Renoprotective effect of *Linum usitatissimum* seeds through haemodynamic changes and conservation of antioxidant enzymes in renal ischaemia-reperfusion injury in rats

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Abstract  Objective: To evaluate an ethanolic extract of seeds of *Linum usitatissimum* (Linn.) (EELU) for its renoprotective role in rats through its antihypertensive effect and conservation of biological oxidation enzymes.

Materials and methods: Male Wistar rats (200–250 g) underwent uninephrectomy on day 0; after 2 weeks of recovery, the nephrectomised rats were divided into four groups of eight each: (I) sham (II); renal ischaemia reperfusion (RIR); (III) RIR + EELU 200 mg/kg; and (IV) RIR + EELU 400 mg/kg. In group II, III and IV the renal artery was occluded for 45 min and reperfused for 4 weeks; the sham group did not undergo RIR.

Results: EELU (400 mg/kg) significantly decreased the haemodynamic changes after 4 weeks of RIR injury. EELU treatment significantly restored the levels of renal endogenous antioxidant enzymes and membrane-bound enzymes. EELU 400 mg/kg restored the levels of blood urea nitrogen and serum creatinine. EELU also decreased the levels of tumour necrosis factor-α and myeloperoxidase activity. A flow-cytometric study confirmed a significant decrease in cellular necrosis and increase in viability after RIR in EELU-treated rats. The anti-apoptotic role of EELU was evident.
Introduction

Hypertension in patients with renal failure is of major importance because it increases the susceptibility to renal and cardiovascular diseases. Renal ischaemia-reperfusion (RIR) is an important cause of renal dysfunction, and renal injury may occur after surgical procedures involving transient renal aortic occlusion [1]. Renal hypoxia and ischaemia can lead to renal hypertension-induced dysfunction in animal models, through activation of the renin-angiotensin aldosterone system (RAAS). Reactive oxygen species (ROS)-mediated cellular activation of the renin-angiotensin aldosterone system accompanies renal injury, which is generally associated with increased microvascular permeability, interstitial oedema, impaired vasoregulation, inflammatory cell infiltration, parenchymal cell dysfunction, necrosis and loss of cell volume [4].

Flaxseed (Linum usitatissimum Linn.) belongs to the family Linaceae, and is commonly known as linseed. Secoisolariciresinol diglucoside (SDG) is the main lignan isolated from flaxseed. The SDG content of flaxseed is about 16.4 mg/g in de-fatted flaxseed meal [5]. SDG has been isolated in pure form [6], and is reported to be a phytoestrogen. Phytoestrogens from dietary soy have been reported to have anti-hypertensive potential [7,8]. Prasad [5] reported that SDG is a long-acting hypotensive agent, with its activity due to stimulation of guanylate cyclase activity. The objectives of the present study were to evaluate the effect of an ethanolic extract of L. usitatissimum (EELU) on haemodynamic and renal function in RIR injury in rats.

Materials and methods

Authenticated seeds of L. usitatissimum (variety NL-97) were obtained from Dr. P.B. Ghorpade (Principal Scientist and Linseed breeder, Punjabrao Deshmukh Krushi Vidyapeeth, College of Agriculture, Nagpur, India, Maharashtra State, India) and a vouched specimen was deposited at the institute. Adrenaline hydrochloride, superoxide dismutase (SOD) and malondialdehyde (MDA) were obtained from Sigma Chemical Co. (St Louis, MO, USA). Reduced glutathione (GSH), 5,5'-dithiobis(2-nitrobenzoic acid) and thiobarbituric acid were obtained from Hi Media, India. All chemicals used were of analytical grade.

The seeds of L. usitatissimum were processed for extraction of lignan-concentrated EELU by the method of Eliasson et al. [9] and extraction of oil at our omega-3 oil unit (Sangammer, Maharashtra, India; set up under National Agriculture Innovation Project funded by Indian Council of Agricultural Research, New Delhi, India). The double cold-pressed flaxseed cake/meal obtained from this oil unit was de-fatted by n-hexane in a soxhlet apparatus to remove residual oil. The de-fatted cake was then hydrolysed with 1 m aqueous sodium hydroxide for 1 h at room temperature with intermittent shaking, followed by extraction with 50% ethanol. The filtrate was acidified to pH 3 using 1 m hydrochloric acid. The filtrate was dried on a tray dryer at room temperature: 12-h dark cycle. The rats had free access to standard food pellets (Chakan Oil Mills, Pune, India) and water was freely available. The experimental protocol was approved by the Institutional Animal Ethics Committee constituted in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision on Experimental Animals, India.

