

Protective effects of *Ginkgo biloba* extract on cultured rat cardiomyocytes damaged by H₂O₂

NIU Yu-Hong, YANG Xue-Yi¹, BAO Wei-Sheng (Shanghai Institute of Cardiovascular Diseases, Zhongshan Hospital, Shanghai Medical University, Shanghai 200032, China)

KEY WORDS *Ginkgo biloba*; oxygen; free radicals; myocardium; cultured cells; hydrogen peroxide

ABSTRACT

AIM: To investigate the influence of *Ginkgo biloba* extract (*GbE*) on cardiomyocytes damaged by H₂O₂. **METHODS:** Cultured rat cardiomyocytes were divided into 3 groups randomly: control group; H₂O₂ (2.5 mmol·L⁻¹) group; H₂O₂ 2.5 mmol·L⁻¹ + *GbE* 150 mg·L⁻¹ group. The cardiomyocytes were cultured in MEM (Eagle's) at 37 °C in the presence of 5 % CO₂ for 4 h. Lactate dehydrogenase (LDH) was assayed by colorimetric method. Lipid peroxidation was determined by measuring thiobarbituric acid-reactive substances. Ultrastructure was viewed under transmission electron microscope. **RESULTS:** Compared with the control group, LDH leakage and malondialdehyde (MDA) content increased in H₂O₂ group. LDH increased from (2166 ± 247) U·L⁻¹ to (5180 ± 648) U·L⁻¹. MDA increased from (3.5 ± 0.2) nmol/10⁶ cells to (7.2 ± 0.4) nmol/10⁶ cells (*P* < 0.01). The ultrastructure was damaged seriously. *GbE* inhibited the increase of LDH leakage and MDA content induced by H₂O₂. In this group, LDH decreased from (5180 ± 648) U·L⁻¹ to (3496 ± 386) U·L⁻¹, MDA decreased from (7.2 ± 0.4) nmol/10⁶ cells to (4.8 ± 0.9) nmol/10⁶ cells (*P* < 0.01). Ultrastructure of cells was also protected by *GbE*. **CONCLUSION:** *GbE* protected the cardiomyocyte against H₂O₂ injury, the protective action was attributed to its antiperoxidative effect.

INTRODUCTION

Ginkgo biloba has been a staple of Chinese medicine for thousands of years, being recommended for coughs, asthma, and acute allergic inflammation. *Ginkgo biloba* extract (*GbE*) can react with free oxygen radicals such as O₂^{·-}, ·OH, 2, 2-DPPH *in vitro*^[1]. *GbE* had a superoxide dismutase-like activity^[2]. However, the report of the effect of *GbE* on cultured cells damaged by free oxygen radicals has not been found. In the present study, the influence of *GbE* on cultured cells damaged by H₂O₂ was studied.

MATERIALS AND METHODS

Rats Sprague-Dawley rats (Certificate No 2-22-11) aging 2 - 3 d (*n* = 60) were obtained from Experimental Animal Center of Shanghai Medical University.

Drug *GbE* was the product of Willmar Schwabe Pharmaceutical Factory, Germany. (lot X940261, 3.5 g·L⁻¹). Per ampoule was standardized to contain *Ginkgo* flavoneglycosides 4.2 mg. Injection solution 1 mL contained sorbitol 40 mg and 3.5 % ethyl alcohol by volume. *GbE* contained 24 % of flavonoid glycosides^[3], the aglycone of which was a flavonol (including quercetin, kaempferol, and isorhamnetine), 6 % of terpene lactones (including ginkgolides A, B, C, J, and bilobalide), and 70 % of other substances (proanthocyanidins, organic acids, sugars, etc).

Preparation of cardiomyocytes Isolation of cardiomyocyte from rat heart was performed^[4], the cardiomyocytes were grown in MEM (Eagle's) culture medium containing 20 % fetal bovine serum. The cell density was adjusted to 5 × 10⁸ cells·L⁻¹, and 1 mL of the suspension was pipeted into each well of 24-well culture plates. Cells were incubated at 37 °C in 5 % CO₂ atmosphere for 4 d.

