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Protective effects of *Ginkgo biloba* extract on gastric mucosa[1]

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KEY WORDS *Ginkgo biloba*; gastric mucosa; oxygen; free radicals

ABSTRACT

AIM: To study the protective effects of *Ginkgo biloba* extract (*Gb*E) on gastric mucosa. **METHODS**: By means of restraint-cold stress (RCS) in rats and 100 % ethanol gavage in mice, the index of gastric mucosal injury was evaluated. The gastric juice was collected using pyloric ligation, and the volume and acidity of juice, and activity of pepsin were determined. The content of malondialdehyde (MDA) was measured by thiobarbituric acid (TBA) method. **RESULTS**: *Gb*E (25, 50, and 100 mg/kg, bid × 5 d, ig) inhibited dose-dependently the gastric mucosal injury induced by RCS and 100 % ethanol gavage. The index of gastric mucosal injury after RCS in groups pretreated with *Gb*E was 58 %, 43 %, and 31 % of control group respectively. The index of gastric mucosal injury induced by ethanol in groups pretreated with *Gb*E was 62 %, 36 %, and 26 % of the control group, respectively. And *Gb*E enhanced the protective effects of cimetidine (Cim) on gastric mucosa. But it did not obviously influence the volume and acidity of gastric juice as well as the activity of pepsin. One hour after the administration of ig 100 % ethanol, the contents of MDA in gastric mucosa and serum in mice increased ($P < 0.01$) *vs* the control group. But pretreatment with *Gb*E (25, 50, and 100 mg/kg, ig) could inhibit this increase of MDA both in gastric mucosa and in serum. **CONCLUSION**: *Gb*E had protective effects on gastric mucosa and *Gb*E plus Cim possessed the synergism in the treatment of acute gastric mucosal lesions.

INTRODUCTION

Ginkgo biloba extract (*Gb*E) is known to have beneficial effects on the pathology of cardiovascular and cerebrovascular diseases[1]. Several recent investigations have shown that *Gb*E has antilipo-peroxidant properties, as it is observed to inhibit the formation of malondialdehyde (MDA)[2,3]. *Gb*E is also effective against small intestinal ischemia-reperfusion injuries of rat[4]. But the relation between *Gb*E and acute gastric mucosal injury has not been investigated as yet. So the present study was designed to examine whether *Gb*E would have protective effects on gastric mucosa. Furthermore, we studied the relation between the anti-lesion effect on gastric mucosa of *Gb*E and its antioxidant action to clarify the possible mechanism of gastric mucosal protective effects of *Gb*E.

MATERIALS AND METHODS

*Gb*E, provided by WanNan DaPeng Nature Production Company (major components: Flavonoids: 24.78 %, terpenoids: 7.9 %) was dissolved in 0.9 % sterile saline prior to the experiment.

Thiobarbituric acid (TBA) was the product of Shanghai Second Reagent Factory.

Cimetidine (Cim) was from Shanghai First Preparation Factory.

Male Wistar rats (200 ± 30) g, healthy Kunming mice (20 ± 2) g of either sex, were obtained from the Experimental Animal Centre of Anhui Medical University (Grade Ⅱ, Certificate No 01). Animals were fasted for 24 h but allowed free access to water before experiments.

Restraint-cold stress (RCS) in rats[5] After being anesthetized with ether, rats were tied to the iron bars. When rats woke, they were stressed at 4 ℃ in the refrigerator. After 3 h, the rats were taken out and killed.

A 100 % ethanol gavage in mice One hour after the last ig *Gb*E or saline, 100 % ethanol 0.5 mL was given ig. The stomach was examined 1 h later.

Assessment of gastric mucosal injury After

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the rat was sacrificed, the stomach was taken out and filled with 10 mL/L formalin 10 mL and immersed in the same concentration of formalin for 10 – 20 min to be fully fixed. Then the stomach was dissected, opened along the greater curvature, spread on a pad, and examined macroscopically. The bleeding spot mass was measured according to Guth's method[6]. Finally the summation of the index of the whole gastric lesion was regarded as the index of gastric mucosal injury of the rat. To exclude error between groups in experiments, each group was equipped with a control group. The index of injury was translated into the percentage of control group in order to compare the results between groups conveniently.

After the mouse was sacrificed, the stomach was taken out and infused with formalin 2 mL and put into the same concentration of formalin to be fully fixed. Finally the stomach was incised along the greater curvature and examined under a binocular microscope with a microruler of 0.01 mm precision for measuring the bleeding spot mass. The summation of area of the whole gastric lesion was regarded as the index of gastric mucosal injury of the mouse. The rest was the same as above in the rat test.