The rats were anaesthetised with thiopental sodium (35 mg/kg, intraperitoneal) on day 0. The abdominal region was shaved and the right kidney removed through a small flank incision. After 2 weeks of recovery the nephrectomised rats were divided into four groups of eight each. In the sham group (group I) the left renal artery was exposed but not subjected to...
RIR. In the RIR groups (groups II–IV) a small incision was
made on the left side of peritoneal cavity of the nephrecto-
mised rat to expose the left kidney. The left renal artery was
occluded for 45 min by using a bulldog clamp; the clamp
was removed and the kidney reperfused for 4 weeks. In groups
I and II, rats received saline treatment for 4 weeks. Group III
rats received EELU 200 mg and in group IV, EELU 400 mg,
aministered once daily by oral gavage for 4 weeks after RIR.

To assess haemodynamic changes, the rats were anaesthe-
tised with urethane (1.25 g/kg, intraperitoneal) and the haemo-
dynamic changes measured using a polyethylene cannula (PE
50) filled with heparinised saline (100 IU/mL) inserted into
the right carotid artery. The cannula was connected to a trans-
ducer and the signal amplified using a bioamplifier. The left
ventricular systolic blood pressure (SBP) was measured using
a microtip transducer catheter (Model SRP-320, Millar Instu-
ment, Inc, Houston, TX, USA) inserted into the left ventricle
via the right carotid artery and connected to a bioamplifier.
The heart rate, maximum first derivative of ventricular pres-
sure (dp/dt max), minimum first derivative of ventricular pres-
sure (dp/dt min) and left ventricular end-diastolic pressure
(EDP) were obtained from primary signals (left ventricular
SBP and blood pressure) using a data-acquisition system
(AD Instruments Pty. Ltd., with LABCHART 6 software,
Bella Vista, NSW, Australia).

Blood was collected from the rats (six) by retro-orbital
puncture after 4 weeks of reperfusion and centrifuged at
10,000g for 15 min at 4 °C. Serum was isolated and used to esti-
mate blood urea nitrogen (BUN) using Jaffe’s method, and cre-
atinine (DAM method) with a standard diagnostics kits (Span
Diagnostics, Gujarat, India). On the last day, rats were housed
in metabolic cages for 24 h and urine flow was measured.

After haemodynamic measurement the left kidney of each
rat was isolated, weighed and two samples were randomly se-
lected for histopathology. The left kidney of the remaining six
rats was cut into four portions; one was individually homoge-
nised and centrifuged. The clear supernatant was collected
after centrifugation and used for the assays of endogenous
antioxidant enzymes. SOD was determined by the method of
Misra and Fridovich [11].

GSH, glutathione S transferase (GST) and glutathione per-
oxidase (GPx) were determined by the method of Moron et al.
[12], Habig et al. [13] and Rotruck et al. [14], respectively. Lipid
peroxidation (MDA formation) was estimated by the method of
Slater and Sawyer [15]. The sediment was resuspended in ice cold
Tris buffer (10 mm, K+, pH 7.4) and was used to estimate mem-
brane-bound enzymes. Na+K+-ATPase and Mg2+-ATPase
were assayed by the methods of Boniting [16] and Ohnishi et al. [17],
respectively. The inorganic phosphorus was estimated by the method of Fiske and Subbarow [18]. Total pro-
tiens were determined by the method of Lowry et al. [19].

Myeloperoxidase (MPO) activity was measured in renal tis-
ue (six rats) by a procedure similar to that previously docu-
mented by Krawisz et al. [20]. The renal homogenate levels of
TNF-α were determined in renal tissue (six rats) with an
ELISA (Thermoscientific, rat TNF-α kit, Cat. number-
ER3TNFA. Pierce Biotech Int., Rockford, IL, USA) accord-
ing to the manufacturer’s instructions.

