collagen content, and

| Table 1 Serum total bilirubin, AST, ALT, LDH and TNF-α levels in control (C), EGb, BDL and BDL+EGb groups |
|--------------------------------------------|
| **Groups**                  | **C (n = 8)** | **EGb (n = 8)** | **BDL (n = 8)** | **BDL+EGb (n = 8)** |
| Total bilirubin (mg/dL)      | 0.36±0.3     | 0.35±0.6       | 10.1±0.6        | 1.68±0.3           |
| AST (mg/dL)                  | 148±16.6     | 150±12.4       | 300±18          | 155±12             |
| ALT (mg/dL)                  | 68±7.3       | 77±6.1         | 135±9.7         | 67±5.7             |
| LDH (U/L)                    | 1 672±240    | 1 499±219      | 5 082±225       | 1 763±226          |

*S*p<0.01, **p<0.001 vs control group. †p<0.001, vs untreated BDL group.

![Figure 1](image1.png)

**Figure 1** Serum TNF-α levels of the control, Ginkgo-biloba-treated, saline-treated BDL and Ginkgo-biloba-treated BDL groups. †p<0.05, ‡p<0.001 vs control; †‡p<0.001 vs saline-treated BDL group.

![Figure 2](image2.png)

**Figure 2** Liver MDA levels of the control, Ginkgo-biloba-treated, saline-treated-BDL, and Ginkgo-biloba-treated BDL groups. †p<0.01 vs control; †‡p<0.01 vs saline-treated BDL group.

![Figure 3](image3.png)

**Figure 3** Liver GSH levels of the control, Ginkgo-biloba-treated, saline-treated-BDL, and Ginkgo-biloba-treated BDL groups. †p<0.001 vs control; †‡p<0.001 vs saline-treated BDL group.

![Figure 4](image4.png)

**Figure 4** Liver MPO activities of the control, Ginkgo-biloba-treated, saline-treated-BDL, and Ginkgo-biloba-treated BDL groups. †p<0.01 vs control; †‡p<0.01 vs saline-treated BDL group.
increase in GSH levels following BDL were reversed by EGB treatment.

Hepatic fibrosis, characterized by an increased production and deposition of extracellular matrix component accompanies most chronic liver disorders and its presence is a major factor contributing to hepatic failure[29,30]. Although the mechanism of liver fibrosis is not fully understood, activated hepatic stellate cells play an important role in connective tissue synthesis and deposition during fibrogenesis[31]. On the other hand, oxidative stress caused by accumulation of hydrophobic bile acids also have a critical role in fibrogenesis[31,33].

Bile duct ligation (BDL) induces a type of liver fibrosis, which etiologically and pathogenetically resembles the biliary fibrosis in the human beings. Injury to hepatocytes results in the generation of lipid peroxides, which may have a direct stimulatory effect on matrix production by activated stellate cells[34,35]. Liu et al., demonstrated excessive production of superoxide radicals and hydroxyl radicals in blood and liver in rats with obstructive jaundice induced by common BDI[36]. In the present study, BDL caused significant increases in the hepatic MDA level -end product of lipid peroxidation- and EGB treatment prevented the increase in MDA, probably in part by scavenging the very reactive hydroxyl and peroxy radicals. Moreover, attenuation of the increase in hepatic luminol- and lucigenin-enhanced CL levels by EGB treatment in BDL animals also supports the scavenging ability of the agent.

An extract of the leaves of *Ginkgo biloba* L., a mixture mainly composed of flavonoid glycosides and terpenoids (ginkgolides and bilobalide), has been shown to exhibit a variety of pharmacological actions. The leaf extract acts as a scavenger of reactive oxygen species, that is, superoxide radical, peroxy radical and nitric oxide[37-39]. It has been reported that the extract suppresses platelet aggregation induced by tert-butyl hydroperoxide and hydrogen peroxide through its antioxidant action[40]. Moreover, the extract and its ingredients (especially ginkgolide C) exhibit a potent antagonistic effect on platelet-activating factor and inhibitory effects on the expression of inducible nitric oxide synthase as well as nitric oxide production[41,42]. It decreased ethanol- or cold stress-induced gastric injury, protected against cerulein-induced acute pancreatitis and hepatic damage evoked by CCL in the rat[43-45]. It has also been demonstrated that EGB has protective effects on aging liver. Thus, these effects suggest that EGB is beneficial in various cardiovascular, cerebrovascular, and neurological disorders in which oxidants are involved.

