The protective effects of *Ginkgo biloba* EGb761 extract against renal ischemia-reperfusion injury in rats

H. AKDERE, E. TASTEKIN¹, M. MERICLILER², K.M. BURGAZLI²

Abstract. – OBJECTIVE: The aim of the study was to investigate the protective effects of *Ginkgo biloba* EGb761 extract on renal ischemia-reperfusion (I/R) injury in rats. MATERIALS AND METHODS: 26 male Wistar albino rats were divided into four groups: First group (n=6), which served as control, received only standard pellet; second group (IR) (n=6) was subjected to renal I/R injury; a third group (Gb) (n=7) was given additional EGb761 extract; and rats in the fourth group (IR-Gb) (n=7) had been treated with EGb761 extract before they were subjected to I/R injury. After rats were euthanized, renal tissues were analyzed microscopically, and tissue malondialdehyde (MDA), catalase (CAT), and superoxide dismutase (SOD) levels were determined. RESULTS: MDA values were significantly high in the IR group when compared with the other groups. No significant difference in MDA values between the Control and Gb groups was observed. SOD enzyme activity was significantly lower in the IR group when compared with other groups. Furthermore, SOD values were found to be comparable in control, Gb and IR-Gb groups. The CAT enzymatic activity was significantly low in the IR group when compared with the other groups. Moreover, although no statistical significance was identified between control group and Gb group, CAT levels in these groups were higher compared to IR-Gb group. Microscopic examination showed no histopathological differences between the control and Gb groups. Cast formation and tubular necrosis in the IR group have been determined to be significantly high when compared with IR-Gb group. We further observed that the histopathological changes in the IR-Gb group were lesser in the advanced levels when compared with the IR group. CONCLUSIONS: *Ginkgo biloba* EgB761 extract applied before renal ischemia-reperfusion decreases the tissue damage.

Key Words: Renal ischemia/reperfusion, Injury, Ginkgo biloba, EGb7.

Introduction

Renal ischemia-reperfusion is a significant injury that has to be taken into consideration at surgical interventions such as suprarenal aortic aneurysm concerning the kidney arteries, partial nephrectomies and renal transplantation¹. The effect of ischemia on the organ is directly related with the duration and reduction of artery flow. Renal transplantation surgery is the best surgical procedure in which ischemia reperfusion can be demonstrated. The ischemia initiates with the kidney acquired from the donor, the reperfusion initiates after the transplantation of the kidney to the patient. This process is important and it affects the patient’s chance to live².

Clinical and experimental studies have showed that the incidents of ischemia-reperfusion are regulated by the reactive oxygen species (ROS)³. The ROS amount increases at the site of ischemia-reperfusion and this indicates destruction of renal tissues. The ROS values in the area decrease with the oxidation of the tissue at reperfusion⁴. In reperfused tissues, xanthine oxidase (XO) in the presence of its substrates hypoxanthine or xanthine reduces molecular oxygen to the superoxide radical (O₂⁻) and hydrogen peroxide (H₂O₂), which can further react to form more reactive hydroxyl radicals (OH⁻)⁵. These free radicals can attack a wide variety of cellular components, including DNA, proteins, and membrane lipids. Cellular defense against free radical injury is provided by enzymatic (catalase, superoxide dismutase, and glutathione peroxidase) and non-enzymatic (melatonin, vitamin E, and vitamin C) free radical scavenging systems⁶.

*Ginkgo biloba* (Salisburia adianthifolia) plant is known by the Chinese for 2000 years and has been used for the treatment of many diseases⁷. The seed of the plant is used both in the manu-
facture of foodstuff and medicine. The standardized and real ginkgo extract is EGb 761. This extract is widely used for the treatment of cardiovascular disorders, diabetes mellitus, aging and various types of cancers8. The effectiveness of these medications come from the antioxidant features of ginkgo8. The organic acids (kynurenic, hydroxykynurenic, and vanillic acid) of the EGb 761 extract are responsible from antioxidant, anti-allergic, anti-inflammatory, anti-proliferative, anti-tumorigenic, anti-anxiety and anti-carcinogenic effects8,9. Many studies in the recent years have been focusing on the neuro-protective effect of Ginkgo biloba on the vascular system as well. The literature acknowledges that it performs its protective effects on vascular and neural systems through nitric oxide and beta-amyloid-derived-peptide8.