To assess DNA fragmentation, genomic DNA was
extracted from renal tissue (two rats) using the phenol–chlooro-
form C-TAB method; 10 μg of DNA were loaded onto 1.5% agarose gel and DNA electrophoresis carried out at 80 V for
1–2 h. The gel was stained with ethidium bromide 0.5 mg/mL
and examined under an ultraviolet transilluminator for the
presence of the amplified DNA. The image of the gel was cap-
tured using a gel documentation instrument.

For flow-cytometric analysis of cellular necrosis and viabil-
ity from kidney, a single-cell suspension from renal tissue (six
rats) of each rat was prepared by the trypsinization method.
Briefly, renal tissue was extensively perfused in situ in PBS
to remove blood, and irrigated in a buffer containing Hapes
(10 mm), KCl (3 mm), NaCl (130 mm), NaH2PO4–H2O
(1 mm), and glucose (10 mm, pH 7.4). The renal tissue was
homogenised and suspended in Hanks balanced salt solution.
Renal tissue homogenate was then treated with trypsin for
10 min and trypsin inhibitor for 5 min. The cell suspension
was obtained by passing the trypsinised renal tissue through
a nylon mesh, washed with cold PBS, and then re-suspending
in 1x binding buffer at a concentration of 1–2 × 108 cells/mL.
An aliquot of 100 μL of cell suspension was transferred to a
Falcon tube and 10 μL of propidium iodide (PI) probe was
added (Sigma). The cell suspension was mixed thoroughly
and incubated for 15 min in the dark at room temperature
(25 °C). Data were acquired and analysed in the processed
samples by flow cytometry using CELLQuest software (Becton
& Dickinson, San Diego, CA, USA) [21].

For the histological evaluation by haematoxylin and eosin
(H&E) staining, kidney (two rats) samples from each group
were cut into small pieces in 4% buffered paraformaldehyde
solution and embedded in paraffin. Sections (3–4 μm) were
cut by microtome and passed through various grades of etha-
nol. Sections were stained with H&E then dehydrated through
graded ethanol, cleared in xylene and mounted with DPX
(Fluka, Switzerland). The stained sections were graded for the
presence of medullary congestion, tubular cell necrosis and
dilation, cytoplasmic vacuolization, nuclear pyknosis, and
cytoplasmic eosinophilia, according to the following scale:
0, no occurrence; I, 0–25% present; II, 25–50% present; and
III, > 50% present.

Data are expressed as the mean (SEM) and analysed statisti-
cally by one-way anova followed by the post hoc Dunnett’s
test; differences with P < 0.05 were considered statistically
significant.

Results
In the nephrectomised and RIR group after 4 weeks there were
significant changes in haemodynamic variable. The heart rate,
SBP, diastolic (DBP), mean arterial blood pressure (MABP),
EDP, dp/dt max, dp/dt min, exponential τ and pressure–time
index were increased significantly. EELU (400 mg/kg) treat-
ment showed a significant decrease in SBP, DBP, MABP,
EDP, dp/dt max (all P < 0.001), dp/dt min (P < 0.05), expo-
nential τ (P < 0.001) and pressure–time index (P < 0.001).
The load-independent index of contractility was significantly
decreased in Group II; in Groups III and IV it was signifi-
cantly higher (P < 0.05 and P < 0.001, respectively) than in
group II (Table 1).

After 4 weeks, rats in group II had a significantly greater
kidney weight and urine flow (P < 0.001, respectively) than
rats in group I, whereas rats treated with EELU 400 mg/kg
had significantly lower kidney weight (P < 0.05) and urine
flow (P < 0.001) than in group II. Both serum creatinine

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and BUN in group II were significantly greater ($P < 0.001$) than in group I. However, the creatinine and BUN levels in group IV were significantly ($P < 0.001$ and $P < 0.01$, respectively) lower than in group II (Table 1).

MPO activity was significantly increased in the renal tissue homogenate of group II, whereas group IV showed a significant decrease in MPO activity ($P < 0.05$). The TNF-$\alpha$ level was significantly increased in group II, whereas in group IV it was significantly decreased ($P < 0.05$; Table 1).

