

GINKGO BILOBA EXTRACT MAINTAINS RENAL PERFUSION IN PARTIAL UNILATERAL URETERAL OBSTRUCTIONS

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Abstract

The aim of this experimental study was to examine the effect on Doppler parameters of Ginkgo biloba glycosides in partial unilateral ureteral obstruction in rabbits. Twelve rabbits were divided into two groups. Baseline renal Doppler ultrasonography was performed. Group 1 received Ginkgo biloba (40 mg/d) for 1 month while group 2, being a sham group, received saline for the same period, prior to partial unilateral ureteral obstruction induction. The left ureter was narrowed by placing a ligature over the catheter. Renal and Doppler ultrasonography was performed to determine kidney structure, resistive (RI) and pulsatility (PI) indices as well as colourisation scores pre-operatively and post-operatively. The sham group had greater RI and PI values and a lower colourisation score than the Ginkgo biloba group. The obstructed kidneys had greater RI and PI values and a lower colourisation score than the non-obstructed kidneys in both groups. The RI value increased and colourisation score decreased to a greater extent for the obstructed kidneys than the non-obstructed kidneys in group 1. The aim of this experimental study was to examine the effect on Doppler parameters of Ginkgo biloba glycosides in partial unilateral ureteral obstruction in rabbits. Twelve rabbits were divided into two groups. Baseline renal Doppler ultrasonography was performed. Group 1 received Ginkgo biloba (40 mg/d) for 1 month while group 2, being a sham group, received saline for the same period, prior to partial unilateral ureteral obstruction induction. The left ureter was narrowed by placing a ligature over the catheter. Renal and Doppler ultrasonography was performed to determine kidney structure, resistive (RI) and pulsatility (PI) indices as well as colourisation scores pre-operatively and post-operatively. The sham group had greater RI and PI values and a lower colourisation score than the Ginkgo biloba group. The obstructed kidneys had greater RI and PI values and a lower colourisation score than the non-obstructed kidneys in both groups. The RI value increased and colourisation score decreased to a greater extent for the obstructed kidneys than the non-obstructed kidneys in both groups. The RI value increased and colourisation score decreased to a greater extent for the obstructed kidneys in both groups.

Key words: rabbits, Ginkgo biloba, ureteral obstruction, Doppler ultrasonography.

Urinary tract obstruction may be partial or complete, unilateral or bilateral, and has been a subject of numerous experimental and clinical studies aimed at identifying underlying factors and determining functional and structural changes in the kidneys (21). Partial unilateral ureteral obstruction (PUUO) causes a reversible or irreversible destruction in kidneys and ureters. Renal blood flow impairment, elevation of intrapelvic pressure, and vasoactive and inflammatory mediators are the important aetiological factors for renal obstructive parenchymal injury (7). These factors may eventually lead to hypoxia and tissue ischaemia (2).

Percutaneous nephrostomy, ureteral stents, endoscopic, laparoscopic, and open surgery represent different treatment modalities in ureteral obstruction. The main aim of treatment is to prevent or reduce renal parenchymal injury due to the obstruction.

The pulsatility index (PI) and resistive index (RI) are calculated from blood flow velocities in vessels during the cardiac cycle and reflect renal haemodynamics (27). PI and RI are also used as duplex Doppler ultrasound (US) measurements of downstream resistance in arteries (12). These indices are valuable in the evaluation of obstructive uropathy (16, 25) and are correlated with the severity of the renal disease (27). The power Doppler US technique allows determining changes in blood flow velocity and renal perfusion status (22). The output is a colour map that displays the intensity of the Doppler signal related to the number of red blood cells, which produce Doppler shifts (30).

The extract of the green leaves of ginkgo trees (Ginkgo biloba) has antioxidant effects and has been used for therapeutic purposes in East Asia for many years (26). The standardised extract, EGb 761, contains flavonoids and terpenoids (17), and is used for treating Alzheimer’s disease and neuronal hypoxia (6). EGb 761 protects the heart from ischaemia (37) and prevents vascular endothelial damage (8) by antagonising platelet aggregation (1) and stimulating blood flow (18).

