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Daidzein Mitigates Oxidative Stress and Inflammation in the Injured Kidney of Ovariectomized Rats: AT1 and Mas Receptor Functions

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Original Paper

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Introduction. Chronic kidney disease (CKD) is a health problem in postmenopausal women, and renal fibrosis is a common feature of CKD. In the renin-angiotensin system, oxidative stress and inflammation are involved in the pathogenesis of renal fibrosis. This study investigated the effect of the phytoestrogen daidzein on oxidative stress and inflammation and the mediation of the angiotensin AT1 and Mas receptors in a fibrotic model of kidney disease of ovariectomized (OVX) rats.
Methods. Unilateral ureteral obstruction (UUO) was performed to induce chronic renal inflammation and fibrosis in 84 OVX rats, which were divided into four main groups (*each* = 21) including sham + Vehicle (Veh.), UUO + Veh, UUO + estradiol (E2), and UUO + daidzein. Each main group composed of three subgroups (*n* = 7), which received saline, losartan (AT1R antagonist), or A779 (Mas receptor [MasR] antagonist) for 15 days after UUO or sham operation. Renal pathology, serum and kidney oxidants and antioxidants, malondialdehyde (MDA), nitric oxide metabolites (NOx), protein carbonyl (PC), and pro-inflammatory and anti-inflammatory cytokines were examined.
Results. UUO increased renal glomerulosclerosis, inflammation, serum and kidney tissue MDA, NOx, and PC together with an increase in TNF-α, IL-1β, and IL-6 expression. Moreover, UUO decreased superoxide dismutase and glutathione peroxidase and catalase activity, total antioxidant capacity, and IL-10 level in the serum and kidney tissue. AT1R blockade reduced and MasR blockade worsened renal impairment. Daidzein and E2 alone and in co-treatment with losartan significantly ameliorated these effects.
Conclusion. Via interaction with AT1R and MasRs, daidzein improved glomerulosclerosis, oxidative stress, and inflammation in UUO-OVX rats. Daidzein may be a candidate for estrogen replacement therapy in postmenopausal or older women against postmenopausal kidney damage.

INTRODUCTION

Renal fibrosis is a crucial hallmark and outcome of chronic kidney disease (CKD), a critical condition in postmenopausal women with a prevalence of 7% in Europe and over 10% in the United States (US).[1] As no treatment modalities are available

that specifically target fibrogenesis, finding new therapeutic modalities is the most important beneficial strategy.

Various signaling pathways and peptides such as renin-angiotensin (RAS) system, transforming growth factor-beta 1 (TGF-β1), and their positive feedback loops are involved in the pathogenesis of renal inflammation and fibrosis through production of oxidative stress and inflammation [2] In the early stages of renal fibrosis, activation and production of reactive oxygen species (ROS) and oxidative stress lead to infiltration of inflammatory cells, epithelial to mesenchymal cell transition (EMT), production of pro-inflammatory cytokines, and extracellular matrix (ECM) accumulation, leading to renal fibrosis and CKD.[3,4] In CKD models such as unilateral ureteral obstruction (UUO), production of ROS and inflammation, and reduced oxidative defense system have been reported, resulting in renal dysfunction, proximal tubular epithelial cell death, autophagy, apoptosis, and fibrosis.[5-8] Inhibition of angiotensin II type 1 receptors (AT1Rs) ameliorates these harmful effects while inhibition of the Ang1-7/MasR axis has the opposite effects.[9-10]

The classical ACE/AT1R axis is one of the main pathways in causing oxidative stress and inflammation in the pathophysiology of renal fibrosis.[9] Conversely, the non-classical ACE2/angiotensin-(1–7) MasR axis has an anti-fibrotic functions.[10] RAS function is gender-dependent; the expression of the classical RAS pathway components is higher in males, and the expression of the non-classical RAS pathway components is higher in females,[11] causing gender differences in CKD progression. These sex differences are partly due to the protective effects of 17beta-estradiol (estrogen, E2) against some diseases after menopause in females. Accordingly, recent studies have shown that E2 deficiency increases the activity of the classical RAS system by increasing ACE and AT1R expression in the lung and heart.[12,13] Therefore, E2-based hormone replacement therapy (HRT) is an effective treatment in reducing CKD. [14] However, HRT has been shown to have many side effects, and finding alternative therapies mimicking the beneficial effects of E2 with fewer side effects is necessary

Given the World Health Organization's recommendation of scientific research on herbal medicines, phytoestrogens that are plant-derived estrogenic components, have attracted attention as new therapeutic targets.[15] These compounds are widely found in soybean, legume-based foods, seeds, cereals, fruits, berries, and vegetables.[16] Their structural similarity with E2, which enables it to mimic estrogenic effects, has led to their use in osteoporosis, menopause symptoms, breast cancer, and prostate diseases. [16] An important member of phytoestrogens is the isoflavone daidzein with antioxidant and anti-inflammatory properties.[16,17] Several studies have reported the beneficial antioxidant and anti-inflammatory effects of daidzein in renal toxicity.[17,18] Currently, there is no evidence for the interaction of daidzein with angiotensin AT1 and Mas receptors in kidney dysfunction and fibrosis in postmenopausal women. Based on these shreds of evidence, this study aimed to investigate the possible mechanisms of the effect of daidzein (and E2, as the positive control) on oxidative stress, inflammation, and fibrosis through modulating AT1R and MasR in a model of UUO nephropathy in OVX rats, as the animal model of postmenopausal condition in women.

MATERIALS AND METHODS
Animals

In the present study, 84 female rats (180 to 220 g) were purchased from the animal center of Kerman University of Medical Sciences, Kerman, Iran, and kept in the animal house with normal humidity and temperature on a 12-h light/dark cycle. Food and water were available *ad libitum.* Experiments were performed following the international guidelines for laboratory animal use and care. The experiment protocol was approved by the Ethics Committee of Kerman University of Medical Sciences, Iran (permission No. IR.KMU.REC.1397.249).