After 4 weeks, rats in group II had a significant decrease in the levels of SOD, GSH, GST and GPx, but rats in group IV had significant restoration of the levels of SOD ($P < 0.05$), GSH ($P < 0.01$), GPx ($P < 0.01$) and GST ($P < 0.01$). MDA levels in group II increased significantly ($P < 0.01$) whereas rats in group IV had significantly lower levels ($P < 0.05$) than in group II.

The levels of the renal membrane-bound enzymes Na$^+$K$^+$-ATPase and Mg$^{2+}$-ATPase in group II decreased significantly (both $P < 0.01$) but rats in group IV had significant restoration in the levels of these enzymes (both $P < 0.05$).

DNA fragmentation in renal tissue was analysed with agarose-gel electrophoresis. In group II there was intra-nucleosomal DNA damage, in the form of typical fragmentation laddering, which indicates cell apoptosis (Fig. 1, lane B), whereas there was less DNA fragmentation in groups III and IV (Fig. 1, lanes C and D, respectively) suggesting anti-apoptotic activity preventing cell death during RIR-induced renal hypertension.

### Table 1  
Haemodynamic changes and left ventricular function, renal function, and endogenous antioxidant enzymes and membrane-bound phosphatase enzymes.

| Mean (SEM) variable                  | Group                     |
|-------------------------------------|---------------------------|
|                                    | Sham (I)                  | RIR (II)                | EELU 200 (III) | EELU 400 (IV) |
| Heart rate, beats/min              | 340.4 (11.52)             | 436.1 (19.9$^{***}$)    | 373.8 (13.80$^{**}$) | 360.4 (16.10$^{***}$) |
| SBP, mmHg                           | 97.7 (2.4)                | 132.1 (2.0$^{***}$)     | 124.6 (4.2$^{**}$)  | 119.9 (3.2$^{**}$)    |
| DBP, mmHg                           | 71.16 (1.57)              | 101.6 (1.15$^{***}$)    | 96.63 (2.79$^{**}$) | 93.91 (2.77$^{**}$)   |
| MABP, mmHg                          | 82.54 (1.32)              | 118.7 (1.48$^{***}$)    | 112.0 (2.87$^{**}$) | 108.5 (2.39$^{**}$)   |
| EDP, mmHg                           | 4.30 (0.27)               | 8.94 (0.40$^{**}$)      | 8.53 (0.32$^{**}$)  | 7.12 (0.57$^{**}$)    |
| Max dp/dt, mmHg/s                   | 1212.1 (184.1)            | 2572.2 (296.1$^{***}$)  | 1788.4 (236.6$^{**}$) | 1245.1 (160.3$^{**}$) |
| Min dp/dt, mmHg/s                   | -1433.3 (132.3)           | -2617.7 (287.6$^{**}$)  | -1926.1 (374.4$^{**}$) | -1511.2 (230.4$^{**}$) |
| Contractility index                 | 18.49 (0.88)              | 8.64 (0.54$^{**}$)      | 12.14 (0.84$^{**}$) | 13.73 (1.45$^{**}$)   |
| Exponential $\tau$, ms              | 18.40 (0.65)              | 26.49 (1.08$^{**}$)     | 22.51 (0.85$^{**}$) | 21.01 (0.99$^{**}$)   |
| Pressure-time index                 | 4.62 (0.048)              | 12.64 (0.71$^{**}$)     | 9.89 (1.23$^{**}$)  | 7.81 (0.87$^{**}$)    |
| **Renal function**                  |                           |                         |                   |                     |
| Kidney weight, mg                   | 756.0 (19.40)             | 900.4 (14.19$^{***}$)   | 847.8 (19.32$^{**}$) | 817.8 (22.77$^{**}$)  |
| Urine flow, $\mu$L/min/kg           | 35.66 (1.18)              | 69.24 (4.29$^{**}$)     | 54.19 (3.72$^{**}$) | 48.97 (3.65$^{**}$)   |
| BUN, mg/dL                          | 19.7 (1.31)               | 63.8 (8.87$^{***}$)     | 47.99 (6.61$^{**}$) | 30.7 (2.70$^{**}$)    |
| Serum creatinine, mg/dL             | 1.06 (0.21)               | 4.08 (0.33$^{**}$)      | 2.58 (0.29$^{**}$)  | 1.94 (0.062$^{**}$)   |
| TNF-$\alpha$, pg/mL                 | 48.7 (13.42)              | 223.5 (20.53$^{***}$)   | 175.4 (18.90$^{**}$) | 132.9 (26.29$^{**}$)  |
| MPO, IU/mg protein                  | 3.13 (0.43)               | 10.0 (1.39$^{**}$)      | 7.41 (2.16$^{**}$)  | 4.95 (0.43$^{**}$)    |
| **Enzymes**                         |                           |                         |                   |                     |
| SOD, unit/mg protein                | 9.67 (1.30)               | 3.85 (0.61$^{**}$)      | 6.65 (1.65$^{**}$)  | 8.16 (0.33$^{**}$)    |
| GSH, $\mu$g/mg protein              | 31.57 (1.59)              | 20.67 (1.27$^{**}$)     | 26.04 (0.52$^{**}$) | 28.47 (1.86$^{**}$)   |
| MDA, mmol/mg protein                | 2.94 (0.08)               | 4.90 (0.42$^{**}$)      | 3.90 (0.37$^{**}$)  | 3.33 (0.37$^{**}$)    |
| GPx, $\mu$mol/min/mg protein        | 24.87 (0.49)              | 19.67 (1.2$^{**}$)      | 21.80 (0.44$^{**}$) | 24.60 (1.0$^{**}$)    |
| GST, $\mu$mol/min/mg protein        | 146.9 (4.71)              | 95.8 (6.66$^{**}$)      | 110.3 (5.93$^{**}$) | 132.7 (6.46$^{**}$)   |
| Na$^+\,$K$^-\,$ATPase, $\mu$mol inorganic P liberated/mg protein | 6.41 (0.76) | 2.95 (0.60$^{**}$) | 4.15 (0.39$^{**}$) | 5.87 (0.70$^{**}$) |
| Mg$^{2+}$-ATPase, $\mu$mol inorganic P liberated/mg protein | 10.69 (1.41) | 4.75 (0.44$^{**}$) | 7.24 (1.26$^{**}$) | 9.86 (1.21$^{**}$) |