¹ Correspondence to Prof YANG Xue-Yi.

Phn 86-21-6404-1990, ext 2514. Fax 86-21-6403-8472.

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Experimental design Cardiomyocytes were used to determine the effect of *GbE* on oxidation induced by H_2O_2 . Solution of H_2O_2 was prepared in PBS buffer. Cardiomyocytes were incubated in medium containing H_2O_2 $2.5 \text{ mmol} \cdot L^{-1}$ with or without *GbE* $150 \text{ mg} \cdot L^{-1}$ at $37^\circ C$ in 5 % CO_2 for 4 h. The cells were viewed under an inverted phase contrast microscope and photographed using a camera attachment.

LDH assay The supernatant was collected from every well for LDH assay. LDH was measured by colorimetric method^[5].

Lipid peroxidation Malondialdehyde (MDA) in membrane was measured. After supernatant was collected, the adherent cells were then dispersed with trypsin/edetic acid (0.1 %/0.04 %) in D-Hanks' solution for 2 min and were ultrasonicated. The cell fragment suspension was collected and the extent of lipid peroxidation was determined by measuring thiobarbituric acid-reactive substances^[6].

Ultrastructure Cardiomyocytes were cultured in culture flask under the same circumstance, after treatment with $H_2O_2 + GbE$, the cells were fixed with 2.5 % glutaraldehyde, then 70-nm ultra-thin sections of cells were made and viewed under transmission electron microscope (TEM).

Statistical analysis Data were expressed as $\bar{x} \pm s$ and analyzed by *t*-test.

RESULTS

Effects of *GbE* on beat and morphologic change of cardiomyocytes injured by H_2O_2 In control culture wells, cardiomyocytes beated synchronously and their appearances were normal. Cells incubated with H_2O_2 $2.5 \text{ mmol} \cdot L^{-1}$ stopped beating, and had a morphologic change; cell pseudopod decreased or contracted, cells tended to be round and granule in cytoplasm increased. After incubation with H_2O_2 $2.5 \text{ mmol} \cdot L^{-1} + GbE$ $150 \text{ mg} \cdot L^{-1}$, cells stopped beating too, but had no morphologic change (Fig 1).

Effect of H_2O_2 and *GbE* on lactate dehydrogenase (LDH) leakage and lipid peroxidation

When cardiomyocytes were incubated without H_2O_2 , LDH leakage maintained at a low level, (2166 ± 247) $U \cdot L^{-1}$. H_2O_2 increased LDH by 239 % vs control