The existence of antioxidant effects of EGb 761 is available through bioflavonoids. These protect the brain, the retina and the vascular system against free radicals and radical generating substances (such as nitric oxide, superoxide, hydroxyl, oxoferryl and peroxyl species) especially formed with tissue damage8,9. It especially has a protective effect against the free radical reactions occurring as a result of lipid peroxidation which especially causes aging of the body11. Apart from this, it prevents cell damage to arise as a result of ischemia and hypoxia by inhibiting the platelet-activating factor (PAF) that causes cell damage, decreased blood circulation and bronchial constrictions. It especially takes its effect on cardiovascular, respiratory, central nervous system and renal system cells12. In the cell cultures formed, it is known that it is effective on the activities of cancer cells by means of proliferation inhibiting factors13.

When the literature is studied, many reports on the effectiveness of Ginkgo biloba on ischemia-reperfusion have been observed to exist. These were mainly on liver, brain, peripheral nerves, testicles, intestines, and lungs. No publications were encountered with regard to the effectiveness of ginkgo extracts on renal ischemia-reperfusion. In the present study we, thus, investigated the protective effects of Ginkgo biloba on the renal ischemia-reperfusion injury in the rats.

**Materials and Methods**

It was planned to form 4 groups from 32 young adult male Wistar albino rats with same biological and physiological features which were bred at the Laboratory Animals Research Department of Trakya University and kept under the standard lab conditions (22±1°C, 12 hours daylight/dark cycle) and which were grouped amongst themselves in our study on the basis of similarity to each other. During the study 2 rats in group I, 2 in group II, 1 in group III and 1 in group IV were died and excluded from the study. Experimental groups were designed as follows:

- **Group I (Control):** The control group fed with standard rat-feed (n=6).
- **Group II (IR):** The group fed with standard rat-feed which the renal ischemia-reperfusion will be applied to (n=6).
- **Group III (Gb):** The group fed with standard rat-feed which Ginkgo biloba (EGb 761) (50 mg/kg) will be applied (n=7).
- **Group IV (IR+Gb):** The group fed with standard rat-feed to which Ginkgo biloba (EGb 761) (50 mg/kg) will be given orally prior to renal ischemia-reperfusion application (n=7).

The 1st Group was planned to be the control group of our study. The renal ischemia was effectuated to the 2nd Group of subjects for 60 minutes and then 60 minutes of reperfusion was applied and the acquisition of the kidney tissues was planned. It was planned to apply Ginkgo biloba (EGb 761) (50 mg/kg) to the 3rd Group subjects for 14 days and 2 hours before the acquisition of kidney tissues. After applying Ginkgo biloba (EGb 761) (50 mg/kg) to the 4th Group subjects for 14 days and 2 hours before forming renal ischemia and it was planned to apply 60 minutes of ischemia and 60 minutes of reperfusion afterwards. At the end of 60 minutes reperfusion, kidney biopsy materials were obtained under the anesthesia of 90 mg/kg ketamine + 10 mg/kg xylazine and these materials to be subjected to standard procedures for light microscopic examination.

For light microscopic examinations; the kidney biopsy materials were fixated in the Bouin solution at the Light Microscopy Laboratory of the Trakya University Department of Pathology and a block was retained by the application of paraffin inclusion and apart from applying hematoxylin and eosin (H&E) stain to the 5 micron paraffin cuts which would reveal the characteristics of the histological structure of the kidney, we planned to research the renal damage following the renal ischemia-reperfusion at the rats with
biochemical parameters (MDA = Malonyl dialdehyde, CAT = Catalase, and SOD = Superoxide dismutase). The tissue samples to be studied were stored at −70 °C.