Measurement of the volume and acidity of gastric juice, and activity of pepsin in rats[7] One hour after the last ig *Gb*E or saline, the abdomen was incised under ether anesthesia, and the pylorus was ligated for 5 h. Then the gastric juice was collected and titrated with NaOH 0.01 mol/L to measure the acidity.

Measurement of MDA contents in mice The contents of MDA in gastric mucosa and serum were determined by TBA method[8].

Statistics Data were expressed as $\bar{x} \pm s$ and compared with t test, the significant level was set at P value < 0.05.

RESULTS

Effects of *Gb*E on gastric mucosal damage induced by RCS and 100 % ethanol Pretreatment with *Gb*E (25, 50, and 100 mg/kg, bid × 5 d, ig) reduced markedly the gastric mucosal damage induced by RCS in rats and 100 % ethanol in mice compared with control ($P < 0.01$, Tab 1).

Tab 1. Effects of *Gb*E on gastric mucosal damage induced by RCS in rats and 100 % ethanol in mice. $^cP < 0.01$ *vs* RCS group. $^fP < 0.01$ *vs* 100 % ethanol group.

Group	Dose/mg·kg^{-1}	Index of injury (% of control)
RCS	—	100 ± 12
*Gb*E + RCS	25	58 ± 8^c
	50	43 ± 14^c
	100	31 ± 10^c
100 % ethanol	—	100 ± 12
*Gb*E + 100 % ethanol	25	62 ± 18^f
	50	36 ± 11^f
	100	26 ± 8^f

Influence of *Gb*E on the volume and acidity of gastric juice, and activity of pepsin in rats After *Gb*E administration (25, 50 and 100 mg/kg, bid × 5 d, ig), the volume of gastric juice decreased, but no significant difference could be detected *vs* control group ($P > 0.05$). And the acidity and activity of pepsin were not influenced (Tab 2).

Influence of *Gb*E on the protective effects of cimetidine on gastric mucosal damage (Cim 140 mg/kg, bid × 5 d, ig) could significantly protect gastric mucosa against the damage induced by restraint-cold stress (RCS). Different doses of *Gb*E (25, 50, and 100 mg/kg, bid × 5 d, ig) plus Cim enhanced the protective effects of Cim. Administration of *Gb*E in combination with Cim reduced the index of injury after RCS ($P < 0.05$) *vs* the Cim + RCS group (Tab 3).

Tab 2. Effects of *Gb*E on the volume and acidity of gastric juice, and activity of pepsin. $n = 6$ rats. $\bar{x} \pm s$.

Group	Dose/mg·kg^{-1}	Gastric juice			Pepsin activity/ U·kg·h^{-1}
		Volume/mL	Free acidity/nmol·L^{-1}	Total activity/ nmol·L^{-1}	
Control	—	8 ± 4	93 ± 23	160 ± 11	199 ± 33
*Gb*E	25	7 ± 1	114 ± 11	152 ± 6	179 ± 15
	50	6 ± 2	111 ± 15	146 ± 30	190 ± 21
	100	4 ± 1	106 ± 23	158 ± 15	195 ± 33

Tab 3. Synergism between the effects of *GbE* and Cim on gastric mucosal damage induced by RCS in rats. $n = 6$ rats. $\bar{x} \pm s$. $^cP < 0.01$ *vs* RCS group. $^eP < 0.05$, $^fP < 0.01$ *vs* Cim + RCS group.

Group	Dose/mg·kg^{-1}	*Index of injury* (% of control)
RCS	—	100 ± 13
Cim + RCS	140	58 ± 15^c
*Gb*E + Cim + RCS	25 ± 140	38 ± 9^{ce}
	50 ± 140	32 ± 9^{cf}
	100 ± 140	27 ± 8^{cf}

Antioxidation effect of *GbE* during damage induced by 100 % ethanol in mice After 100 % ethanol ig, the contents of MDA in gastric mucosa and serum increased. But pretreatment with *Gb*E (25, 50, and 100 mg/kg, bid × 5 d, ig) markedly inhibited the increment of MDA formation both in gastric mucosa and in serum induced by ethanol (Tab 4).

Tab 4. Effects of *GbE* on change of MDA level in gastric mucosa and serum after ig 100 % ethanol in mice. $n = 8$ mice. $\bar{x} \pm s$. $^cP < 0.01$ *vs* control. $^eP < 0.05$, $^fP < 0.01$ *vs* 100 % ethanol group.