Materials

17β-estradiol was purchased in vials from Aboureyhan Pharmaceutical Company (Tehran, Iran). Daidzein and losartan (AT1 receptor blocker) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) (sc-24001A and sc-353662, respectively). The other materials and resources were: BCA protein quantification kit (DB9684, DNA Biotech Co., Iran), A779)Mas receptor antagonist, SML1370) (Sigma-Aldrich, St. Louis, MO, USA), polyvinylidene difluoride (PVDF) membrane (162-017777; Bio-Rad Laboratories, CA, USA), 5%

BSA (A-7888; Sigma Aldrich, MO, USA), Anti-IL-1β (MBS2004054, Biocompare), anti-TNF alpha (ab205587, Abcam), anti-IL-10 (ab192271, Abcam), anti-IL-6 (MBS2002878, Biocompare), anti-beta actin loading control antibodies (ab8227; Abcam), goat anti-rabbit IgG H&L (HRP) (ab6721; Abcam), and goat anti-mouse IgG H&L (HRP) (ab6789; Abcam).

Bilateral Ovariectomy and UUO Procedure

For ovariectomy (OVX), the animal was anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg) intraperitoneally (IP), and a transverse incision (3 to 4 cm) was made in the lower abdominal region. The fat and intestines were pulled aside to visualize the uterus and its tubes. After identifying both ovaries, the ovarian arteries were ligated, and ovaries were extracted. Then 1–2 ml of sterilized saline solution was poured into the abdomen, and the muscles and skin were sutured continuously with 4.0 sterile sutures. The location of the wound was disinfected using potassium iodide, and the animals were cared for two weeks to ensure estrogen depletion.[19] On day 15, unilateral ureteral obstruction (UUO), an approved model of progressive chronic renal inflammation and fibrosis, was induced in OVX rats.[20] The animals were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg), and a left flank incision was made. The left ureter was ligated with 4.0 silk suture at two points along its length, and then the mid-portion was cut and removed. In the sham group, the flank incision was made, but the ureter was not ligated. The incision was sutured as explained above, and the animals were transferred to their cages with access to food and water *ad libitum*.[20]

Experimental Groups and Sample Collection

The OVX/UUO rats were divided into four main groups (*n* = 21): sham + Veh. (DMSO), UUO + Veh. , UUO + 17β-estradiol (5 µg/kg)[21] (positive control), and UUO + daidzein (1 mg/kg). This dose of daidzein was determined in a preliminary dose/response study using five different daidzein doses against UUO-induced kidney inflammation and fibrosis. In each main group, consisting of three subgroups (*n* = 7), one subgroup received saline, one received A779 (744 µg/kg),[22] and one received losartan (10 mg/kg)[23] for 15 days. All treatments were injected intraperitoneally. On the 16th day,

the animals were anesthetized with ketamine+ xylazine (80/10 mg/kg), and blood samples were taken from the left ventricle and centrifuged (4000 g, 5 min). Serum samples were frozen at -80 °C for the determination of serum redox status. The left kidney was harvested; one part was immersed in 10% formalin for morphological studies, and the other part was frozen in liquid nitrogen and stored at -80 °C for tissue redox and western blot studies.

Renal Histology

Left kidney tissues fixed with 4% buffered paraformaldehyde were embedded in paraffin, and 5-µm-thick sections were prepared. Masson's trichrome staining was performed on the sections for glomerulosclerosis assay and hematoxylin and eosin (H&E) staining for the inflammation assay. An expert pathologist blind to the experimental groups examined the sections with an Olympus microscope (CX41, Tokyo, Japan). Collagen deposition was considered glomerulosclerosis and was assessed in 20 randomly selected fields of cortical glomeruli (magnification: x 400). Glomerular damage score of 0 to 4 were used as 0 = normal, 1 = sclerosis up to 25%, 2 = sclerosis of 26 to 50%, 3 = sclerosis of 51 to 75%, 4 = sclerosis of > 75% of the glomeruli.[24] For quantitative scoring of inflammation, six fields in each sample were selected, and scores 0 to 4 were used (magnification x 100), with score 0 = normal glomeruli, tubules, and interstitium, 1 = inflammatory cells present in < 25%, 2 = inflammation in < 50%, 3 = inflammation in < 75%, and 4 = inflammation in > 75% of the fields of view.[25]

Assessment of Serum and Kidney Malondialdehyde (MDA)

The homogenate of the kidney was prepared by adding 100 mg tissue in 1 mL phosphate buffer (50 mmol/l; pH 7.5) containing 1 mM EDTA.[26]

For MDA assessment, the method of Rao *et al.* was used with a slight modification. Briefly, kidney tissue homogenate or serum was mixed with trichloroacetic acid and thiobarbituric acid (TBA) and then placed in a boiling water bath for 45 minutes. After cooling, n-butanol was added, and the mixture was centrifuged for 10 minutes. Finally, the pink-colored mixture was separated, and the absorbance was measured at 534 nm. [27]

Serum and Kidney Total Antioxidant Capacity (TAC) and Protein Carbonyl (PC)

The ferric reducing antioxidant power (FRAP) assay procedure (Benzie and Strain, 1996) was used to measure TAC.[28] Briefly, serum or kidney tissue homogenate and FRAP reagent were mixed and incubated at 37 ℃ for 5 minutes, and absorbance was read at 593 nm.

PC levels were measured by Levine *et al.*'s method,[29] based on the reaction of 2,4-dinitrophenylhydrazine (DNPH) with carbonyl groups in the oxidatively damaged proteins. The absorbance of the 2, 4-dinitrophenyl (DNP) hydrazones was measured at 370 nm.

The Determination of Serum and Kidney Antioxidants

The superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities in the serum and kidney tissue homogenate were determined according to the Randox kit instructions (UK; Cat NO.RS504; UK; Cat NO.SD125, respectively). In this procedure, the absorbance of SOD and GPX was measured at 560 and 340 nm, respectively.[30,31] Sinha's method with minor modifications was used to determine catalase (CAT) activity.[32] Samples and reagents were mixed and heated for 10 min and placed in a boiling water bath. Then the absorbance of samples was measured at 570 nm.