**Enzyme Assay**

The renal tissue samples were homogenized with 150 mM ice-cold KCl for the determination of MDA and glutathione levels. Homogenates were centrifuged at 2600 g for 10 min at 4°C. The MDA concentrations in the renal tissue contents, an indicator of lipid peroxidation, were assayed in the form of thiobarbituric acid reacting substances. MDA was quantified using an extinction coefficient of 1.56 x 10⁵ M⁻¹ cm⁻¹ and expressed as nanomoles of MDA per milligram tissue⁻¹. All enzyme activities were determined after the renal tissue homogenization with phosphate buffered saline (PBS) at the 7.4 of pH. The total (Cu-Zn and Mn) SOD activity was determined according to the method of Sun et al.¹⁴ It is based on the inhibition of nitroblue tetrazolium (NBT) reduction by the xanthine-xanthine oxidase system as a superoxide generator. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. The specific activity is expressed in units per milligram protein. The CAT activity was measured as previously described.¹⁵ It is based on the determination of the rate constant (k; /s) of hydrogen peroxide decomposition rate at 240 nm. The results were expressed as the rate constant per milligram homogenate protein.

**Surgical Procedure**

All surgical procedures were carried out under xylazine/ketamine (10/90 mg/kg, i.p.) anesthesia. The rat was incised at mid-line and the intestines were eliminated and the left kidney was accessed. The pedicle was released with the elimination of the fatty tissue on it. In the ischemia group, 5 mm bulldog clasper pedicle was placed and with a 60 minutes ischemia followed by a 60 minutes reperfusion, the kidney tissues were removed out.

**Renal Pathology**

The left kidney was longitudinally cut from the middle into two and fixated in 10% formaldehyde. The tissues were embedded in paraffin wax and 4 µm cuts were taken and stained with H&E. The pathologist recorded the pathological (tubular necrosis and cast formation) differences in the tissues as percentages without knowing the groups he was examining. The degree of tubulointerstitial damage in the cortex was determined using a semi-quantitative graded scale, where 0 = no abnormality, 1 = minimal damage (involvement of 25% of cortex), 2 = mild damage (involvement of 25-50% of cortex), 3 = moderate damage (involvement of 50-75% of cortex), and 4 = severe damage (involvement of 75% of cortex).¹⁶ These analyses were performed in two sections from each animal at 4000 magnification in at least ten different regions for each section.

**Statistical Analysis**

The results are expressed as mean ± standard deviation. The Kruskal-Wallis test was used to compare the four groups. In two group comparisons, Mann-Whitney U test was performed; p values below 0.05 were considered as statistically significant.

**Results**

MDA, CAT and SOD enzyme values are shown in Table I. MDA values were found to be statistically higher in the IR group when compared with the other groups. There were no significant differences in MDA values between the Control and Gb groups. Additionally, levels of MDA were higher in IR-Gb group than in both control and Gb groups (p < 0.05).

---

**Table I.** Enzyme results of experimental groups.

<table>
<thead>
<tr>
<th></th>
<th>Control Group (n=6)</th>
<th>Gb Group (n=7)</th>
<th>IR Group (n=6)</th>
<th>IR-Gb Group (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA</td>
<td>0.473 ± 0.013</td>
<td>0.468 ± 0.014</td>
<td>0.739 ± 0.050</td>
<td>0.521 ± 0.047</td>
</tr>
<tr>
<td>SOD</td>
<td>8.266 ± 0.430</td>
<td>8.301 ± 0.351</td>
<td>5.877 ± 0.465</td>
<td>7.896 ± 0.717</td>
</tr>
<tr>
<td>CAT</td>
<td>0.356 ± 0.020</td>
<td>0.360 ± 0.017</td>
<td>0.208 ± 0.058</td>
<td>0.304 ± 0.031</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation.
SOD enzyme activity was significantly lower in the IR group when compared with other groups ($p < 0.05$). Yet SOD values were comparable in control, Gb and IR-Gb groups ($p > 0.05$).

The CAT enzymatic activity was significantly low in the IR group when compared other groups ($p < 0.05$). Besides, although no statistical significance was identified between control group and Gb group ($p > 0.05$), CAT levels in these groups were higher compared to IR-Gb group.

No histopathological differences have been observed in the control and Gb groups (Figure 1). Cast formation and necrotic pathologies in the IR group have been determined to be significantly higher when compared with IR-Gb group ($p < 0.05$) (Table II). We further observed that the histopathological changes in the IR-Gb group were lesser in the advanced levels when compared with the IR group (Figures 2 and 3).

