Protective effects of *Ginkgo biloba* extract on the ethanol-induced gastric ulcer in rats

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INTRODUCTION

*Ginkgo biloba*, a member of the family Ginkgoaceae, was cultivated in China in the mid-1700s. Originating in the southeastern China some 200 million years ago, it has been living to an age of 1,000 years and is the last remaining member of its order. It is used medically today as a standardized preparation *GbE* (EGb 761) which contains 240 mg/g flavonoids (ginkgo-flavone glycosides) and 60 mg/g terpenoids (ginkgolides and bilobalides). Those are the most important active ingredients in the extract. The flavonoids act as free radical scavengers, especially for oxygen-derived free radicals, such as OH·, O2·, RO·, and ROO·, and to neutralize ferryl ion-induced peroxidation[2,3]. The terpenoids is known as an antagonist of platelet-activating factor, which implicates in the processes of platelet aggregation and arterial thrombosis, acute inflammation, allergic reactions and cardiovascular insufficiency[4,5].

Recently *GbE* is used to improve cardiovascular circulation and to lessen cerebrovascular insufficiency in western countries clinically[6].

On the other hand, ethanol is well-known as a damaging agent to gastric mucosa in animal and clinical studies. At concentrations greater than 400 mL/L, it causes marked mucosal hyperemia, necrosis, edema and mucosal or submucosal hemorrhage[7,8]. The formation of lesions may be mediated by oxygen-derived free radicals[9,10].

GbE is well-known as a strong free radical scavenger. The gastric mucosal injury after ethanol treatment may be mediated through: (1) inhibition of lipid peroxidation; (2) preservation of gastric mucus and NP-SH; and (3) blockade of cell apoptosis.

RESULTS: The findings of our study are as follows: (1) *GbE* pretreatment was found to provide a dose-dependent protection against the ethanol-induced gastric ulcers in rats; (2) the *GbE* pretreatment afforded a dose-dependent inhibition of ethanol-induced depletion of stomach wall mucus, NP-SH contents and increase in the lipid peroxidation (increase MDA) in gastric tissue; (3) gastric ulcer induced by ethanol produced an increase in JNK activity in gastric mucosa which also significantly inhibited by pretreatment with *GbE*; and (4) *GbE* alone had no inhibitory effect on gastric secretion in pylorus-ligated rats.

CONCLUSION: The finding of this study showed that *GbE* significantly inhibited the ethanol-induced gastric lesions in rats. We suggest that the preventive effect of *GbE* may be mediated through: (1) inhibition of lipid peroxidation; (2) preservation of gastric mucus and NP-SH; and (3) blockade of cell apoptosis.

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Key words: *Ginkgo biloba* extract; Ethanol; Gastric ulcer; c-Jun kinase

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Abstract

AIM: To evaluate the preventive effect of *Ginkgo biloba* extract (*GbE*) on ethanol-induced gastric mucosal injuries in rats.

METHODS: Female Wistar albino rats were used for the studies. We randomly divided the rats for each study into five subgroups: normal control, experimental control, and three experimental groups. The gastric ulcers were induced by instilling 1 mL 50% ethanol into the stomach. We gave *GbE* 8.75, 17.5, 26.25 mg/kg intravenously to the experimental groups respectively 30 min prior to the ulcerative challenge. We removed the stomachs 45 min later. The gastric ulcers, production of gastric juice and its acidity were also measured.

RESULTS: The findings of our study are as follows: (1) *GbE* pretreatment was found to provide a dose-dependent protection against the ethanol-induced gastric ulcers in rats; (2) the *GbE* pretreatment afforded a dose-dependent inhibition of ethanol-induced depletion of stomach wall mucus, NP-SH contents and increase in the lipid peroxidation (increase MDA) in gastric tissue; (3) gastric ulcer induced by ethanol produced an increase in JNK activity in gastric mucosa which also significantly inhibited by pretreatment with *GbE*; and (4) *GbE* alone had no inhibitory effect on gastric secretion in pylorus-ligated rats.

CONCLUSION: The finding of this study showed that *GbE*...
showed the GbE protective effects on the ethanol-induced gastric mucosal lesion. Thus, the present study was intended to evaluate the possible mechanisms of GbE protective effect on the ethanol-induced gastric ulcer in rats.

**MATERIALS AND METHODS**

**Animals and the experimental design**

Female Wistar albino rats (Animal Center of National Taiwan University, Taipei, Taiwan) about 180-200 g were used for the study. We maintained the animals in air conditional room and allowed them free access to tap water. Food was deprived but free access to tap water was allowed 36 h before the experiment to ensure an empty stomach.