The Determination of Serum and Kidney Nitric Oxide Metabolites (NO$_x$)

The Griess method was used to measure the NOx levels. Deproteinization was performed using ZnSO$_4$ in the presence of 0.3 M NaOH. Then vanadium (III) chloride (VaCl$_3$) and the Griess reagent were mixed with the deproteinated sample, and the mixture was incubated at 37 °C for 30 min. Finally, optical density (OD) was determined at 540 nm.[33]

Western Blotting

Western blot analyses were performed as previously described, with some modifications.[34] For this, tissues were lysed with RIPA buffer. The lysates were removed by centrifugation at 14,000 rpm for 20 min at 4 °C. Protein concentration was determined by the BCA Protein Quantification kit according to the manufacturer's instructions. The tissue lysates were mixed with an equal volume of 2x Laemmli sample buffer. Lysates (20 μg) were then subjected to SDS-PAGE after 5 minutes of boiling and were subsequently transferred to a 0.2 μm Immune-Blot™ polyvinylidene difluoride (PVDF) membrane. The membranes were then blocked with 5% BSA in 0.1% Tween 20 for 1 hour. Then, the membranes were incubated with anti-IL-1β, anti-TNF alpha, anti-IL-10, anti-IL-6, and anti-beta actin loading control antibodies for 1 hour at room temperature. Subsequently, the membranes were washed three times with TBST and incubated with goat anti-rabbit IgG H&L (HRP) and goat anti-mouse IgG H&L (HRP) secondary antibodies. The membranes were then incubated with enhanced chemiluminescence (ECL) for 1 to 2 min. β-actin was used for normalization of protein expression. Gel Analyzer version 2010a (NIH, USA) was used for performing densitometry of protein bands. The percentage area under the curve (PAUC) of each band was divided by the PAUC of its corresponding actin band, and the results were compared between groups as described previously.[35]

RESULTS

Effect of Daidzein on Renal Morphology

In the sham + Veh. groups, histology of kidney tissue was normal, but in the UUO + Veh. groups, severe glomerulosclerosis and inflammation were observed ($P < .001$) (Figure 1A-D). Treatment with E2 and daidzein alone and in co-treatment with A779 or losartan reduced glomerulosclerosis and inflammation significantly ($P < .05$). Daidzein + A779 co-treatment was more effective on inflammation than E2 + A779 co-treatment ($P < .01$).

Effect of Daidzein on Serum Oxidant and Antioxidant Levels

As seen in figure 2A, the serum level of MDA in the UUO + Veh. groups increased significantly compared to the sham+ Veh. groups ($P < .001$). Treatment with E2 and daidzein alone and in co-treatment with A779 or losartan reduced MDA levels, significantly ($P < .05$). Daidzein was more effective than E2 in reducing MDA ($P < .001$). The serum levels of TAC and antioxidant enzymes (SOD, GPX, and CAT activity) decreased in the UUO + Veh. groups compared to the sham+ Veh. groups ($P < .05$) (Figure 2 B-E). Treatment with E2 and daidzein alone and co-treatment with A779 or losartan increased TAC and antioxidant enzyme levels ($P < .05$).
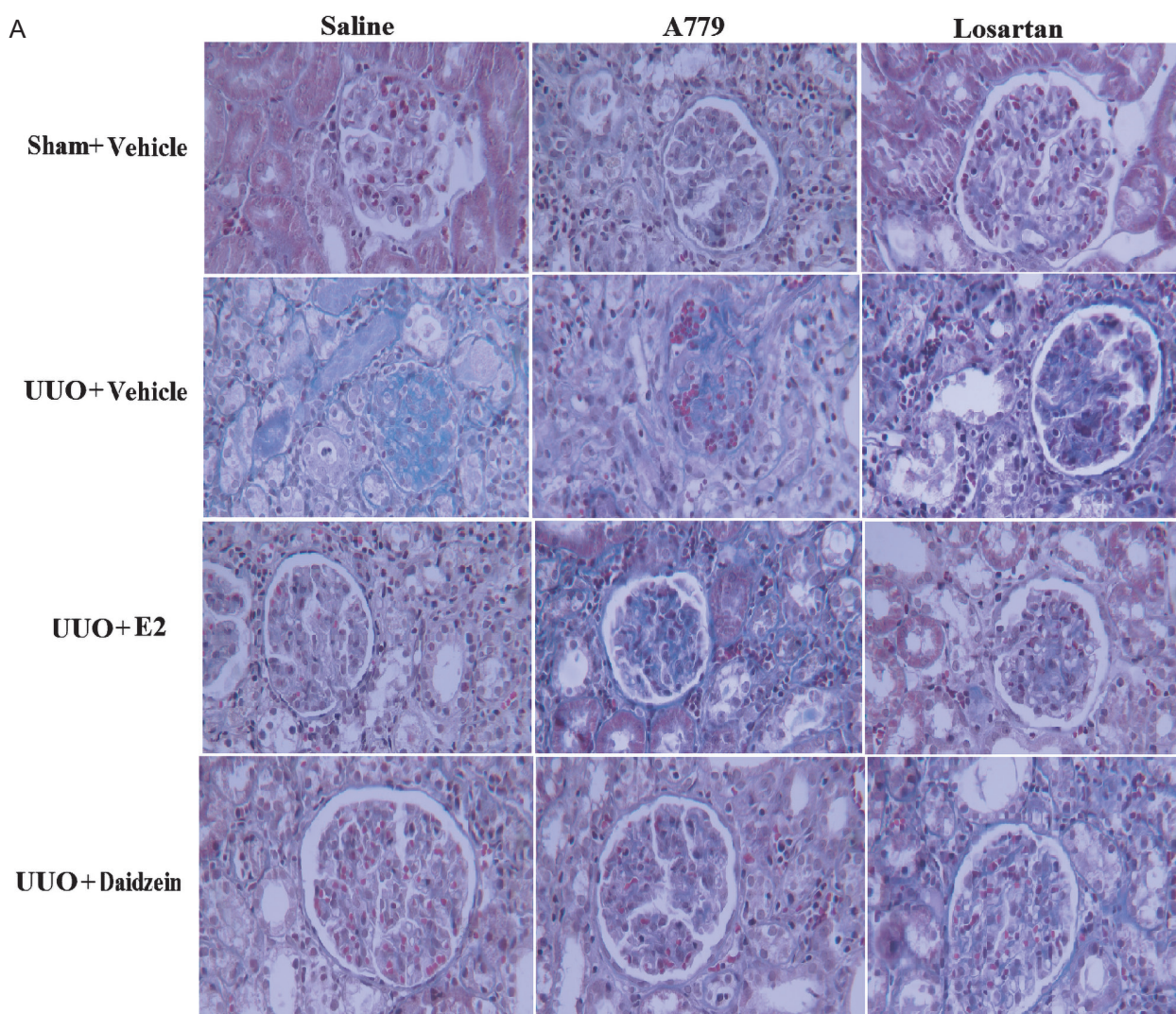
Figure 1. Representative Masson's trichrome staining (magnification ×400) (A), and H&E staining (magnification ×100) (B) of kidney sections from studied groups received saline, A779, or Losartan. Quantitative (mean ± SEM) analysis of the glomerular injury (C) and inflammtion (D). Red arrows :Glomeruli; yellow arrows: Foci of chronic inflammatory cells aggragation (n = 7 in each group; *** P < .001 *vs.* sham+ Veh, ## P < .01, ### P < .001 vs. UUO+ Veh, $$ P < .01 *vs.* UUO+E2).