### Discussion

Renal ischemia-reperfusion injury is an important problem in renal vein operations, partial nephrectomy and renal transplantations. The studies show that *ginkgo* compounds show antioxidant effect on the body and that they have protective effects against oxidative injuries formed as a result of ischemia-reperfusion at various organs and tissues [8-11]. No studies on *Ginkgo biloba* (Gb) with regard to renal ischemia-reperfusion have been encountered in the literature. The Gb dose of (50 mg/kg) given to the rats in our study have been calculated as the highest dose that can be given proportionate with the human dose unlike all the other studies [17]. This is because the duration of the ischemia is longer than the ischemia durations given in the literature and complies with renal transplantation [17, 18]. Apart from this, 2 weeks of *Ginkgo* application is longer than that of periods in the literature. The average period in the literature is 1 week or less [17-19]. Besides, single sided ischemia-reperfusion and nephrectomy have been applied to avoid systemic tissue injury. In addition, giving Gb 2 hours before the ischemia in our study, makes us different from the other IR models [17-19].

In the renal ischemia free radicals generated as a result of lipid peroxidation on the kidney cell membrane causes tissue damage. The best indicative of this is the tissue MDA values [9, 10]. The tissue damage developed after the 60 minutes ischemia-reperfusion in our study has been observed in the IR group the most. The MDA values which indicate the tissue damage in this group was substantially significant when compared with the other groups ($p < 0.05$). In the studies of Aktozet et al [18] and Kadkhodae et al [19], 10 minutes of reperfusion has been performed after the 30 minutes of ischemia in the IR groups.

### Table II. Histopathological findings.

<table>
<thead>
<tr>
<th></th>
<th>IR Group (n=8)</th>
<th>IR-Gb Group (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Necrosis (%)</td>
<td>11.38 ± 2.06</td>
<td>4.04 ± 1.06</td>
</tr>
<tr>
<td>Cast formation (%)</td>
<td>12.77 ± 2.79</td>
<td>5.86 ± 1.61</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation.
In this study of ours, while the MDA values decreased in the IR group, CAT and SOD values decreased significantly. These values are indicative of ischemia. All tissue enzymes have recovered in the IR-Gb group in our study, unlike the other studies, whereas no recovery due to the antioxidant deployed was observed in the SOD values in literature. The same results apply for the CAT values in various studies. Antioxidant activity irregularity and ROS production arising during the reperfusion causes the renal damage. The antioxidant repair this damage. SOD accomplishes this by removing superoxide by means of enzymatic dismutase. The same effect can be seen on CAT. In our report, the presence of Gb effect more in the two enzymes have reduced the organ damage. Whereas the Control and Gb group’s SOD and CAT values have decreased the most in the IR group where the tissue damage was the highest, the drop in the IG-Gb group has decreased significantly. We think that this is due to the positive effect of Gb on this.

The literature data shows that the best criteria indicating renal ischemia pathology are tubular necrosis and cast formation rate. In the histopathological examination of our study, similarly, we have used the tubular necrosis and cast formation rates. The extremely high histopathological percentages have been observed in the IR group and lowest in the control and Gb groups, and furthermore significantly low values were demonstrated in the IR-Gb group when compared with the IR group. In addition, histopathological recovery at a significant rate was observed in the IR-Gb group when compared with the control and Gb group. These results are similar with the other antioxidants effects on renal ischemia-reperfusion studies. As there was no study on the effect of Gb on renal IR, we couldn’t carry out a comprehensive comparison.

Conclusions

These results are parallel with the enzyme results and we have shown that the Gb (Egb 761 extract) applied before renal ischemia-reperfusion decreases the tissue damage developing due to ischemia and that it has a protective effect. This gives the impression that the regular application of Gb Egb 761 extract to kidney donors for 2 weeks prior to kidney transplantation shall reduce the rejection ratio of the transplanted kidney. For the inclusion of Gb in the medical treat-
Ginkgo biloba and renal ischemia-reperfusion injury

It is needed that further detailed experiments and clinical examination should be carried out in the long run.

Conflict of Interest
The Authors declare that they have no conflict of interests.

References