Data were analysed by one-way anova followed by post hoc Dunnett’s tests, with $P < 0.05$ considered to indicate significance.

* $P < 0.05$.  
** $P < 0.01$.  
*** $P < 0.001$ vs. sham group.  
# $P < 0.05$.  
## $P < 0.01$ vs RIR group.  
+ $P < 0.05$.  
++ $P < 0.01$.  
+++ $P < 0.001$ vs. RIR group.

Figure 1  
Effects of EELU on DNA fragmentation in RIR-induced injury in rats: (A) sham, (B) RIR, (C) EELU 200 mg/kg, (D) EELU 400 mg/kg groups.
Figure 2  Effect of EELU on cellular necrosis and viability detected in renal cells by flow cytometry using the PI method; PI fluorescence intensity was measured using FL-1. Representative dot-plot images from four experimental groups: (A) sham, (B) RIR, (C) EELU 200 mg/kg, and (D) EELU 400 mg/kg. A histogram of cellular necrosis (E) and viability (F) were plotted; each bar represents the mean ± SEM of four experimental groups. **P < 0.001 vs. RIR group.

Figure 3  The effect of EELU treatment on morphological changes assessed by histological examination of the renal cortex of rats by H&E stain (×100). Representative histological images (A) sham group; regular renal tissue with glomeruli (arrow) and tubuli (☆). (B) RIR group, cellular vacuolisation (arrowhead), congestion (arrow), and necrosis (☆); (C) EELU 200 mg/kg, reduced congestion (arrow); and (D) EELU 400 mg/kg showing reduced glomeruli necrosis (arrow) and tubular dilation (☆).
Cellular necrosis in group II was increased significantly \((P < 0.001)\) whereas groups III and IV had a significant (both \(P < 0.001)\) decrease in cellular necrosis. The viability of renal cells was significantly (\(P < 0.001)\) decreased in group II and significantly (\(P < 0.001)\) greater in groups III and IV than in group II (Fig. 2).