The objective of this experiment was to investigate the effect of ginkgo glycosides on renal functions as assessed by colourisation score, RI, and PI using power and duplex Doppler in PUUO-induced rabbits with ureteral ligation.
**Material and Methods**

**Animals, management, and grouping.** Twelve New Zealand rabbits, weighing an average of 2,800 g (2,500-3,500 g) were obtained from the Ankara University Experimental Animal Research Unit. The rabbits were housed under standard conditions in individual cages in a temperature and light controlled room and allowed consumption *ad libitum* of pellet feed with free access to water (5). The rabbits were randomly assigned into one of two experimental groups. The rabbits in group 1 received *Ginkgo biloba* extract (EGb 761; Tebokan Tablet 40 mg, Abdi İbrahim, Turkey) for 1 month prior to PUUO induction. Group 2 was defined as the sham group and received no drug. EGb 761 tablets were diluted in 10 ml of distilled water to form a homogeneous solution and then administered at a dose of 10 mg/d through drinking water. The animals’ drinking water was checked but not replaced during the day to ensure that the animals received the daily dose of the drug. The drug was administered for 30 d pre-operatively and continued for 7 d post-operatively. All the experiments were accepted by the local Ethical Committee, in compliance with the Principles of Laboratory Animal Care (NIH publication No.85-23, revised 1985).

**Surgical procedure.** Unilateral partial ureteral obstruction was induced according to the method described by Ayyıldız *et al* (2). After a month of administration of *Ginkgo biloba* extract or saline, all rabbits in both groups underwent laparotomy under general anaesthesia (xylose HCl, 20 mg/kg i.m.; 2% Rompun®, Bayer, Turkey and ketamine HCl, 50 mg/kg i.m.; 10% Ketamidor®, Pfizer, Turkey). During laparotomy, the left ureter was exposed and isolated in both groups. PUUO was then performed by inserting a 24-gauge (19 x 0.7 mm) intravenous catheter into the proximal ureter and ligating the ureter and catheter around the ureter using a 3-0 silk suture (Fig. 1). The left ureters were subsequently narrowed by placing a ligature over the catheter, which was then carefully removed in order not to injure the ureter. This was followed by closure of the abdominal incision. Right ureters were left intact for comparison.

Postoperatively, excretory urography was performed following the injection of 1.5 ml/kg of iopromide (Ultravist 370; Schering, Germany) to confirm the establishment of PUUO (Fig. 2).

**Imaging.** Ultrasonography (US) for renal morphology, power Doppler US for colourisation, and duplex Doppler US for RI and PI values were performed pre-operatively in both kidneys at the beginning of the study and after starting 1-month delivery of *Ginkgo biloba* extract and saline. The same procedures were also performed post-operatively at the 4th h and days: 1, 3, and 7. An Esaote US device, model AU5 (ESAO TE BIOMEDICA, Italy) with a 7.5-10.0 MHz multifrequency linear probe was used for ultrasonographic examinations. The abdominal region was shaved and ultrasound gel applied to the prepared area for clearer images. No sedatives or anaesthetics were used since these might have interfered with imaging parameters.

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![Fig. 1. Left ureter after partial ureteral obstruction.](image-url)
Fig. 2. Excretory urography of the obstructed kidney exhibiting dilatation in the proximal ureter. The asterisk indicates the location of the obstruction established in the proximal ureter. The white arrow indicates confirmation of the partial ureteral obstruction.

Renal shape, size, and width, echogenicity of the parenchyma and renal pelvis diameters were the renal morphology measurements employed. The colourisation score used to evaluate parenchymal perfusion was categorised from 1 to 4 as follows: 1 - poor perfusion (reduced colourisation in interlobar vessels and no cortical colourisation), 2 - mild perfusion (reduced cortical and interlobar vessel colourisation), 3 - good perfusion (presence of irregular colourisation) and 4 - high perfusion (homogeneous coloration of the cortical parenchymal perfusion and the medullary region) (13). Doppler signals were generally obtained from the interlobar arteries along the border of the medullary pyramids and from the arcuate arterioles along the corticomедullary border by duplex-wave. Care was taken to ensure that spectral spikes were similar between the 3 and 5 wave forms and these were determined with the same set-up at 60°. Peak systolic and end-diastolic artery velocities were recorded and the RI (peak systolic velocity - end diastolic velocity) / peak systolic velocity and PI (maximum systolic velocity - minimum diastolic velocity) / average velocity values calculated.