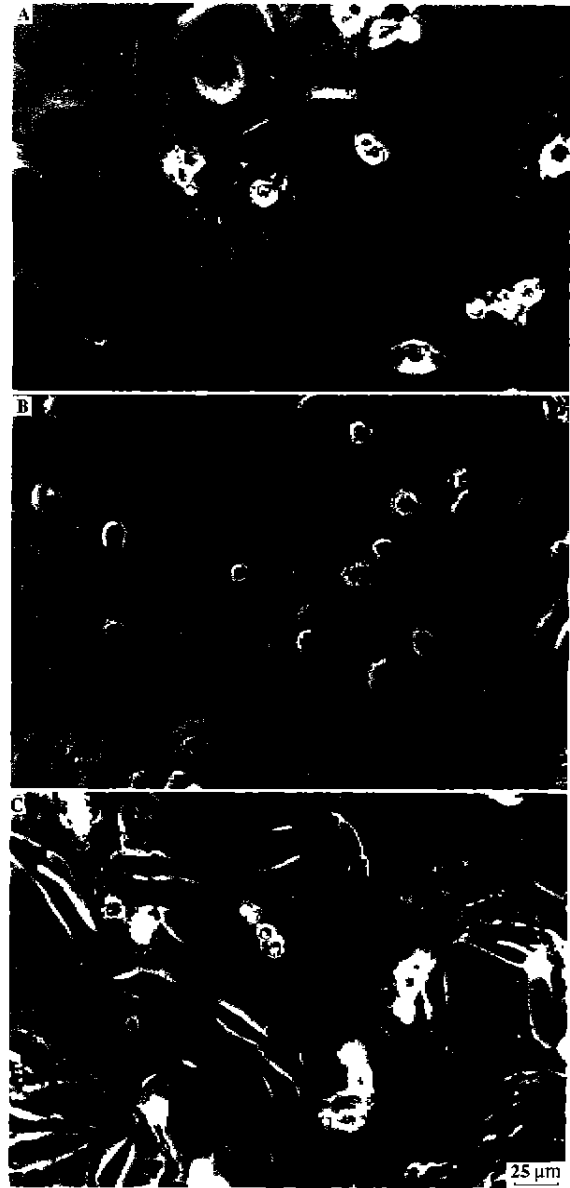


Fig 1. Micrographs of cultured cardiomyocytes. $\times 400$. A: Control group. B: H_2O_2 group. C: *GbE* group.

($P < 0.01$). Incubation with *GbE* $150 \text{ mg} \cdot L^{-1}$ resulted in a decline of LDH release in H_2O_2 treated cells (Tab 1).

Effects of *GbE* on lipid peroxidation of cardiomyocytes were measured by the content of MDA. H_2O_2 increased MDA vs control group ($P < 0.01$). When cells were pretreated with *GbE* $150 \text{ mg} \cdot L^{-1}$, MDA was reduced markedly (Tab 1).

Effect of *GbE* on ultrastructure of cardiomyocytes injured by H_2O_2 Cell ultrastructure in

Tab 1. Effect of GbE on cardiomyocyte injured by H₂O₂. n = 15 experiments, $\bar{x} \pm s$. *P < 0.01 vs control group, [†]P < 0.01 vs H₂O₂ group.

	LDH (U·L ⁻¹)	MDA (nmol/10 ⁶ cells)
Control	2 166 ± 247	3.48 ± 0.24
H ₂ O ₂	5 180 ± 648 ^c	7.2 ± 0.4 ^c
H ₂ O ₂ + GbE	3 496 ± 386 [†]	4.8 ± 0.9 [†]

control group was normal. The ultrastructure of cardiomyocyte demonstrated regular arrangement of myofibril and clear mitochondria structure. The cell membrane was integrated. The nucleus was ellipse and chromatin distributed homogeneously in the nucleus. After exposure to H₂O₂, cell membrane was unintegrated, volume of cell organ decreased. Mitochondrial structure was not clear, the density of its crista was diminished. Myofibrils were broken down. GbE diminished damage at mitochondrial crista and matrix induced by H₂O₂, and distended the sarcoplasmic reticulum. The membrane was integrated, almost normal (Fig 2).

DISCUSSION

H₂O₂ *in vitro* decreased ATP concentration and mediate cell injury^[7,8]. In our present study, cultured cardiomyocytes were damaged seriously by H₂O₂, too.

In our experiment, GbE decreased LDH leakage from cardiomyocyte and MDA content caused by H₂O₂, and prevented the injury of cell structure. But GbE 150 mg·L⁻¹ could not keep the cells injured by H₂O₂ 2.5 mmol·L⁻¹ beating. It indicated that not all of the funtion injury caused by H₂O₂ 2.5 mmol·L⁻¹ could be liminated by GbE 150 mg·L⁻¹. However, GbE 150 mg·L⁻¹ was able to prevent the further injury to the cell structure. The potence contributed to *Ginkgo* flavonoids in GbE. In order to find the best protective result, more dosage of GbE should be used in our present experiment.

In conclusion, GbE protected cardiomyocyte against H₂O₂ injury. The protective action may be

attributed to its antiperoxidative effect, suggesting that GbE should prevent and treat free radical-induced disorders.