We purchased *Ginkgo biloba* extract (GbE, Cerenin® ampule, 3.5 mg GbE/mL) from DR. Willmar Schwabe Karlsruhe F.R.G. (Germany), and used normal saline as a vehicle.

The animals were divided into five subgroups for each study. GbE 8.75, 17.5, 26.25 mg/kg were given intravenously to the experimental groups respectively 30 min prior to the ulcerative challenge (50% ethanol, 1 mL) by orogastric gavage to the stomach of fasted rats. Each control and experimental group consisted of five rats.

We sacrificed animals under ether anesthesia 45 min after treatment with ethanol, and removed their stomachs which were opened along the greater curvature and examined for lesions developed in the glandular portion under dissecting microscope (×10) with a square grid. The numbers of ulcer lesions (U. No.) in the glandular portion under dissecting microscope were noted. The ulcer area (mm²) were measured and expressed as the ulcer index (U.I.). We calculated the protective ratio (%) according to the following formula:

\[
\text{Preventive ratio} (%) = \frac{(a-b)}{a} \times 100
\]

where 

- \(a\): the ulcer index of the control group
- \(b\): the ulcer index of the experimental group

**Determination of gastric mucus**

The glandular stomach was removed and weighted. We transferred the glandular segments immediately to 0.1% Alcian blue solution in 0.16 mol/L sucrose solution with 0.05 mol/L sodium acetate to pH 5.0 and stained for 2 h at room temperature. After having rinsed with sucrose solution, we extracted the dye complexed with the gastric mucus with 0.05 mol/L magnesium chloride solutions. The aliquot of magnesium chloride solution (4 mL) was further extracted with equal volume of diethyl ether and centrifuged (3 600 r/min, 10 min). Then, we calculated the quantity of Alcian blue extracted/g (net) of glandular tissue.

**Estimation of non-protein sulfhydryl groups (NP-SH) and malondialdehyde (MDA) contents**

After the rats were killed, we opened the glandular stomachs and rinsed them in ice-cold saline, then stored them rapidly in a dry ice bath until analyzed. For the determination of NP-SH, the tissues were homogenized in ice-cold 50% (g/L) aqueous TCA and centrifuged. We determined the NP-SH by measuring the supernatants and 5,5’-dithiobis (2-nitrobenzoic acid) in phosphate buffer (pH 8.0). We read the absorbance (412 nm) 5 min after it is being incubated.

To determine the MDA, we incubated the supernatants with N-methyl-2-phenylindole and we read absorbance at 586 nm according to the manufacturer’s instructions (BIOXYTECH LPO-586 kits, OXIS International Inc., Portland, USA).

**Determination of anti-secretary activity in pylorus-ligated rats**

We fasted animals for 36 h. The following procedures were carried out under ether anesthesia. We gave GbE intravenously 15 min before pylorus ligation, and collected gastric juice 3 h after pylorus ligation. After its volume was measured and expr-esssed in mL/L, the gastric juice was centrifuged at 3 000 r/min for 10 min. We also determined the total acidity of gastric juice by titrating it with 0.01 mol/L NaOH to pH 7.0.

**Western blotting and JNK kinase activity assay**

Gastric mucosa was homogenized in Gold lysis buffer (40 mmol/L Tris-NaOH pH 7.5, 500 mmol/L NaCl, 0.1% NP-40, 6 mmol/L EDTA, 6 mol/L EGTA, 10 mmol/L β-glycerophosphate, 10 mmol/L NaF, 10 mmol/L PNPP, 300 μmol/L sodium orthovanadate, 1 mmol/L benzamidine, 2 μmol/L PMSF, 10 μg/mL aprotinin, 1 μg/mL leupeptin, and 1 mmol/L DTT) and centrifuged. We collected the supernatant as total tissue lysate. Equal amounts of total tissue lysate (50 μg) were resolved by SDS-polyacrylamide gel electrophoresis (PAGE), and transferred onto Immobilon-P membrane (Millipore, Bedford, MA) as described previously.