Effect of Daidzein on Serum Protein Carbonyl and NOx Concentrations

The serum levels of PC and NOx increased significantly in the UUO + Veh. groups compared to their corresponding sham + Veh. groups (P < .05) (Figure 3 AB). Treatment with E2 and daidzein alone and in co-administration with A779 or losartan reduced PC and NOx significantly (P < .001).

Effect of Daidzein on Oxidant and Antioxidant Levels in the Left Kidney

The kidney levels of oxidant MDA in the UUO + Veh. groups increased significantly compared to their corresponding sham+ Veh. groups (P < .001) (Figure 4A). Treatment with E2 and daidzein alone

and in co-treatment with A779 or losartan reduced MDA levels significantly (P < .001). Daidzein was more effective than E2 in reducing MDA (P < .001). In UUO + Veh. groups, the kidney levels of TAC and antioxidant enzymes (SOD, GPX, and CAT activity) decreased compared to the sham + Veh. groups (P < .001) (Figure 4 B-E). Treatment with E2 and daidzein alone and in co-administration with A779 or losartan increased TAC and antioxidant enzyme levels (P < .001).

Effect of Daidzein on Left Kidney Protein Carbonyl and NOx Concentrations

In the sham + Veh. groups, PC and NOx levels were normal. In UUO + Veh. groups, the kidney
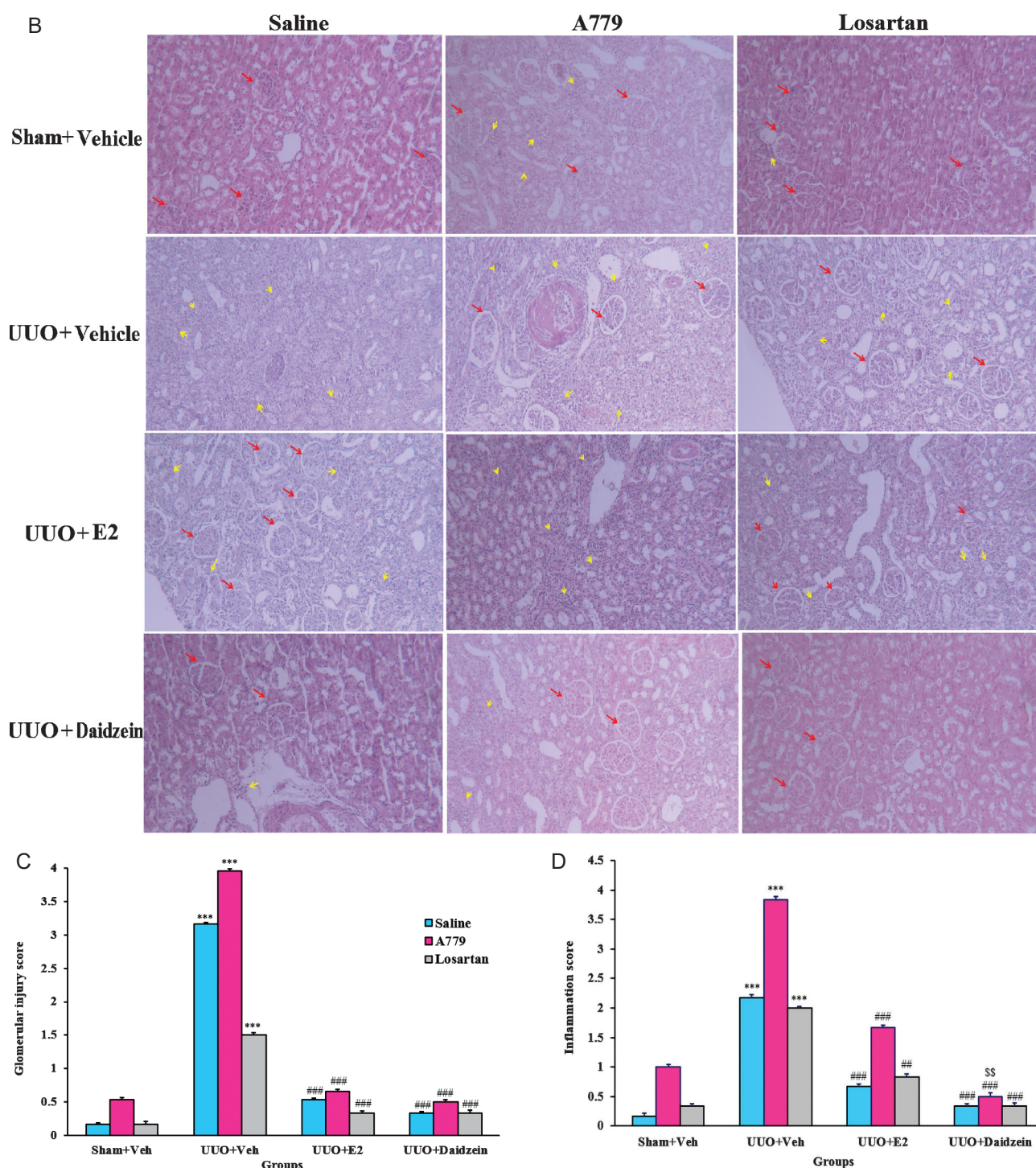
Figure 1. Continued

