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BRIEF COMMUNICATION

# Effects of Oral *Ginkgo biloba* Supplementation on Cataract Formation and Oxidative Stress Occurring in Lenses of Rats Exposed to Total Cranium Radiotherapy

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**Abstract**

**Purpose:** To determine the antioxidant role of *Ginkgo biloba* (GB) in preventing radiation-induced cataracts in the lens after total-cranium irradiation of rats with a single radiation dose of 5 Gy.

**Methods:** Sprague-Dawley rats were randomly divided into three groups. Group 1 received neither GB nor irradiation (control group). Group 2 was exposed to total-cranium irradiation of 5 Gy in a single dose [radiation therapy (RT) Group], and group 3 received total cranium irradiation from a cobalt-60 teletherapy unit, plus 40 mg/kg per day GB (RT+GB group). At the end of the tenth day, the rats were killed and their eyes were enucleated to measure the antioxidant enzymes, the activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), and the lipid peroxidation level [malondialdehyde (MDA)].

**Results:** Irradiation significantly increased both the MDA level and the activity of GSH-Px, and significantly decreased the activity of SOD in the rat lenses. GB supplementation significantly increased the activities of SOD and GSH-Px enzymes and significantly decreased the MDA level. Total cranium irradiation of 5 Gy in a single dose promoted cataract formation, and GB supplementation protected the lenses from radiation-induced cataracts.

**Conclusions:** We suggest that *Ginkgo biloba* is an antioxidant that protects the rat lens from radiation-induced cataracts. **Jpn J Ophthalmol** 2004;48:499–502 © Japanese Ophthalmological Society 2004

**Key Words:** antioxidant enzymes, cataract, gamma-irradiation, lens, oxidative stress

## Introduction

Ionizing radiation, such as X- and γ-rays and ultraviolet light, is known to be a cataractogenic factor in rat lenses.[1–3] The destructive action of ionizing radiation is predominantly due to reactive oxygen species (ROS), including the superoxide radical ($O^{\bullet}_2^-$), the hydroxyl radical ($OH^-$), and hydrogen peroxide ($H_2O_2$), generated by the decomposition of water. Superoxide dismutase (SOD) enzyme catalyzes the dismutation of $O^{\bullet}_2^-$ into $H_2O_2$. $H_2O_2$ can then be transformed into $H_2O$ and $O_2$ by the enzymes catalase (CAT) and glutathione peroxidase (GSH-Px). One of the indices of oxidative damage is the level of malondialdehyde (MDA), which is formed as an end product of lipid peroxidation.[1,2]

*Ginkgo biloba* extract was prepared from the leaves of the *Ginkgo biloba* tree according to a well-defined procedure. This extract has been reported to be a potent

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scavenger for several ROS, including $O^{\bullet}_{2}^{-}$, $OH^{-}$, $H_2O_2$, and peroxyl radicals.[4]

## Materials and Methods

Twenty-seven albino female Sprague-Dawley rats (195 ± 32 g body weight, 8 to 12 weeks old) were randomly divided into three equal groups. Prior to total cranium irradiation, the rats were anesthetized with 80 mg/kg ketamine HCl (Pfizer İlaç, İstanbul, Turkey) and placed on a plexiglass tray in the prone position. Group 1 served as the control and received sham irradiation. Groups 2 and 3 (RT group and RT+GB group, respectively) were exposed to total cranium irradiation of 5 Gy in a single dose from a cobalt-60 teletherapy unit (Picker C-9, Picker, Cleveland, OH, USA) with output 0.59 Gy/min to a 0.5 cm depth from a source-to-surface distance of 80 cm on a 5 × 5 cm anterior field. A bolus material 0.5 cm thick was placed on the rats eyes. The rats in the RT+GB group received 40 mg/kg GB diluted in 1 ml physiological saline containing 40 mg/ml *Ginkgo biloba* extract (Tebokan drops, Abdi İbrahim Pharmaceutical Company, İstanbul, Turkey) daily orally through an orogastric tube, starting 3 days before irradiation and continuing for 7 days after irradiation (total, 10 days). The rats in the control group and the RT group received 1 ml of physiological saline as a placebo. All procedures involving Sprague-Dawley rats adhered to the ARVO Resolution on the Use of Animals in Research.

The lenses were graded by slit-lamp biomicroscopy (Nikon, Zoom-Photo Slit-Lamp, FS-3V, Tokyo, Japan) as follows: grade 0, normal, clear lenses; grade 1, lenses showing visible posterior sutures; grade 2, lenses displaying isolated vacuoles; grade 3, coalescing vacuoles; grade 4, peripheral coalescing vacuoles and radial streaks extending into central crystalline opacity.[1] At the beginning of the experiment, the lenses of all the rats were classified as grade 0.