We also incubated the membrane with an anti-JNK1 antiserum (Transduction Laboratories, Lexington, KY). The membranes were subsequently probed with anti-mouse IgG antibody conjugated to horseradish peroxidase (Santa Cruz Biotechnology) and visualized using enhanced chemiluminescence’s kits (ECL, Amersham). For kinase assay, we immunoprecipitated equal amounts of total tissue lysate (400 μg) with JNK1 specific antibody and protein A/G-PLUS agarose for 15 h at 4 °C. Kinase assay was carried out in 45 μL of kinase buffer (40 mmol/L Tris-NaOH pH 7.5, 500 mmol/L NaCl, 0.1% NP-40, 6 mmol/L EDTA, 6 mol/L EGTA, 10 mmol/L β-glycerophosphate, 10 mmol/L NaF, 10 mmol/L PNPP, 300 μmol/L sodium orthovanadate, 1 mmol/L benzamidine, 2 μmol/L PMSF, 10 μg/mL aprotinin, 1 μg/mL leupeptin, and 1 mmol/L DTT) containing 5 μmol/L cold ATP, 10 μCi [γ-32P] ATP (5 000 Ci/mmol, Amersharn), and 1 μg GST-c-Jun fusion protein (Santa Cruz Biotechnology) as substrate, and incubated for 20 min at 25 °C. We mixed each sample with 8 μL of 5X Laemmli’s loading buffer to stop the reaction, heated for 10 min at 100 °C, and subjected to 8% SDS-PAGE. The gels were dried, visualized by autoradiography.

**Statistical analysis**

Results were expressed as mean±SE for each experiment. We analyzed the data with Student’s *t*-test. If a *P* value was less than 0.05, the differences were considered statistically significant.

**RESULTS**

Effect of GbE on ethanol-induced gastric mucosal damage
Table 1 Effect of Ginkgo biloba extract on the induction of gastric ulcers by 50% ethanol in rats

| Treatment and dose                  | n  | U. Number | U.I. (mm²) | Preventive ratio (%) |
|-------------------------------------|----|-----------|------------|----------------------|
| Control (NS)                        | 5  | 0         | 0          | 0                    |
| NS + 50% EtOH (1 mL/rat)            | 5  | 10±4      | 20.3±1.23  |                      |
| GbE (8.75 mg/kg)+50% EtOH           | 5  | 8±3       | 15.2±3.21  | 24.91                |
| GbE (17.5 mg/kg)+50% EtOH           | 5  | 3±3       | 8.8±3.59   | 56.52                |
| GbE (26.25 mg/kg)+50% EtOH          | 5  | 2±2       | 6.3±1.22   | 68.64                |

Table 2 Effect of Ginkgo biloba extract on the induction of changes in gastric wall mucus by gavages with 50% ethanol

| Treatment and dose                  | n  | Gastric wall mucus (μg Alcian blue/g wet tissue, mean±SE) |
|-------------------------------------|----|---------------------------------------------------------|
| Control (NS)                        | 5  | 442.1±28.36                                            |
| NS + 50% EtOH (1 mL/rat)            | 5  | 356.7±30.67                                            |
| GbE (8.75 mg/kg)+50% EtOH           | 5  | 348.8±32.37                                            |
| GbE (17.5 mg/kg)+50% EtOH           | 5  | 395.2±25.91                                            |
| GbE (26.25 mg/kg)+50% EtOH          | 5  | 410.8±29.16                                            |

Effect of GbE on ethanol-induced gastric mucus

There was a significant decrease in the gastric mucus after treatment with 1 mL 50% ethanol. As shown in Table 2, pretreatment of GbE (8.75-26.25 mg/kg) significantly protected the decline in the gastric mucus levels which were induced by ethanol.

Table 3 Effect of Ginkgo biloba extract on the levels of NP-SH in the stomachs of rats treated by gavages with 50% ethanol

| Treatment and dose                  | n  | NP-SH concentrations (μg/100 mg wet tissue, mean±SE) |
|-------------------------------------|----|-----------------------------------------------------|
| Control (NS)                        | 5  | 12.21±1.03                                           |
| NS + 50% EtOH (1 mL/rat)            | 5  | 2.82±1.32                                            |
| GbE (8.75 mg/kg)+50% EtOH           | 5  | 5.03±1.25                                            |
| GbE (17.5 mg/kg)+50% EtOH           | 5  | 5.66±1.82                                            |
| GbE (26.25 mg/kg)+50% EtOH          | 5  | 11.32±2.01                                           |

Effect of GbE on ethanol-induced gastric mucosal NP-SH and MDA contents

Ethanol (50%, 1 mL) treatment significantly reduced the NP-SH concentration in the gastric mucosa as compared with control in rats. As shown in Table 3, GbE pretreatment significantly prevented the decrease in NP-SH concentrations in the dose of 8.75-26.25 mg/kg. However, during the GbE treatment alone there was no change in the concentration of NP-SH in gastric mucosa. Moreover, pretreatment of GbE significantly prevented lipid peroxidation induced by ethanol, the MDA concentrations were 209.27±10.48 or 118.8±8.26 in the rats that were treated with ethanol alone or combined with GbE, respectively (Table 4).
also inhibited ethanol-induced depletion in the NP-SH concentrations (Table 3) and MDA production in gastric mucosa (Table 4). All those findings in our study suggest that GbE has its role of being a free radical scavenger, decreases the lipid peroxidation, and blocks the loss of mucus, NP-SH, resulting in having protective effect on ethanol-induced mucosal injury.