Histological evaluation of the kidney in group I showed regular morphology of renal parenchyma with well-defined glomeruli and tubuli. Rats in group II showed severe (>50% area of the kidney) vascular congestion, tubular cell necrosis, cytoplasmic vacuolization and nuclear pyknosis. In groups III and IV, despite the presence of mild (0-25% area of the kidney) vasocongestion and tubular cell necrosis, the glomeruli maintained a better morphology than in group II (Fig. 3).

Discussion

The occlusion of renal artery for 45 min followed by reperfusion results in RIR-mediated renal damage [2,22,23]. RIR can arise as enhanced oxidative stress and possibly the activation of the RAAS [24]. Aunapuu et al. [2] reported that hypertension is the major factor determining progression of acute renal diseases, and showed an increase in SBP after 4 weeks of RIR. In the present study EELU treatment at 400 mg/kg was associated with a significant decrease in SBP, DBP, MABP, EDP, dp/dt max and dp/dt min after 4 weeks of RIR in nephrectomised rats. The antihypertensive activity of SDG was studied by Prasad [5], who reported that SDG is a long-acting hypotensive agent in normotensive rats, and hypothesised that hypotensive activity might be due to stimulation of guanylate cyclase activity (an action similar to that of nitric oxide). These findings support our assumption and explain our results.

It was reported that the kidneys appear to be hypertrophic and their weight is increased after 24 h and 4 weeks of RIR injury [2,25]. Our results are in accordance with a previous study [2], and showed a significant increase in kidney weight in group II after 4 weeks of RIR injury. Treatment with EELU 400 mg/kg significantly reduced the hypertrophic growth of the kidney.

The decline in renal function after 4 weeks of RIR was reflected in our results, showing increased urine flow. Several reports showed an increase in renal function indicators such as BUN and creatinine after 4 and 6 h of RIR injury in nephrectomised rats [22,23]. The present study showed a significant increase in the levels of BUN and creatinine after 4 weeks of RIR injury. Increases in serum creatinine and BUN after 4 weeks might be due to the absence of one kidney in addition to RIR injury in the other kidney. Treatment with EELU (400 mg/kg) significantly restored the levels of BUN and creatinine.

Michels et al. [26] reported that the kidney is extremely sensitive to changes in oxygen tension within its complex architecture, making it very prone to hypoxic injury when the renal artery is temporarily occluded. GST, GPx and SOD are the most important endogenous antioxidant enzymes, and they are present in high concentrations in kidney cells. GSH scavenges superoxide radicals and protects protein thiol (–SH) groups from oxidation [27]. Our results showed a significant decrease in the levels of SOD, GSH, GST and GPx, and increased lipid peroxidation in group II after 4 weeks. Treatment with EELU (400 mg/kg) significantly increased the level of protective antioxidant enzymes and decreased lipid peroxidation. This suggests that EELU 400 mg/kg protects the kidney against RIR-induced injury through its antioxidant role.

There was a significant reduction in the activity of Na⁺K⁺-ATPase and Mg²⁺-ATPase in the renal homogenate of group II after 4 weeks of RIR injury. Decreased activity of Na⁺K⁺-ATPase in renal ischaemia reperfusion might be due to its interaction at the cytoplasmic surface of the basolateral membrane. The decrease in Mg²⁺-ATPase might be due to RIR-induced destruction of renal parenchyma [28]. In group IV there was a significant increase in levels of Na⁺K⁺-ATPase and Mg²⁺-ATPase, which might be due to the ability of EELU to protect the –SH group from oxidative damage through the inhibition of peroxidation of membrane lipids [29].

The process of cellular apoptosis and necrosis has been reported to contribute to extensive cell loss in many pathological states, such as RIR-induced renal failure [30]. Treatment with EELU (400 mg/kg) for 4 weeks was associated with a significant decrease in cellular necrosis, increase in viability of renal cells and reduction in DNA fragmentation after RIR, which indicated prevention of renal cell death by apoptosis. The histopathology in group II showed morphological changes such as severe tubular cell swelling, necrosis, pyknotic nuclei and medullary congestion. In contrast, sections of the kidney of rats in group IV showed preservation of the architectural structure of the kidney, which further supports the protective action of EELU against RIR injury.