At the end of the tenth day, the rats were anesthetized with 80 mg/kg ketamine HCl, and then an intracardiac withdrawal of blood was performed. Their eyes were enucleated, and the lenses were dissected out immediately. Lenses were rinsed in ice-cold distilled water, and immediately placed in three times their volume of cold 1.15% KCl (5 ml) containing 0.2% Triton X-100. Lenses were homogenized by an OMNI TH International homogenizer (model TH 220, OMNI, Warrenton, VA, USA) for 10 s at the first speed level. Then, the homogenate was centrifuged at 10000 *g* for 60 min at 4°C. The supernatant was stored at −80°C in aliquots for biochemical measurements. The activities of SOD and GSH-Px enzymes and the MDA level were determined in these supernatants spectrophotometrically (CE 3041, Cecil, Cambridge, UK). The MDA level was expressed as nmol/mg protein. The activities of SOD and GSH-Px enzymes were expressed as U/mg protein of lens sediment.

The Mann Whitney *U* test and χ-squared test were used for statistical analyses with the significance level set at $P < 0.05$.

## Results

Lens grades determined by slit-lamp biomicroscopy and the level of MDA and the activities of SOD and GSH-Px in the rat lens in the control, RT, and RT+GB groups are presented in Table 1. At the end of the tenth day, the lenses in the control group were classified as grade 0 and those in the RT group as grades 1 or 2 ($P = 0.02$). Although grade 1 cataract development was detectable in seven rats in the RT group, it was detectable in only two rats in the RT+GB

**Table 1.** Lens grades by slit-lamp microscopy, level of malondialdehyde (MDA), the superoxide dismutase (SOD) activity, and glutathione peroxidase (GSH-Px) activity in the rat lens in control, radiation therapy (RT), and RT plus *Gingko biloba* (RT+GB) groups

| | Control group ($n = 9$) | RT group ($n = 9$) | RT+GB group ($n = 9$) |
|---|---|---|---|
| Grade of cataract | | | |
| 0 | 9 | 1 | 7 |
| 1 | 0 | 7 | 2 |
| 2 | 0 | 1 | 0 |
| 3 | 0 | 0 | 0 |
| 4 | 0 | 0 | 0 |
| Antioxidants | | | |
| MDA | | | |
| Median (mean ± SD) nmol/mg proteins | 17.73 ± 4.41[b] | 25.33 ± 4.75[a,c] | 21.32 ± 4.88[b] |
| SOD | | | |
| Median (mean ± SD) U/mg protein | 35.44 ± 11.82[b] | 23.75 ± 7.87[a,c] | 35.67 ± 13.22[b] |
| GSH-Px | | | |
| Median (Mean ± SD) U/mg protein | 0.16 ± 0.04[b,c] | 0.28 ± 0.12[a] | 0.33 ± 0.15[a] |

Grade 0, normal, clear lenses; grade 1, lenses showing visible posterior sutures; grade 2, lenses displaying isolated vacuoles; grade 3, coalescing vacuoles; grade 4, peripheral coalescing vacuoles and radial streaks extending into central crystalline opacity.

[a] $P < 0.05$ compared with control group; [b] $P < 0.05$ compared with RT group; [c] $P < 0.05$ compared with RT+GB group.