Apoptosis has been implicated in causing ethanol-induced gastric mucosal injury[27-29]. Previous studies indicated that JNK kinase activity was elevated during the process of apoptosis and blocking of JNK activity was able to prevent cell apoptosis. The data from this study showed that ethanol significantly increased the JNK kinase activity, resulting in cell apoptosis and gastric mucosal damage. As shown in Figure 1, blocking of JNK activity may contribute to GbE’s gastric mucosal protection.

The finding of this study further showed that GbE did not significantly decrease the gastric acid secretion (Table 5). Our finding here is similar to the report by Wang et al in 2000[10].

In conclusion, the data of this study suggest that GbE can inhibit ethanol-induced gastric lesions in rats. The possible mechanisms of GbE’s antiulcer benefit may be due to its oxygen radicals scavenging by inhibition of lipid peroxidation, preventing the loss of gastric mucus and NP-SH, and blockade of ethanol-induced apoptosis. Further studies are warranted to evaluate GbE at the pharmacological effective dosage before any consideration for clinical trials.

REFERENCES

1. Kleijnen J, Knipschild P. Ginkgo biloba. Lancet 1992; 340: 1136-1139
2. Pincemail 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
3. Gardés-Albert M, Ferradini C, Sekaki A, Droy-Lefaix MT. Oxygen-centered free radicals and their interactions with EGB 761 or CP 202. In: Ferradini C, Droy-Lefaix MT, eds. Advances in Ginkgo biloba extract research: Ginkgo biloba extract (EGB 761) as a free-radical scavenger. New York Elsevier Science 1993: 1-11
4. Akiba S, Kawachi T, Oka 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 1998; 46: 1243-1248
5. Akisu M, Kultursay N, Coker I, Huseyinov A. Platelet-activating factor is an important mediator in hypoxic ischemic brain injury in the newborn rat. Flunarizine and Ginkgo biloba extract reduce PAF concentration in the brain. Biol Neonate 1998; 74: 439-444
6. Kleijnen J, Knipschild P. Ginkgo biloba for cerebral insufficiency. Br J Clin Pharmacol 1992; 34: 352-358
7. Oates PJ, Hakkinen JP. Studies on the mechanism of ethanol-induced gastric damage in rats. Gastroenterology 1988; 94: 10-21
8. Szabo S, Goldberg I. Experimental pathogenesis: drugs and chemical lesions in the gastric mucosa. Scand J Gastroenterol Suppl 1990; 174: 1-8
9. Pihan G, Regillo C, Szabo S. Free radicals and lipid peroxidation in ethanol- or aspirin-induced gastric mucosal injury. Dig Dis Sci 1987; 32: 1395-1401
10. Szeleynyi I, Brune K. Possible role of oxygen free radicals in ethanol-induced gastric mucosal damage in rats. Dig Dis Sci 1988; 33: 865-871

Table 5 Effect of Ginkgo biloba extract on the gastric secretion and acidity in pylorus-ligated rats

| Treatment and dose | n | Volume of gastric secretion (ml) | Titratable acidity (mEq/L) |
|--------------------|---|---------------------------------|---------------------------|
| Control (NS)       | 5 | 12.35±0.98                      | 63.82±8.83                |
| GbE (8.75 mg/kg)   | 5 | 12.67±0.81                      | 61.98±7.34                |
| GbE (17.5 mg/kg)   | 5 | 11.98±0.53                      | 58.14±9.28                |
| GbE (26.25 mg/kg)  | 5 | 10.76±0.91                      | 56.28±8.91                |

1 NS, normal saline; GbE, Ginkgo biloba extract; ETOH, ethanol. Each value repessed with mean±SE.
11 Wang Q, Zhao WZ, Ma CG. Protective effects of Ginkgo biloba extract on gastric mucosa. Acta Pharmacol Sin 2000; 21: 1153-1156