EELU at 200 mg/kg had a limited renoprotective role in RIR-induced renal injury when compared with 400 mg/kg. Oral administration of 200 mg/kg EELU significantly reduced urine flow and serum creatinine levels, increased GSH, decreased cellular necrosis, increased renal cell viability and preserved the architectural structure of the kidney after RIR-induced injury. Its effect on the other variables assessed was insignificant when compared with group II. These differences between the doses might be dose-related but further investigations are needed to determine the appropriate renoprotective dose of EELU.

We conclude that treatment with EELU (400 mg/kg) for 4 weeks after RIR significantly decreased blood pressure, dp/dt max, dp/dt min and pressure–time index. EELU decreased kidney weight, urine flow, and creatinine and BUN levels in the nephrectomised rat. EELU also restored MPO activity and TNF-α levels, and attenuated the RIR-induced oxidative damage, as shown by a significant increase in the levels of endogenous antioxidants SOD, GSH, GST and GPx, the membrane-bound enzymes Na⁺K⁺-ATPase and Mg²⁺-ATPase, and significant decrease in the level of the lipid peroxidation product, MDA. The anti-apoptotic role of EELU was evident from the decrease in DNA fragmentation. The flow cytometric study confirmed a decrease in cellular necrosis after RIR. The histopathology of kidney provided structural evidence for the renoprotective effect of EELU.

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References

[1] Ozcan AV, Sacar M, Aybek H. The effects of iloprost and vitamin C on kidney as a remote organ after ischemia-reperfusion of lower extremities. *J. Surg. Res.* 2007;**140**:20.

[2] Aunapuu M, Pechter U, Kuhnel W, Ots M, Arend A. Morphological changes in experimental post ischemic rat kidney. A pilot study. *Ann. Anat.* 2005;**187**:63–70.

[3] Chatterjee PK, Thiemermann C. An in vivo model of ischemia/reperfusion and inflammation of the kidneys of the rat. *Meth. Mol. Biol.* 2003;**225**:223–37.

[4] Williams P, Lopez H, Brit D. Characterization of renal ischemia-reperfusion injury in rats. *J. Pharmacol. Toxicol. Method.* 1997;**37**:1–7.

[5] Prasad K. Antihypertensive activity of secoisolariciresinol diglucoside (SDG) isolated from flaxseed: role of guanylate cyclase. *Int. J. Angiol.* 2004;**13**:7–14.

[6] Westcott ND, Muir AD. Process for extracting lignans from flaxseed. US Patent no. 5705618 1998.

[7] Teede HJ, Dalais FS, Kotsopoulos D, Liang YL, Davis S, McGrath BP. Dietary soy has both beneficial and potentially adverse cardiovascular effects: a placebo-controlled study in men and postmenopausal women. *J. Clin. Endocrinol. Metab.* 2001;**86**:3053–60.

[8] Washburn S, Burke GL, Morgan T, Anthony M. Effect of soy protein supplementation on serum lipoproteins, blood pressure, and menopausal symptoms in perimenopausal women. *Menopause* 1999;**6**:7–13.

[9] Eliasson C, Kamal-Eldin A, Andersson R, Aman P. High-performance liquid chromatographic analysis of secoisolariciresinol diglucoside and hydroxycinnamic acid glucosides in flaxseed by alkaline extraction. *J. Chromatograph. A* 2003;**1012**:151–9.

[10] Zanwar AA, Hegde MH, Bodhankar SL. Cardioprotective activity of flav lignan concentrate extracted from seeds of *Linum usitatissimum* in isoprenaline induced myocardial necrosis in rats. *Interdiscip. Toxicol.* 2011;**4**:90–7.

[11] Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for SOD. *J. Biol. Chem.* 1972;**247**:3170–5.

[12] Moran MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochem. Biophys. Acta* 1979;**582**:67–78.

[13] Habig WH, Jakoby WB. Assay of differentiation of glutathione S-transferases. *Meth. Enzymol.* 1981;**77**:398–405.