Statistical analysis. Renal Doppler parameters (RI, PI, and colourisation score) were subjected to 2-way ANOVA using the SAS PROC GLM Procedure (32) as repeated measures, time being subplot. The linear model was used to test the effect of treatment and PUUO: $Y_{ijk}=\mu+G_i+K_j+(G*K)_ij+t_k+(G*t)_ik+(K*t)_{ik}+(G*K*t)_{ijk}+\epsilon_{ijk}$, where $y$=response variable, $\mu$=population mean, $G_i$=i-th group (control or treated), $K_j$=j-th kidney (obstructed or non-obstructed kidney), $t_k$=k-th (time relative to induction), $e$=residual error. Data were analysed separately for the pre-operative and post-operative periods. Statistical significance was set at $P<0.05$.

Results

B-mode measurements. Pre-operative US examination for the left and right kidneys revealed a renal longitudinal length of 32.09 ±2.14 and 32.83 ±2.51 mm, vertical width of 18.58 ±2.19 and 19.28 ±2.59 mm, and cortical thickness of 3.08 ±0.25 and 3.09 ±0.21 mm, respectively. The cortex and medulla were normal in appearance. No rabbits had any renal or pararenal mass, pelvis renalis dilatation or pathologies (i.e., fluid) in the pararenal and/or abdominal cavity.

As expected, there was no post-operative renal pelvis dilatation in the right (non-obstructed) kidney. However, the renal pelvis of the left (obstructed) kidney was dilated on day 7 post-operatively (11.43 ±2.52 mm).

The obstructed kidneys were elongated and enlarged, whereas no morphological changes occurred in the non-obstructed kidneys. Cortical thickness and echogenicity remained unchanged in both kidneys. Mean renal longitudinal length was 41.64 ±3.11 and 33.75 ±2.77 mm, vertical width was 26.85 ±2.20 and 20.74 ±2.66 mm, and cortical thickness was 2.97 ±0.16 and 3.26 ±0.25 mm for the obstructed and non-obstructed kidneys, respectively.

Doppler US measurements. The control group had greater RI (0.56 vs. 0.50; $P<0.0001$) and PI (1.47 vs 1.42; $P<0.001$) values and lower colourisation (2.89 vs 3.35; $P<0.0001$) than the treatment group (Table 1). The obstructed-control kidney had greater RI (0.55 vs. 0.50; $P<0.0001$) and PI values (1.50 vs 1.40; $P<0.0001$) and a lower colourisation score (2.94 vs 3.29, $P<0.001$) than the non-obstructed-control kidney. There was an interaction on PI values in terms of kidneys between the groups ($P<0.0001$). Although there was a significant difference in PI values between the control and treated groups (0.19), this difference declined in the treated group (0.01) (Table 1; $P<0.0001$). Ginkgo biloba did not alter the magnitude of changes in RI and colourisation score in obstructed or non-obstructed kidneys.

There were no pre-operative differences in RI (Fig. 3A) and PI (Fig. 3B) values between groups and kidneys. Post-operatively, RI value increased over time (Fig. 3A; $P<0.0001$), at a greater extent in the obstructed-control kidney than in the obstructed-treatment kidney (Fig. 3A; $P<0.001$). Moreover, the RI value for the obstructed-control kidney increased at a greater extent than that for the non-obstructed-control kidney (Fig. 3A; $P<0.005$).

The PI response in the groups and kidneys was similar to the RI response. Mean pre-operative PI values did not differ between groups and kidneys. Post-surgically, PI values increased over time (Fig. 3B; $P<0.05$) regardless of the experimental group. The PI values for obstructed treated kidneys decreased, whereas those in untreated kidneys remained high post-operatively (Fig. 3B; $P<0.001$).
Table 1
Effect of Ginkgo glycoside on renal Doppler parameters in unilateral partial obstruction

<table>
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<th>Group</th>
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<th>Variable</th>
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<td>Non-obstructed</td>
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<td>Group x kidney</td>
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RI = resistive index; PI = pulsative index.

1Time effect, P<0.0001. 2Time effect, P<0.05. 3Group x time interaction, P<0.001. 4Group x time interaction, P<0.05.
5Group x time interaction, P<0.57. 6Kidney x time interaction, P<0.005. 7Kidney x time interaction, P<0.06. 8Kidney x
time interaction, P<0.001.