GSH, a key antioxidant, is an important constituent of intracellular protective mechanisms against various noxious stimuli, including oxidative stress. On the other hand, reduced GSH, which constitutes the main component of endogenous non-protein sulfhydryl pool, is known to be a major low molecular weight scavenger of free radicals in the cytoplasm[46]. Because of their exposed sulfhydryl groups, non-protein sulfhydryls bind a variety of electrophilic radicals and metabolites that may be damaging to the cells[47]. Huang et al., studied the mitochondrial functions in bile duct-ligated rats and suggested that biochemical and molecular changes are related to oxidative stress in the liver[48]. In accordance with the previous reports, our results also support the notion that depletion of tissue GSH, as observed in the BDL-induced hepatic injury, is one of the major factors that permit lipid peroxidation and subsequent tissue damage. Since administration of *Ginkgo biloba* extract prevented the hepatic GSH depletion, it appears that the protective effect of the extract involves the maintenance of antioxidant capacity in protecting the hepatic tissue against oxidative stress.

Excessive production of hydroxyl radicals in blood and liver has previously been demonstrated by Liu et al., in rats with obstructive jaundice induced by common BDI[36]. It has been demonstrated by various investigators that circulating
proinflammatory cytokines, such as TNF-α, interleukin-1-β (IL-1β) and IL-6, which trigger hepatic injury, were increased, at least in part, by a free radical-mediated apoptotic mechanism. Therefore, it seems reasonable to propose that antioxidants or free radical scavengers counteract the oxidant stress produced by cholestasis. In this study, increased TNF-α levels of BDL rats tended to decrease following the treatment by EGb, also supports the notion that EGb ameliorates oxidative liver injury caused by BDL through its antioxidant effect.

Observations suggest that reactive oxygen metabolites (ROM) play a role in the recruitment of neutrophils into damaged tissue, but activated neutrophils are also a potential source of ROM. Although it is not certain whether neutrophil accumulation and activation are the causes or the results of injury, increasing evidence suggests that mesangial cells and neutrophils release chemotactic substances (e.g., IL-8), which further promote neutrophil migration and activation. Several methods have been used to define the role of neutrophils in the tissue injury. MPO, which is an essential enzyme for normal neutrophil function, is released as a response to various stimulatory substances. In the present study, increased hepatic MPO activity due to BDL, which indicates that tissue injury involves the contribution of neutrophil infiltration, was effectively reversed by EGb treatment. Moreover, BDL-induced increase in fibrotic activity, as assessed by hepatic collagen content, was also reduced by EGb treatment. These findings suggest that EGb has an additional protective effect on inflammation-induced production and deposition of extracellular matrix components, which result in hepatic fibrosis.

In conclusion, the findings of our present study demonstrate that Ginkgo biloba extract, with its potent free radical scavenging and antioxidant properties, seems to be a highly promising agent in protecting hepatic tissue against oxidative damage and in preventing hepatic fibrosis and dysfunction due to obstructive jaundice.