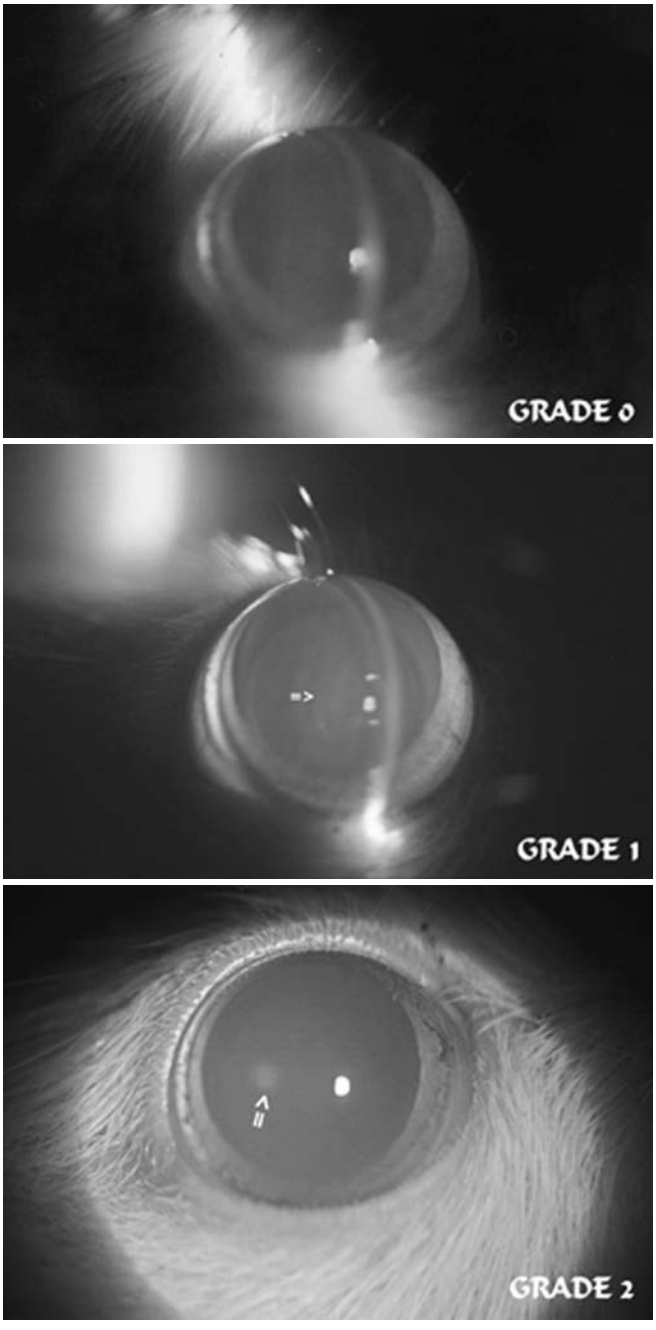


**Figure 1A–C.** Slit-lamp biomicroscopy images of three grades of lenses (*grades 0*, *1*, and *2*). **A** Slit-lamp biomicroscopy image of a normal lens (*grade 0*). The lenses are clear. **B** The grade 1 image illustrates a lens with visible posterior sutures (*arrow*). **C** The grade 2 image illustrates a lens displaying isolated vacuoles (*arrow*).

group. On the other hand, one grade 2 cataract was detected in one rat in the RT group, but none was detected in the RT+GB group (Fig. 1). Compared with rats in the RT group, a significant reduction of cataract formation was observed in rats of the RT+GB group ($P = 0.008$).

In the RT group, the MDA level was significantly higher than in the control group ($P < 0.05$). In the RT+GB group, the MDA level was significantly lower than in the RT group ($P < 0.05$). There was no significant difference between the control group and the RT+GB group. In the control group and RT+GB groups, SOD activity was significantly higher than in the RT group ($P < 0.05$). In the control group, the GSH-Px activity was significantly lower than in the other groups ($P < 0.05$). In the RT+GB group, the GSH-Px activity was higher than in the RT group, but there was no significant difference between the two groups.

## Discussion

Ionizing radiation damages biological tissues by producing free radicals in aqueous solutions such as cell cytoplasm, which in turn can cause oxidative damage to biological molecules such as nucleic acids, proteins, and lipids, leading to cataract development. Ionizing radiation also initiates lipid peroxidation. Although the mechanisms of radiation cataract development have not been clearly demonstrated, the theory of oxidative damage is of interest, because oxygen necessity and use seem to be most important for radiation effects, as well as for other situations in which oxidative injury may occur.[1,2] Consequently, Bardak et al.[1] found that 1 week after exposure, SOD and GSH-Px activities in rat lenses were lower in an ultraviolet-B group than in controls, and the MDA level was higher than in controls ($P < 0.05$). Thus, the MDA level served as an index of cellular damage by free radicals. They suggested that the depletion of important intracellular antioxidant stores by ultraviolet radiation in the lenses of the animals might have been the main cause of lens opacification. It has been reported that irradiation of 5 Gy in a single dose to the whole rat body significantly increases grade 3 cataract formation at 8 weeks after irradiation.[3]

In the present study, we found that the MDA level in the rat lenses of the RT group was significantly higher than in the control group, which is consistent with the hypothesis that ionizing radiation generates oxidative stress. We also found that SOD and GSH-Px activities in the RT group were lower and higher, respectively, than in the control group. These results also indicate oxidative stress and an early protective response to oxidative damage. Irradiation with 5 Gy to the total cranium in a single dose caused cataracts to form in the rat lenses (Table 1).

*Ginkgo biloba* extract (GBE) acts as an antioxidant, which can counteract the deleterious effects of the oxidative damage caused by free radicals and related ROS. It scavenges reactive oxygen and nitrogen radicals. Because GBE enters intact cells, it protects cells from alloxan- or light-induced stress, and the nuclear DNA from single strand breaks. In addition, GBE effectively inhibits chemically induced apoptosis and accelerates corneal wound healing. It protects against retinal ischemia-reperfusion injury and retinal damage and increases ocular blood flow velocity in the ophthalmic artery. The antioxidant, antiapoptotic, and cytoprotective properties of GBE are apparently responsible for its beneficial effects, protecting the lens against selenite-induced lens opacification.[4]

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Moreover, GBE enhanced the radiation effect on a C3H mouse fibrosarcoma, probably by increasing tumor blood flow without increasing acute normal tissue radiation damage. Based on this action, it has been suggested that GBE may be a radiosensitizer.[5] These are only a few of the reasons why we used GBE as a radioprotector in the present study.

In our study, in the RT group, the MDA level was higher than in the RT+GB group. This result was statistically significant. The SOD activity was significantly higher in the RT+GB group than in the RT group, and GSH-Px activity was higher in the RT+GB group than in the RT group, but not statistically significant. These results suggest that GBE supplementation might accelerate the activity of the lens SOD enzyme, enabling the antioxidant system to gradually clear away free radicals by lowering the superoxide dismutase level. We also found that GBE supplementation decreased lipid peroxidation. We succeeded in demonstrating that total cranium irradiation enhances cataract formation, and that GBE supplementation protects the lenses from radiation-induced cataracts.

In conclusion, we suggest that *Ginkgo biloba* extract is an antioxidant that can protect the lenses from radiation-induced cataracts.

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