levels of PC and NOx increased significantly compared to the sham + Veh. groups ($P < .001$) (Figure 5 AB). Treatment with E2 and daidzein alone and in co-administration with A779 or losartan reduced kidney PC and NOx significantly ($P < .001$).

Effect of Daidzein on the Expression of Pro-inflammatory and Anti-inflammatory Cytokines

Figures 6A–C show a significant increase in the expression of the protein of inflammatory cytokines TNF-α, IL-1β, and IL-6 in the obstructed kidney in UUO + Veh. groups compared to sham + Veh.
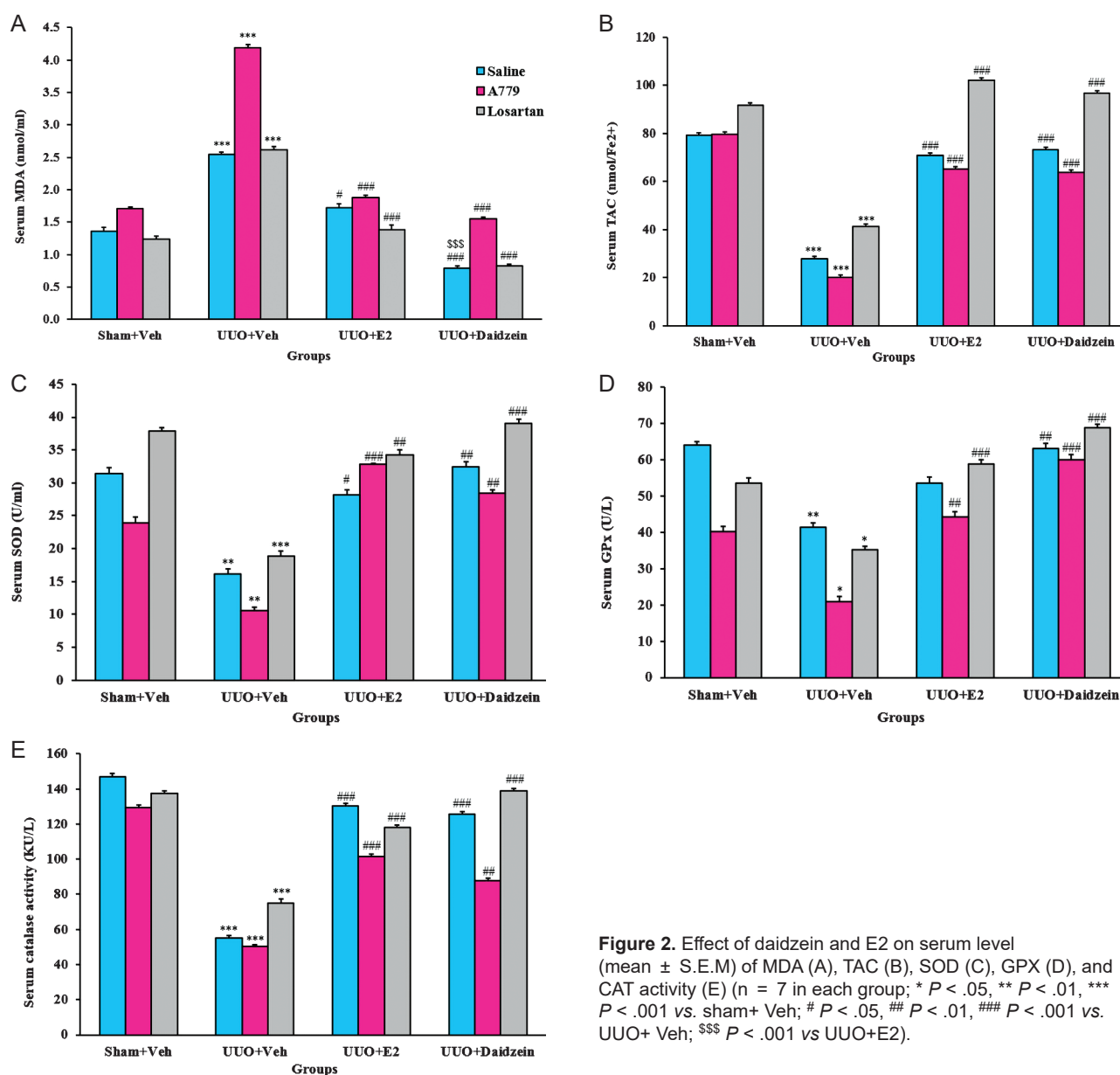
Figure 2. Effect of daidzein and E2 on serum level (mean ± S.E.M) of MDA (A), TAC (B), SOD (C), GPX (D), and CAT activity (E) (n = 7 in each group; * P < .05, ** P < .01, *** P < .001 *vs.* sham+ Veh; # P < .05, ## P < .01, ### P < .001 *vs.* UUO+ Veh; $$$ P < .001 *vs* UUO+E2).
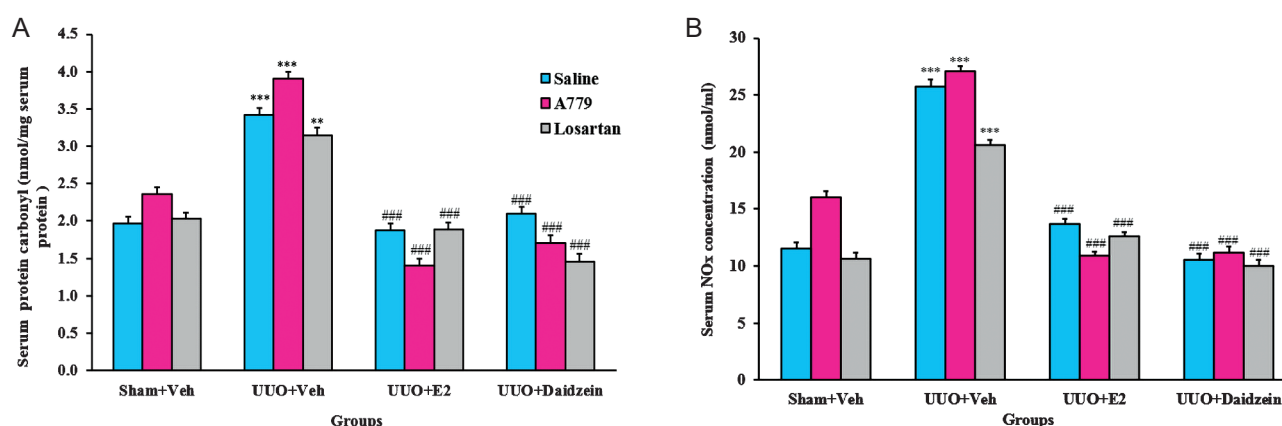