Group	Dose/ mg·kg^{-1}	MDA In gastric mucosa/ μmol·g^{-1}(protein)	MDA In serum/ nmol·L^{-1}
Control	—	2.0 ± 0.6	2.3 ± 0.6
100 % Ethanol	—	3.3 ± 0.4^c	4.0 ± 0.6^c
*Gb*E + 100 % Ethanol	25	2.6 ± 0.6^f	3.1 ± 0.8^e
	50	2.3 ± 0.3^f	3.0 ± 0.8^e
	100	2.2 ± 0.5^f	2.4 ± 0.6^f

DISCUSSION

Pretreatment with *Gb*E (25, 50, 100 mg/kg, bid × 5 d, ig) inhibited dose-dependently the acute gastric mucosal lesion induced in two kinds of models. Moreover *Gb*E could enhance the protective effects of Cim (H_2-receptor blocker) on gastric mucosa. These results implied that *Gb*E had significant protective effects on acute gastric mucosal lesions and the synergism between *Gb*E and Cim could contribute to the drug therapy of acute gastric mucosal lesion.

This study also showed that *Gb*E did not obviously influence the volume and acidity of gastric juice as well as activity of pepsin. It indirectly implicates that *Gb*E might have a cytoprotective action. *Gb*E had anti-lesion effects on gastric mucosa, but it did not disturb the normal digestive function. This suggests that *Gb*E might have the similar cytoprotection on gastric ulcer as other flavonoids[9].

Increasing evidence suggests that various experimental acute gastric mucosal lesions are related to oxygen-derived free radicals[10,11]. In 1989 Lu[12] proposed that MDA generation could be used as an indicator of ·OH-caused lipid peroxidation and indirectly reflected the amount of ·OH formed. And ·OH is a major cause in gastric mucosal cell injury induced by ethanol *in vitro*[13]. A recent study done by Xia *et al*[14] also demonstrated that administration of 75 % or 95 % ethanol, ig to mice, could induce acute gastric mucosal lesions accompanied with an increase in MDA in gastric mucosa. And it has also been reported that oxygen-derived free radical is an important pathogenic factor of RCS[15]. Moreover, *Gb*E could block lipo-peroxidation and inhibit the formation of MDA with a positive relation to *Gb*E concentration[2]. Thus, as our results showed, *Gb*E could protect gastric mucosa against damage induced by RCS and ethanol. This implied that these protective effects of *Gb*E on gastric mucosal injury might be associated with its antioxygen-derived free radical scavenging property. The increased MDA contents indicated that during the development of mucosal injury by ethanol, the formation of free-radicals increased. Pretreatment with *Gb*E (25, 50, and 100 mg/kg, bid ig × 5 d) inhibited the ethanol-induced increase in MDA contents both in gastric mucosa and in serum. This suggested that *Gb*E was not only involved in the local gastric mucosa protective mechanism but also enhanced the antioxidant activity of the whole body.

In conclusion, *Gb*E (25, 50, and 100 mg/kg, ig) had protective effects on gastric mucosa and *Gb*E plus Cim possessed synergism in the treatment of acute gastric mucosal lesions. These effects may be related to its antioxidant action.

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银杏叶提取物的胃粘膜保护作用[1]

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关键词 银杏; 胃粘膜; 氧; 自由基

目的: 研究银杏叶提取物的胃粘膜保护作用. **方法**: 采用大鼠束缚-冷冻应激(RCS)模型和小鼠无水乙醇损伤模型观察 *Gb*E 对胃粘膜损伤指数的影响; 采用幽门结扎法收集胃液, 观察 *Gb*E 对胃液分泌量, 胃液酸度和胃蛋白酶活性的影响; 采用硫代巴比妥酸(TBA)法测定胃粘膜及血清中丙二醛(MDA)含量. **结果**: *Gb*E (25, 50, 100 mg/kg, bid × 5 d, ig)剂量依赖性地抑制 RCS 和无水乙醇引起的胃粘膜损伤. 用药组应激后的胃粘膜损伤指数分别为对照组的 58 %, 43 %和 31 %; 用药组乙醇诱发的胃粘膜损伤指数降至对照组的 62 %, 36 %和 26 %; *Gb*E 尚能增强西米替丁对胃粘膜的保护作用, 但对大鼠胃液分泌量、胃液酸度及胃蛋白酶活性 *Gb*E 并无明显影响. 小鼠经无水乙醇 ig 后 1 h, 胃粘膜和血清中的 MDA 含量显著升高($P < 0.01$), 而 *Gb*E (25, 50, 100 mg/kg, ig)预处理则可以明显抑制 MDA 的升高. **结论**: *Gb*E 具有胃粘膜保护作用, 并且与西米替丁在治疗急性胃粘膜损伤方面具有协同作用.

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