Fig. 3. A- Effect of treatment on resistive index (RI) values of the kidneys (SEM=0.02). B- Effect of treatment on pulsative index (PI) values of the kidneys (SEM=0.04). C- Effect of treatment on colourisation scores of the kidneys (SEM=0.24).
Fig. 4. A-B. Pre-operative colourisation in the left and right kidneys. Cortical tissue and interlobar vessels are well-coloured. C. On day 7 post-operatively, colourisation of the obstructed kidney in the treatment group decreased compared to the pre-operative situation. There was no change in interlobar vessel colourisation. D. On day 7 post-operatively, cortical colourisation of the obstructed kidney in the control group was negligible and colourisation of the interlobar vessels decreased.

Pre-operative colourisation score did not differ between groups or kidneys. There was an overall decrease in colourisation during the post-operative period (Fig. 3C; P<0.0001), greater for the control group than for the treatment group (Fig. 3C; P<0.05). Moreover, in the obstructed treated kidney, colourisation tended to be similar to that in the non-obstructed treated kidney compared to the control group (P<0.06; Fig. 3C and Fig. 4A-D).

Discussion

Urinary tract obstruction leads to renal parenchymal damage and can also result in permanent renal dysfunction unless the obstruction is treated (36). Progressive chronic renal disease is accompanied by a non-specific renal scarring process, such as interstitial fibrosis, loss of capillaries and glomeruli, and results in a reduction in the number and area of renal vessels (31). Similar pathologies (i.e., decreased renal function and increased fibrosis, tubular apoptosis, and cellular proliferation) as well as decreased renal blood flow (RBF) and glomerular filtration rate are also observed in unilateral ureteral obstruction (3). A decrease in RBF is more notable in the 3rd h following obstruction due to release of angiotensin II, thromboxane A2, and antidiuretic hormone caused by preglomerular vasoconstriction (29) and increased intrarenal vascular resistance (28). This is followed by increased pressure in the renal pelvis, which declines in later stages of obstruction (19). Increased intravascular resistance reflects the degree of intrarenal damage, which can be determined using Doppler US (28). The RI and PI values obtained from duplex Doppler are used for evaluating perivascular diseases (11), renovascular function (23), and in the diagnosis of ureteral obstruction (15, 35). For instance, a reduction in diastolic flow relative to systolic flow in hydronephrotic kidneys is accompanied by elevated intrarenal RI (24).

An earlier increase in renal RI in ureteral obstruction is related to a decreased RBF and increased intrarenal vascular resistance (33). PI is actually correlated with renal vascular resistance increased by angiotensin II infusion (4). In the present experiment, RI and PI values of obstructed kidneys increased significantly relatively to their baseline values. However, this elevation was suppressed by EGb 761 (Table 1, Figs 3A and 3B). This extract has an anti-aggregation effect by antagonising platelet activating factor (PAF). Moreover, it reduces thrombocyte aggregation and suppresses neutrophil degranulation and superoxide production by inhibiting PAF (10). EGb 761 exerts vasorelaxing and anti-aggregation effects, through scavenging superoxide anions that increase the half-life of endothelium-derived relaxing factor (17). The vasoregulatory effects may also be related to increasing blood viscosity and flow (14). In patients with intermittent claudication, Doppler parameters for local arterial and capillary perfusion were improved by EGb 761 (20). In another study, similar improvements were reported in ophthalmic arteries by elevation in end-diastolic blood flow assessed by Doppler US in response to this extract (9).

Power Doppler imaging can identify slowed blood flow. As power Doppler imaging displays the total integrated Doppler power, the subtle change in renal perfusion can therefore be detected by using this imaging modality (34). The advantages of power Doppler US over colour Doppler US include relative
angle independence, absence of aliasing and increased sensitivity to slow blood flow (22). In the presented experiment, perfusion status was evaluated by monitoring colourisation in the kidneys using power Doppler. Colourisation of the obstructed kidney decreased more dramatically in the control group in comparison with the obstructed kidney in the treatment group during the post-operative period (Table 1; Fig. 3C). At the same time, RI and PI responses stood in contrast to the colourisation (Figs 3A, 3B). Decreased RI value and increased colourisation in the treatment group compared to the control group may be related to the vasorelaxing and anti-aggregation effects of Ginkgo biloba extract.

In conclusion, ginkgo glycosides may protect and restore renal perfusion in partial unilateral ureteral obstructions, as reflected by a decrease in RI and increase in colourisation score obtained using power Doppler US. They may also be recommended in order to minimise renal parenchymal damage and maintain kidney function.

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References