Figure 3. Effect of daidzein on serum level of protein carbonyl (A), and NOx (B), concentrations (Mean ± S.E.M, n = 7; ** P < .01, *** P < .001 *vs.* sham+ Veh; ### P < .001 *vs.* UUO+ Veh) [NOx: Nitric oxide metabolites].
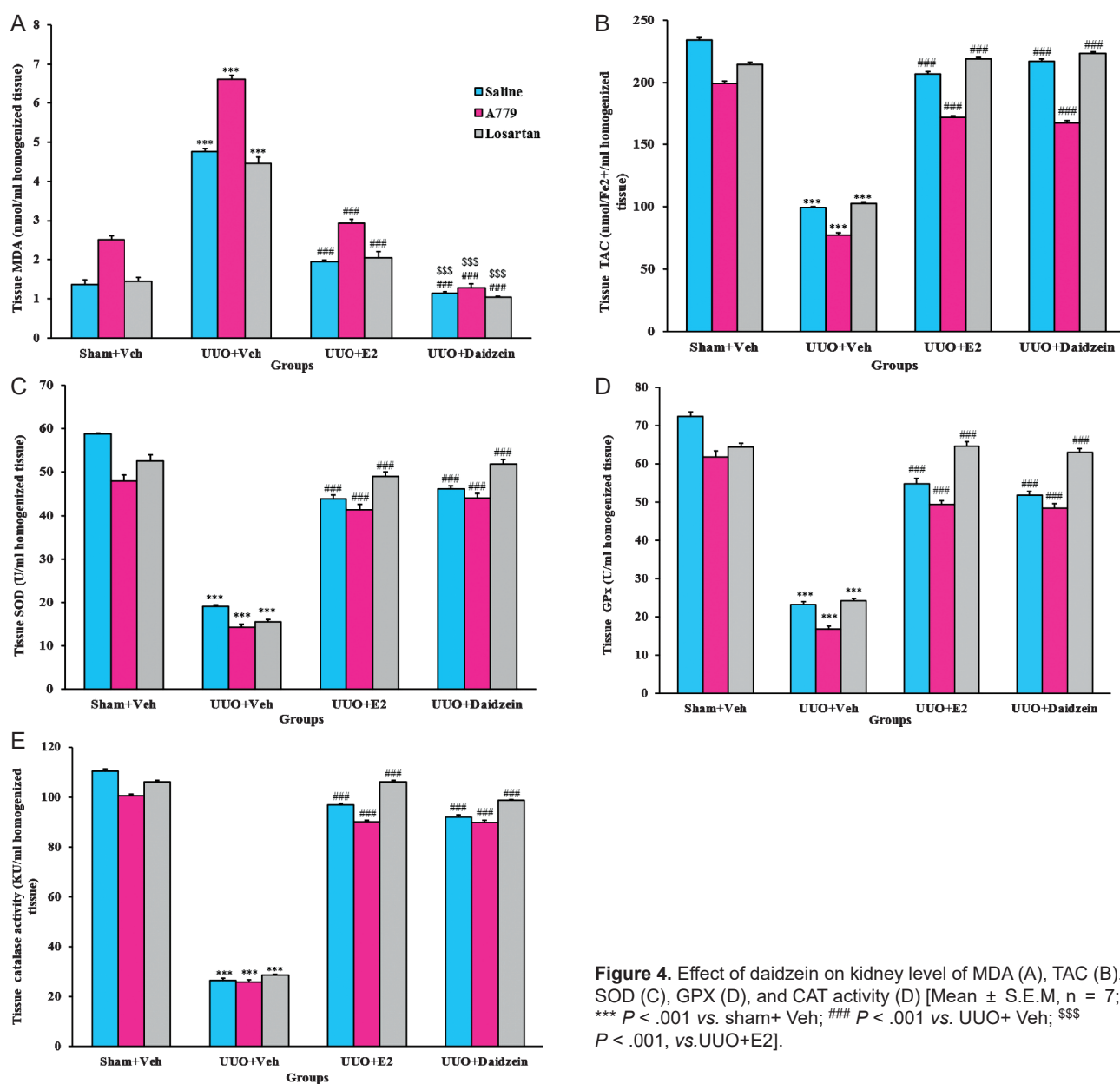
Figure 4. Effect of daidzein on kidney level of MDA (A), TAC (B), SOD (C), GPX (D), and CAT activity (D) [Mean ± S.E.M, n = 7; *** *P* < .001 *vs*. sham+ Veh; ### *P* < .001 *vs*. UUO+ Veh; $$$ *P* < .001, *vs*.UUO+E2].
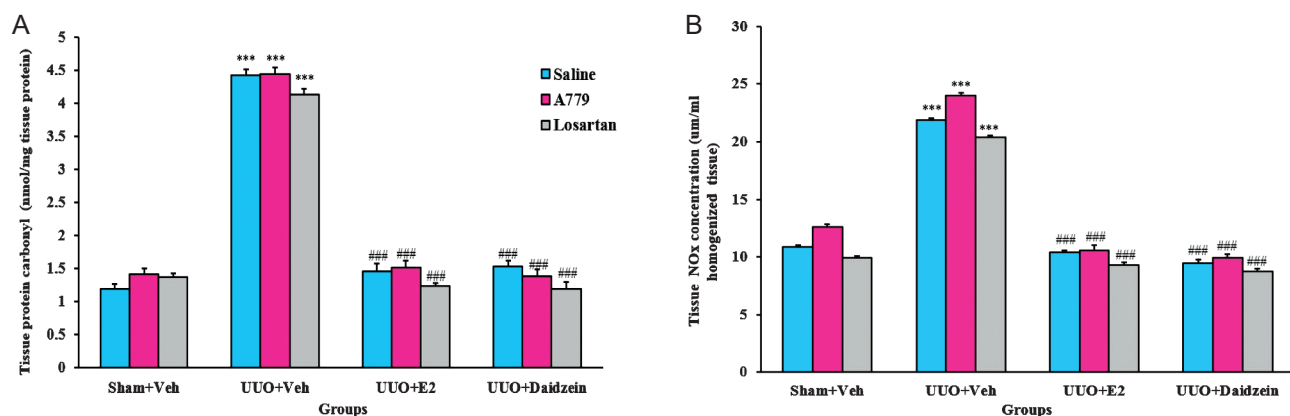