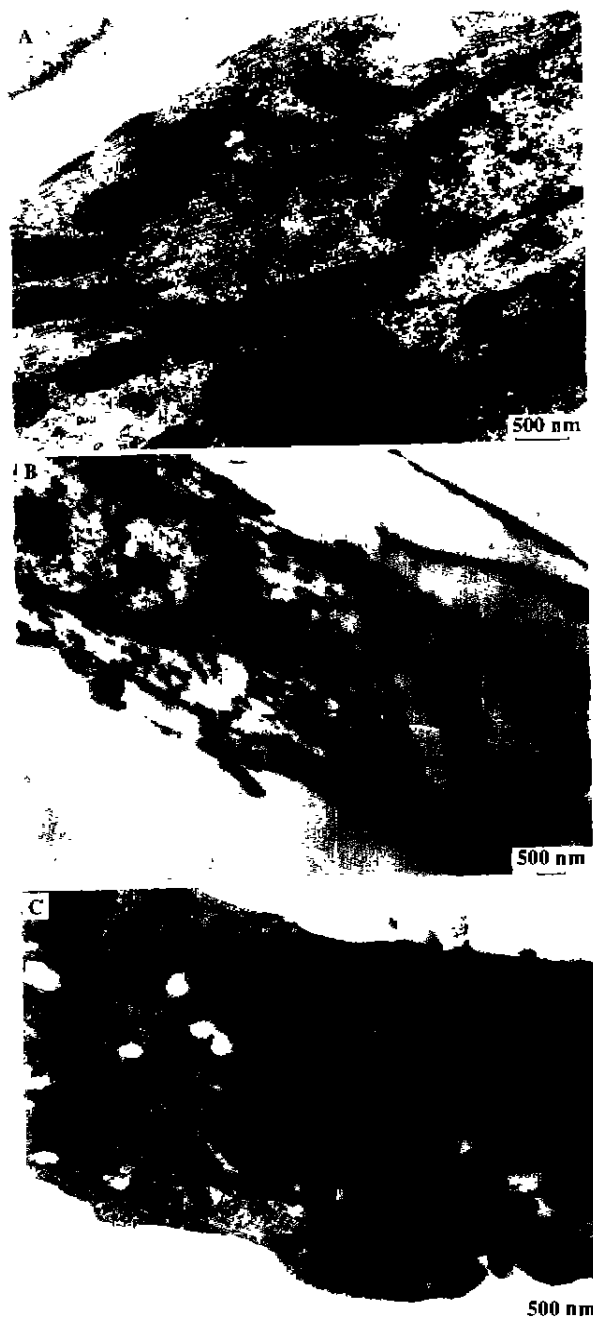


Fig 2. Electron micrographs of cultured cardiomyocytes. Lead citrate, uranyl acetate double stain. A (× 20 000): Control group. B (× 12 000): H₂O₂ group. C (× 20 000): GbE group.

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银杏提取物对过氧化氢损伤的培养大鼠心肌细胞的保护作用

牛玉宏, 杨学义¹, 鲍伟胜

(上海医科大学中山医院, 上海市心血管病研究所, 上海 200032, 中国)

关键词 银杏; 氧; 自由基; 心肌; 培养的细胞; 过氧化氢

目的: 探讨银杏叶提取物 (*GbE*) 对过氧化氢损伤的培养心肌细胞的保护作用及其机制。 **方法:** 比色法测定乳酸脱氢酶活性; 戊巴比妥酸法测定细胞内脂质过氧化物含量; 透射电镜下观察细胞超微结构。 **结果:** 过氧化氢导致心肌细胞 LDH 释放从 $(2166 \pm 247) \text{ U} \cdot \text{L}^{-1}$ 增至 $(5180 \pm 648) \text{ U} \cdot \text{L}^{-1}$, MDA 含量从每 10^6 细胞 $(3.5 \pm 0.2) \text{ nmol}$ 增至 $(7.2 \pm 0.4) \text{ nmol}$; 心肌细胞超微结构受到严重损伤。加 *GbE* 使 LDH 释放从 $(5180 \pm 648) \text{ U} \cdot \text{L}^{-1}$ 降至 $(3496 \pm 386) \text{ U} \cdot \text{L}^{-1}$; MDA 生成由每 10^6 细胞 $(7.2 \pm 0.4) \text{ nmol}$ 降至 $(4.8 \pm 0.9) \text{ nmol}$ 并减轻心肌超微结构的损伤。 **结论:** *GbE* 通过清除氧自由基保护过氧化氢损伤的心肌细胞。

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读者注意

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