[14] Rotruck JT, Pope AL, Canther HE, Swanson AB, Hafeman DC, Hoekstra WC. Selenium. Biochemical role as a component of glutathione peroxidase. *Science* 1979;**179**:588–90.

[15] Slater TF, Sawyer BC. The stimulatory effects of carbon tetrachloride and other halogenoalkanes or peroxidative reactions in rat liver fractions in vitro. *Biochem. J.* 1971;**123**:805–14.

[16] Bonting SL. *Presence of Enzyme System in Mammalian Tissues Membrane and Ion Transport*. Wiley Inter Science, London 1970: 257–263.

[17] Ohnishi T, Suzuki T, Suzuki Y, Ozawa K. A comparative study of plasma membrane Mg²⁺-ATPase activities in normal, regenerating and malignant cells. *Biochim. Biophys. Acta* 1982;**684**:67–74.

[18] Fiske CH, Subbarow Y. Colorimetric determination of phosphorus. *J. Biol. Chem.* 1925;**66**:375–400.

[19] Lowry OH, Rosenbrough NJ, Farr AC, Randell RJ. Protein measurement with folinphenol reagent. *J. Biol. Chem.* 1951;**193**:265–73.

[20] Krawisz JE, Sharon P, Stenson WF. Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. *Gastroenterology* 1984;**87**:1344–50.

[21] Urbanits SB, Griesmacher A, Hopfengere G, Stockhammer G, Karimib A, Mullerb MM. FACS analysis a new and accurate tool in the diagnosis of lymphoma in the cerebrospinal fluid. *Clinica Chimica Acta* 2002;**317**:101–7.

[22] Korkmaz A, Kolankaya D. Protective effect of rutin on the ischemia/reperfusion induced damage in rat kidney. *J. Surg. Res.* 2010;**160**:309–15.

[23] Hagiwara S, Koga H, Iwasaka H, Kudo K, Hasegawa A, Kusaka J et al. ETS-GS, A New antioxidant, ameliorates renal ischemia reperfusion injury in a rodent model. *J. Surg. Res.* 2010. doi:10.1016/J.JSS.2010.01.039 (in press).

[24] Atlas SA. The renin-angiotensin aldosterone system, pathophysiological role and pharmacologic inhibition. *J. Manag. Care. Pharm.* 2007;**13**:59–S20.

[25] Sener G, Tugtepe H, Yuksel M, Cetinel S, Gedik N, Yegen BC. The renin-angiotensin aldosterone system. pathophysiological role and pharmacologic inhibition. *J. Manag. Care. Pharm.* 2007;**13**:59–S20.

[26] Sener G, Tugtepe H, Yuksel M, Cetinel S, Gedik N, Yegen BC. The renin-angiotensin aldosterone system. pathophysiological role and pharmacologic inhibition. *J. Manag. Care. Pharm.* 2007;**13**:59–S20.

[27] Siems WG, Grunescker C, Hopfengere G, Stockhammer G, Karimib A, Mullerb MM. FACS analysis a new and accurate tool in the diagnosis of lymphoma in the cerebrospinal fluid. *Clinica Chimica Acta* 2002;**317**:101–7.

[28] Michels C, Dorai T, Chander P, Choudhury M, Grasso M. Hypoxic preconditioning in a rat renal ischemia model. An evaluation of the use of hydralazine. *World J. Urol.* 2009;**27**:817–23.

[29] Siems WG, Grune T, Estrahuer B. 4-Hydroxynonenal formation during ischemia and reperfusion of rat small intestine. *Life Sci.* 1995;**57**:785.

[30] Upagamlawar A, Farswan M, Rathod S, Balaraman R. Modification of biochemical parameters of gentamicin nephrotoxicity by Coenzyme Q10 and green tea in rats. *Indian J. Exp. Biol.* 2006;**44**:416–8.

[31] Floreani M, Bonetti AC, Carpudo F. Increase of Na⁺-K⁺ ATPase activity in intact rat brain synaptosomes after their interaction with phosphatidyl serum vesicles. *Biochem. Biophys. Res. Commun.* 1981;**101**:1337–44.

[32] Liebertal W, Koh JS, Levine JS. Necrosis and apoptosis in acute renal failure. *Semin. Nephrol.* 1998;**18**:505–18.