Figure 5. Effect of daidzein on kidney level of protein carbonyl (A), and NOx concentration (B) (Mean ± S.E.M, n = 7; *** *P* < .001 *vs*. sham+Veh; ### *P* < .001 *vs*. UUO+ Veh) [NOx: Nitric oxide metabolites concentration].
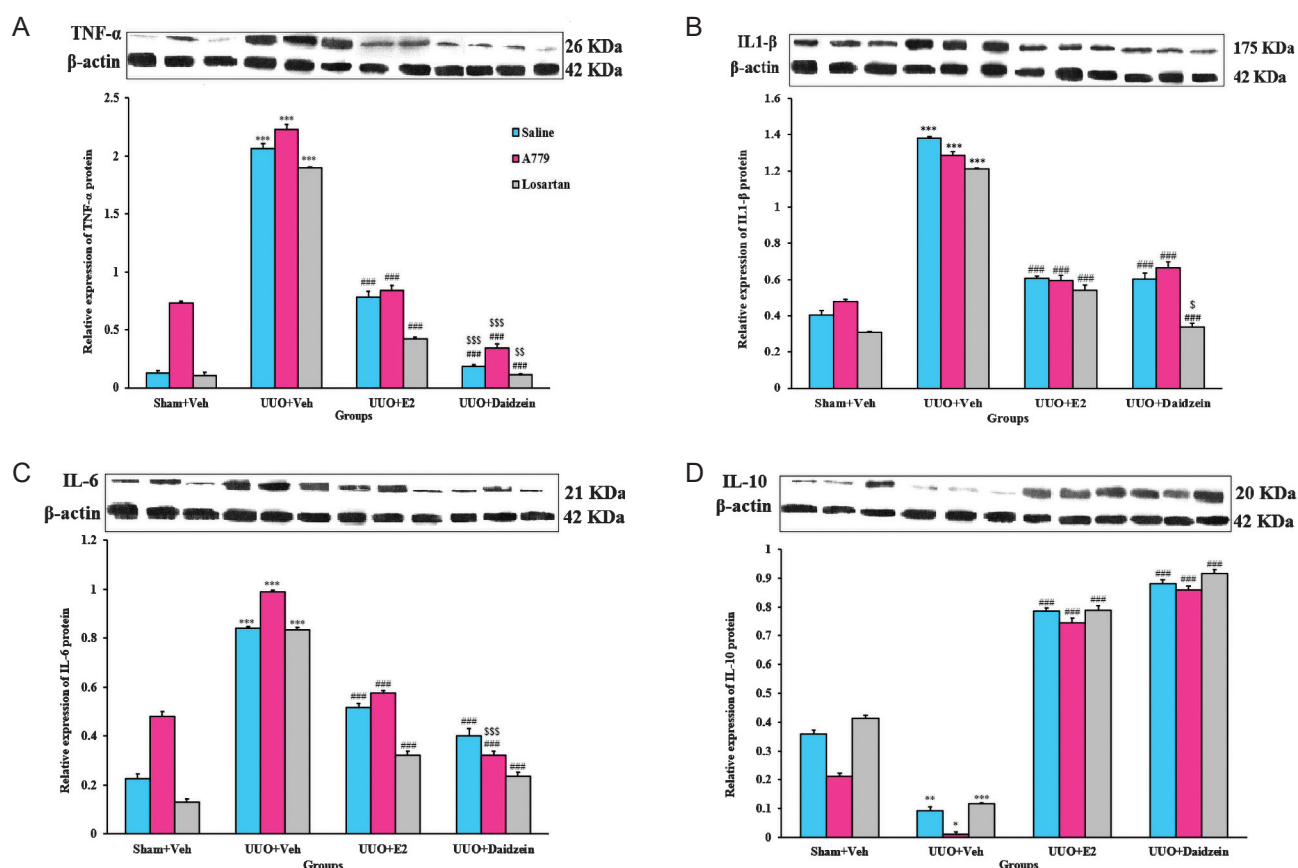
Figure 6. Effect of daidzein on relative protein expression of inflammatory cytokines TNF-α (A), IL-1β (B), IL-6 (C), and anti-inflammatory cytokine IL-10 (D) in the kidney tissue of study groups (Data are mean ± S.E.M, n = 7; * $P < .05$, ** $P < .01$, *** $P < .001$ *vs.* sham+ Veh; ### $P < .001$ *vs.* UUO + Veh; $ $P < .05$, $$ $P < .01$, $$$ $P < .001$ *vs.* UUO+E2).

groups ($P < .001$). Daidzein and E2 treatment alone and in co-treatment with A779 or losartan significantly reduced the inflammatory cytokine levels ($P < .001$). Daidzein was more effective than E2 in reducing the expression of TNF-α, IL-1β, and IL-6 proteins ($P < .05$). The expression of anti-inflammatory IL-10 significantly decreased in the UUO + Veh. groups compared to the sham + Veh. groups ($P < .05$). Treatment with E2 and daidzein alone and in co-treatment with A779 or losartan increased the expression of IL-10 protein significantly ($P < .001$).