REFERENCES

1 Kullak-Ublick GA, Meier PJ. Mechanisms of cholestasis. Clin Liver Dis 2000; 4: 357-385
2 Bruck R, Shirin H, Aeed H, Matas Z, Hochman A, Pines M, Avni Y. Prevention of hepatic cirrhosis in rats by hydroxyl radical scavengers. J Hepatol 2001; 35: 457-464
3 Zhong Z, Froh M, Lehnert M, Schoonhoven R, Yang L, Lind H, Lemasters JJ, Thurman RG. Polyphenols from Camellia sinensis attenuate experimental cholestasis-induced liver fibrosis in rats. Am J Physiol Gastrointest Liver Physiol 2003; 285: G1004-1013
4 Sokol RJ, Winkhoffer-Roob BM, Devereaux MW, McMickin JM. Generation of hydroperoxides in isolated rat hepatocytes and hepatic mitochondria exposed to hydrophobic bile acids. Gastroenterology 1995; 109: 1249-1256
5 Orellana M, Rodrigo R, Thielemann L, Guajardo V. Bile duct ligation and oxidative stress in the rat: effects in liver and kidney. Comp Biochem Physiol C Toxicol Pharmacol 2000; 126: 105-111
6 Pastor A, Collado PS, Almar M, Gonzales-Gallego J. Antioxidant enzyme status in biliary obstructed rats: effects on liver and kidney. Pediatr Res 1998; 44: 397-401
7 Sokol RJ, Devereaux MW, Khandwala R. Effect of oxyphenurin, a xanthine oxidase inhibitor, on hepatic injury in the bile duct-ligated rat. Pediatr Res 1998; 44: 397-401
8 Serviddio G, Pereda J, Pallardo FV, Carretero J, Borras C, Cutrin J, Vendemiale G, Poli G, Vina J, Sastre J. Ursodeoxycholic acid protects against secondary biliary cirrhosis in rats by preventing mitochondrial oxidative stress. Hepatology 2004; 39: 711-720
9 Yang S, Tan TM, Wee A, Leow CK. Mitochondrial respiratory function and antioxidant capacity in normal and cirrhotic livers following partial hepatectomy. Cell Mol Life Sci 2004; 61: 220-229
10 Park EJ, Ko G, Kim J, Sohn DH. Antifibrotic effects of a polysaccharide extract from Ganoderma lucidum, glycyrhizin, and penstifoline in rats with cirrhosis induced by biliary obstruction. Biol Pharm Bull 1997; 20: 417-420
11 Park EJ, Nan JX, Kim JY, Kang HC, Choi JH, Lee SJ, Lee BH, Kim SJ, Lee JH, Kim YC, Sohn DH. The ethanol-soluble part of a hot-water extract from Artemisia iwayomogi inhibits liver fibrosis induced by carbon tetrachloride in rats. J Pharmacol Pharmacol 2000; 52: 875-881
12 Nan JX, Park EJ, Kim JJ, Ko G, Sohn DH. Antifibrotic effects of the methanol extract of Polygonum aviculare in fibrotic rats induced by bile duct ligation and scission. Biol Pharm Bull 2000; 23: 240-243
13 Wasser S, Ho JM, Ang HK, Tan CE. Salvia miltiorrhiza reduces experimentally-induced hepatic fibrosis in rats. J Hepatol 1998; 29: 760-771
14 Li D, Friedman SL. Liver fibrogenesis and the role of hepatic stellate cells: new insights and prospects for therapy. J Gastroenterol Hepatol 1999; 14: 618-633
15 Shimizu I, Ma YR, Mizobuchi Y, Liu F, Miura T, Nakai Y, Yasuda M, Shiba M, Horiie T, Amagaya S, Kawada N, Hori H, Ito S. Effects of Sho-saiko-to, a Japanese herbal medicine, on hepatic fibrosis in rats. Hepatology 1999; 29: 149-160
16 van Beek TA. Chemical analysis of Ginkgo biloba leaves and extracts. J Chromatogr A 2002; 967: 21-35
17 Akiba S, Kawachi T, Hashizume T, Sato T. Inhibitory effect of the leaf extract of Ginkgo biloba L. on oxidative stress-induced platelet aggregation. Biochem Mol Biol Int 1996; 46: 1243-1248
18 Lamant V, Maugo C, Braquet P, Chap H, Douse-Blazly L. Inhibition of the metabolism of platelet activating factor (PAF-acether) by three specific antagonists from Ginkgo biloba. Biochem Pharmacol 1987; 36: 2749-2752
19 Kobuchi H, Droy-Lefaix MT, Christen Y, Packer L. Ginkgo biloba extract (EGb 761): inhibitory effect on nitric oxide production in the macrophage cell line RAW 264.7. Biochem Pharmacol 1997; 53: 897-903
20 Shen J, Wang J, Zhao B, Hou J, Gao T, Xin W. Effects of EGb 761 on nitric oxide and oxygen free radicals, myocardial damage and arrhythmia in ischemia-reperfusion injury in vivo. Biochem Biophys Acta 1998; 1406: 228-236
21 Hu B, Sun S, Mei G, Chen L, Tong E. Protective effects of Ginkgo biloba extract on rats during cerebral ischemia/ reperfusion. Chin Med J 2002; 115: 1316-1320
22 Martinez RG, LeBlanc RA, Bidschad S, Shirey RJ, Maisey MN. Antifibrotic effect of Ginkgo biloba extract on liver fibrosis induced by bile duct ligation and scission. J Pharmacol Methods 1999; 40: 285-295
23 Lopez De Leon A, Nieto RM, Becker G, Saltzman A, Ackerman R, Ho J. Microsomal lipid peroxidation. Methods Enzymol 1979; 52: 302-311
24 Beutler E. Glutathione in red blood cell metabolism. A Manual of Biochemical Methods, Grune & Stratton, New York 1975: 112-114
25 Hillegas LM, Griswole D, Brickson B, Albrightson-Winslow C. Assessment of myeloperoxidase activity in whole rat kidney. J Pharmacol Methods 1999; 40: 285-295
26 Lopez De Leon A, Rojkind M. A simple micromethod for collagen and total protein determination in formalin-fixed paraffin-embedded sections. J Histochem Cytochem 1985; 33: 737-743
27 Haklar G, Özeri ES, Yüksel M, Akta AO, Yalçın AS. Different kinds of reactive oxygen and nitrogen species were detected in colon and breast tumors. Cancer Lett 2001; 165: 219-224
28 Fort J, Oberti F, Pilette C, Veall N, Gallois Y, Douay O, Rousselet MC, Renesbaum J, Cales P. Antifibrotic and he-
modynamic effects of the early and chronic administration of octreotide in two models of liver fibrosis in rats. *Hepatology* 1998; 28: 1525-1531