12 Liang YC, Huang YT, Tsai SH, Lin-Shiau SY, Chen CF, Lin JK. Suppression of inducible cyclooxygenase and inducible nitric oxide synthase by apigenin and related flavonoids in mouse macrophages. Carcinogenesis 1999; 20: 1945-1952

13 Body SC, Sasame HA, Body MR. High concentrations of glutathione in glandular stomach: possible implications for carcinogenesis. Science 1979; 205: 1010-1012

14 Videla LA, Valenzuela A. Alcoholic ingestion, liver glutathione and lipoperoxidation: metabolic interrelations and pathological implications. Life Sci 1982; 31: 1295-1297

15 Li T, Zhang XJ. Involvement of sulfhydryls in the protective mechanism of gastric mucosa. ShengLi XueBao 1990; 42: 571-577

16 Allen A, Cunliffe WJ, Pearson JP, Sellers LA, Ward R. Studies on gastrointestinal mucus. Scand J Gastroenterol Suppl 1984; 93: 101-113

17 Salim AS. Sulphydryl-containing agents and the prevention of duodenal ulcer relapse. Pharmacology 1993; 46: 281-288

18 Garner A, Flenstrom G, Allen A, Heylings JR, McQueen S. Gastric mucosal protective mechanisms: roles of epithelial bicarbonate and mucus secretions. Scand J Gastroenterol Suppl 1984; 101: 79-86

19 Farre AJ, Colombo M, Alvarez I, Glavin GB. Some novel 5-hydroxytryptamine 1A (5-HT1A) receptor agonists reduce gastric acid and pepsin secretion, reduce experimental gastric mucosal injury and enhance gastric mucus in rats. J Pharmacol Exp Ther 1995; 272: 832-837

20 Grisham MB, Von Ritter C, Smith BF, Lamont JT, Granger DN. Interaction between oxygen radicals and gastric mucus. Am J Physiol 1987; 253: G93-G96

21 Cross CE, Halliwell B, Allen A. Antioxidant protection: a function of tracheobronchial and gastrointestinal mucus. Lancet 1984; 1: 1328-1330

22 Gong DH, Turner B, Bhaskar KR, Lamont JT. Lipid binding to gastric mucin: protective effect against oxygen radicals. Am J Physiol 1990; 259: G681-G686

23 Hiraiishi H, Terano A, Ota S, Mutoh H, Sugimoto T, Harada T, Razandi M, Ivy KJ. Role for mucous glycoprotein in protecting cultured rat gastric mucosal cells against toxic oxygen metabolites. J Lab Clin Med 1993; 121: 570-578

24 Terano A, Hiraishi H, Ota S, Shiga J, Sugimoto T. Role of superoxide and hydroxyl radicals in rat gastric mucosal injury induced by ethanol. Gastroenterol Jpn 1989; 24: 488-493

25 Mutoh H, Hiraishi H, Ota S, Ivy KJ, Terano A, Sugimoto T. Role of oxygen radicals in ethanol-induced damage to cultured gastric mucosal cells. Am J Physiol 1990; 258: G603-G609

26 Slomiany A, Morita M, Sano S, Piotrowski J, Skrodzka D, Slomiany BL. Effect of ethanol on gastric mucus glycoprotein synthesis, translocation, transport, glycosylation, and secretion. Alcohol Clin Exp Res 1997; 21: 417-423

27 Piotrowski J, Piotrowski E, Skrodzka D, Slomiany A, Slomiany BL. Gastric mucosal apoptosis induced by ethanol: effect of antiulcer agents. Biochem Mol Biol Int 1997; 42: 247-254

28 Mizushima T, Tsutsui S, Rokutan K, Tsuichiya T. Suppression of ethanol-induced apoptotic DNA fragmentation by geranylgeranylacetone in cultured guinea pig gastric mucosal cells. Dig Dis Sci 1999; 44: 510-514

29 Hoshino T, Takano T, Tsutsui S, Tomisato W, Tsuichiya T, Mizushima T. Effects of prostaglandin E2 on gastric irritant-induced apoptosis. Dig Dis Sci 2002; 47: 2370-2379

30 Marucci 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

31 Kim HK, Son KH, Chang HW, Kang SS, Kim HP. Inhibition of rat adjuvant-induced arthritis by ginkgetin, a biflavone from ginkgo biloba leaves. Planta Med 1999; 65: 465-467

32 Yoshikawa T, Naito Y, Kondo M. Ginkgo biloba leaf extract: review of biological actions and clinical applications. Antioxid Redox Signal 1999; 1: 469-480