DISCUSSION

The present study investigated the effect of daidzein and E2 and the interaction with the RAS system in UUO-induced renal oxidative stress and inflammation in OVX female rats. The main findings were that daidzein and E2 alone and in co-treatment with A779 or losartan, improved the pathology of inflammation and glomerulosclerosis

in the obstructed kidney, balanced the circulatory and kidney redox state by decreasing serum and tissue MDA and increasing serum and tissue TAC and antioxidant enzymes, reduced reactive nitrogen species (RNS) of protein carbonyl and NOx concentration in the serum and UUO kidney, and reduced the expression of inflammatory cytokines TNF-α, IL-1β, and IL-6 and increased the expression of anti-inflammatory cytokine IL-10.

A significant increase in hydrostatic pressure in UUO triggers ROS and RNS production, the release of cytokines, and inflammatory cell infiltration, leading to extracellular matrix metalloprotein (EMT) expression and myofibroblast activation and expression of fibrogenic genes.[4,36] Several studies have proved a positive feedback loop between ROS, inflammatory cytokines, and signaling pathways such as RAS and TGF-β1 in the progression of renal fibrosis.[4,36,37] In the present study, UUO increased oxidative stress, inflammation, and glomerulosclerosis in OVX female rats by elevation

of MDA, protein carbonyl, NOx, and inflammatory cytokines and reduction in TAC, antioxidant enzymes, and anti-inflammatory cytokine IL-10.

The classical and non-classical RAS axes have important functions in the progression and development of renal fibrosis.[36] Ang II, the main peptide of the classical RAS axis, triggers ROS activation and inflammation through a positive loop with TGF-β1, inducing remodeling,[38,39] but Ang1-7 counteracts these effects.[40, 41] In the present study, UUO and the blockade of Mas receptors by A779 revealed the highest renal injury and inflammation score and serum and tissue MDA levels and the lowest serum and tissue antioxidant capacity and antioxidant enzyme activity (Figures 1–4, UUO + Veh. group). Conversely, UUO and the blockade of AT1 receptors by losartan produced the lowest renal injury and inflammation scores and the highest serum TAC, SOD, and catalase levels (Figures 1-3, UUO + Veh. group). While treatment with E2 and daidzein almost completely inhibited the effect of UUO on renal injury and inflammation, daidzein seems to be more effective than E2 (Figure 2, UUO + daidzein and UUO + E2 groups). Reduction of serum and kidney tissue MDA, protein carbonyl and NOx, and raising of antioxidant balance are associated with these improving effects (Figures 3-5). Again, A779 worsened the situation, but losartan helped to improve the situation in some aspects. It seems that the antioxidant and anti-inflammatory activity of daidzein and E2 have prominent effects because their combination with losartan or with A779 potentiates or reduces their effects, respectively, but in only some aspects. Investigating the effects of E2 and daidzein on the expression of AT1 and Mas receptors in the kidney tissue could help further clarify the nature of the interaction of E2 and daidzein with these classical and non-classical angII receptors.

In addition to the above mechanisms of daidzein and E2 in inhibition of renal injury and inflammation, assessing the expression of inflammatory and anti-inflammatory cytokines in UUO kidney tissue revealed that TNF-α, IL-1β, and IL-6 were highly up-regulated, and anti-inflammatory cytokine IL-10 was highly down-regulated by the process of UUO-induced renal injury (Figure 6). Daidzein (and E2) significantly recovered the up-regulation of inflammatory cytokines and reversed the down-regulation of IL-10. Except for IL-1β, for the other

three cytokines, daidzein was more effective than E2. Co-treatment with losartan seems to increase the improving effects of daidzein on the expression of inflammatory cytokines (Figure 6).

Inconsistent with our study, in UUO mice, disruption of the ACE2 gene impaired the ACE2/Ang 1–7 axis but enhanced the ACE/Ang II/AT1 axis leading to activation of the NF-κB pathway and expression of renal TNF-α, IL-1β, and MCP-1.[42] However in that study the effect of daidzein and E2 and their interaction with AT1 and Mas receptors were not investigated. More recently, in 2020, in two animal models of kidney damage by cisplatin and diabetes, treatment with daidzein suppressed the NF-κB pathway, NOx2, TNFα, MCP-1, and IL6 production, and lipid peroxidation but increased antioxidant enzyme activities.[18,43] These findings follow the antioxidant and anti-inflammatory effects of daidzein found in our UUO model in ovariectomized rats. Also, in the mice model of angiotensin II-induced abdominal aortic aneurysm, daidzein exhibited anti-inflammatory and antioxidant effects via decreasing pro-inflammatory cytokines and increasing anti-inflammatory cytokines.[44] Although losartan and A779 monotherapy showed significant effects on kidney injury, inflammation, and related oxidative stress and inflammatory factors, the powerful effects of daidzein and E2 masked the effects of losartan and A779 to some extent when they were used in combination with daidzein and E2.

CONCLUSION

According to the present study, daidzein and E2 improve renal oxidative stress and inflammation in the OVX (postmenopausal model) rats by augmenting the redox system and anti-inflammatory cytokine balance. Our results suggest that MasR agonist(s) may be more beneficial than ATIR blockers in protection against renal fibrosis. However, more studies are needed to clarify the interaction of daidzein and RAS receptors. Daidzein therapy is recommended as a natural alternative to estrogen hormone replacement therapy (HRT) in postmenopausal or older women against renal dysfunction.

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