30 Friedman SL. Liver fibrosis-from bench to bedside. *J Hepatol* 2003; 38(Suppl 1): S38-S53

31 Muriel P, Moreno MG. Effects of silymarin and vitamins E and C on liver damage induced by prolonged biliary obstruction in the rat. *Basic Clin Pharmacol Toxicol* 2004; 94: 99-104

32 Lee KS, Houglum K, Chojkier M. Activation of hepatic stellate cells by TGF alpha and collagen type I is mediated by oxidative stress through c-myb expression. *J Clin Invest* 1995; 96: 2461-2468

33 Liu TZ, Lee KT, Chern CL, Cheng JT, Stern A, Tsai LY. Free radical-triggered hepatic injury of experimental obstructive jaundice of rats involves overproduction of proinflammatory cytokines and enhanced activation of nuclear factor kappaB. *Ann Clin Lab Sci* 2001; 31: 383-390

34 Pinembre J, Dupuis M, Nasr C, Hans P, Haag-Berrurier M, Anton R, Deby C. Superoxide anion scavenging effect and superoxide dismutase activity of Ginkgo biloba extract. *Experientia* 1989; 45: 708-712

35 Maitra I, Marcocci L, Droy-Lefaix MT, Packer L. Peroxyl radical scavenging activity of Ginkgo biloba extract EGb 761. *Biochem Pharmacol* 1995; 49: 1649-1655

36 Marcocci L, Maguire JJ, Droy-Lefaix MT, Packer L. The nitric oxide-scavenging properties of Ginkgo biloba extract EGb 761. *Biochem Biophys Res Commun* 1994; 201: 748-755

37 Cheung F, Siow YL, Chen WZ, O K. Inhibitory effect of Ginkgo biloba extract on the expression of inducible nitric oxide synthase in endothelial cells. *Biochem Pharmacol* 1999; 58: 1665-1673

38 Cheung F, Siow YL, O K. Inhibition by ginkgolides and bilobalide of the production of nitric oxide in macrophages (THP-1) but not in endothelial cells (HUVEC). *Biochem Pharmacol* 2001; 61: 503-510

39 Wang Q, Zhao WZ, Ma CG. Protective effects of Ginkgo biloba extract on gastric mucosa. *Acta Pharmacol Sin* 2000; 21: 1153-1156

40 Soybir G, Koksoy F, Ekiz F, Yalcin O, Fincan K, Haklar G, Yulsel M. The effects of free oxygen radical scavenger and platelet-activating factor antagonist agents in experimental acute pancreatitis. *Pancreas* 1999; 19: 143-149

41 Ozenerler S, Dincer S, Akylol G, Ozogul C, Oz E. The protective effect of Ginkgo biloba extract on CCl4-induced hepatic damage. *Acta Physiol Hung* 1997-1998; 85: 277-285

42 Luo YJ, Yu JP, Shi ZH, Wang L. Ginkgo biloba extract reverses CCl4-induced liver fibrosis in rats. *World J Gastroenterol* 2004; 10: 1037-1042

43 Ross D. Glutathione, free radicals and chemotherapeutic agents. Mechanisms of free-radical induced toxicity and glutathione-dependent protection. *Pharmacol Ther* 1988; 37: 251-249

44 Szabo S, Nagy L, Plebani M. Glutathione, protein sulphhydrils and cysteine proteases in gastric mucosal injury and protection. *Clin Chim Acta* 1992; 206: 95-105

45 Huang YT, Hsu YC, Chen CJ, Liu CT, Wei YH. Oxidative-stress-related changes in the livers of bile-duct-ligated rats. *J Biomed Sci* 2003; 10: 170-178

46 Zimmerman BJ, Grisham MB, Granger DN. Role of oxidants in ischemia-reperfusion induced granulocyte infiltration. *Am J Physiol* 1990; 258: G185-G190

47 Granger DN, Korthuis RJ. Physiologic mechanisms of postischemic tissue injury. *Annu Rev Physiol* 1995; 57: 311-332

48 Donnahoo KK, Meng X, Ayala A, Cain MP, Harken AH, Meldrum DR. Early kidney TNF-α expression mediates neutrophil infiltration and injury after renal ischemia-reperfusion. *Am J Physiol* 1999; 277: R922-929

49 Winterbourn CC, Kettle AJ. Biomarkers of myeloperoxidase-derived hypochlorous acid. *Free Radic Biol Med* 2000; 29: 403-409