

## Preventive Role of Estradiol on Kidney Injury Induced by Renal Ischemia-Reperfusion in Male and Female Rats

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**How to cite this article:** Iran-Nejad A, Nematbakhsh M, Eshraghi-Jazi F, Talebi A. Preventive role of estradiol on kidney injury induced by renal Ischemia-Reperfusion in male and female rats. *Int J Prev Med* 2015;6:22.

### ABSTRACT

**Background:** Renal ischemia-reperfusion (RIR) is the main cause of renal failure. The incidence of RIR injury seems to be gender-related due to female sex hormone; estrogen. This study was designed to investigate the protective role of estrogen against RIR injury in male and ovariectomized female rats.

**Methods:** Thirty-nine Wistar rats were used in this study as male and ovariectomized female rats in the sham-operated, RIR, and estradiol-treated plus RIR groups. The RIR was induced by clamping the renal vessels for 45 min and then 24 h of reperfusion. All animals finally were sacrificed for the measurements.

**Results:** The serum levels of creatinine and blood urea nitrogen and kidney tissue damage score significantly increased in both male and female RIR rats ( $P < 0.05$ ). Estradiol however significantly attenuated these parameters ( $P < 0.05$ ) toward normal levels in female ( $P < 0.05$ ), but not in male rats. Kidney weight increased in both genders and estradiol intensified it in the male rats ( $P < 0.05$ ). Uterus weight was increased by estradiol in female rats ( $P < 0.05$ ) and testis weight did not alter in male rats.

**Conclusions:** Estradiol demonstrated a protective role against RIR injury in female rats; however, estradiol as an antioxidant could not protect the male kidney from RIR injury.

**Keywords:** Estrogen, gender, rat, renal ischemia-reperfusion

## INTRODUCTION

Acute renal failure is an important disturbance in many patients<sup>[1]</sup> and renal ischemia-reperfusion (RIR)

injury is the most common cause of acute renal failure. RIR injury induces renal dysfunction characterized by increasing levels of creatinine (Cr) and blood urea nitrogen (BUN).<sup>[2-4]</sup> In addition, studies have shown that the incidence of renal diseases is gender-related, and males exhibit a more rapid reduction in renal function than females.<sup>[5,6]</sup> Mature male rats are more vulnerable to RIR injury than female rats, and females have higher survival rates than males.<sup>[7]</sup> Sex hormones; testosterone and estrogen play an important role in this phenomenon.<sup>[8-13]</sup> Studies on experimental animal models have shown that male and female sex hormones affect renal injury.<sup>[13-18]</sup> In

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10.4103/2008-7802.153537

addition, it is reported that estradiol replacement therapy attenuates renal dysfunction in diabetic ovariectomized female rats.<sup>[19]</sup> Estradiol therapy in brain injury in rats provides a protective effect against ischemia<sup>[20,21]</sup> and improves spatial learning and memory.<sup>[21]</sup> In addition, administration of estradiol ameliorates liver dysfunction in aging male rats<sup>[22]</sup> while estradiol did not protect the kidney against confers protection nephrotoxicity.<sup>[23-25]</sup> To the best of our knowledge, the role of estradiol in RIR injury in males and females was not compared yet. Accordingly, this study was designed to determine whether estradiol could prevent RIR-induced renal injury in male and ovariectomized female rats.

## METHODS

### Animals

Sixteen adult female (weighing  $175 \pm 5.2$  g) and 19 adult male (weighing  $222.4 \pm 8.6$  g) Wistar rats (Animal Center, Isfahan University of Medical Sciences, Isfahan, Iran) were used in this study. The animals were housed under standard conditions with 12 h light/12 h dark cycle. Prior to the experiment, the protocols were confirmed to be in accordance with the Guidelines of Animal Ethics Committee of the Isfahan University of Medical Sciences.

### Ovariectomy surgery

One week before estradiol administration, all female rats were anesthetized, and an incision was made in the abdominal middle line above the urinary outlet. The ovaries were removed, and the skin was sutured.

### Experimental protocol

The animals were randomly divided into six experimental groups;

- Group 1 ( $n = 5$ , the sham-operated group): Male rats received sesame oil (intramuscularly) and after 3 days underwent the surgical procedure without RIR surgery
- Group 2 ( $n = 8$ ): Male rats received regimen the same as group 1 and underwent RIR surgery
- Group 3 ( $n = 6$ ), male rats received single dose of estradiol ( $500 \mu\text{g}/\text{kg}$ ; intramuscularly) and 3 days later underwent RIR surgery
- Groups 4, 5, and 6 ( $n = 5, 6, \text{ and } 5$ ): Ovariectomized female rats received treatment/regimen the same as groups 1, 2, and 3, respectively.

### Renal ischemia-reperfusion surgery

To induce the RIR model, the animals were anesthetized by ketamine ( $75 \text{ mg}/\text{kg}$ , i.p.) and xylazine ( $10 \text{ mg}/\text{kg}$ , i.p.). Two small incisions were made on the flanks and when the kidneys were visible, the kidney arteries and veins on both sides were clamped to stop the renal blood circulation. After 45 min, the clamp was removed for recirculation of blood flow. 24 h after the RIR surgery, all rats were anesthetized again. Blood samples were

obtained via heart puncture. Serum samples were removed and stored at  $-20^\circ\text{C}$  until measurement. Finally, all animals were killed, and kidneys and testes or uterus were removed and weighed immediately. In addition, the left kidney was fixed in 10% formalin solution for pathological assessments.

### Measurements

Serum levels of Cr and BUN were measured using quantitative kits (Pars Azmoon, Iran) and automatic analyzer RA-1000 (Technicon, Ireland).

### Histopathological procedures

The left kidney was fixed in 10% formalin solution and embedded in paraffin. The tissue slices were stained by hematoxylin and eosin to examine the tissue damage based on the presence of tubular atrophy, hyaline cast, ischemic necrosis, vacuolization, and debris. According to the damage intensity, the samples were scored as 1–4 and 0 was assigned to normal tissue.

### Statistical analysis

The data were reported as mean  $\pm$  standard error of the mean. The uterus, kidney, and testis weights; as well as BUN and Cr levels in the ischemia group were compared with the values obtained for the ischemia plus estradiol group or the sham-operated group using the Student's *t*-test. To compare the groups with regard to the kidney tissue damage score (KTDS), the Mann–Whitney test was applied.  $P < 0.05$  was considered as statistically significant.

## RESULTS

### Serum creatinine and blood urea nitrogen levels

Renal ischemia-reperfusion injury increased the serum Cr and BUN levels in both genders ( $P < 0.05$ ). However, estradiol decreased these parameters toward normal values in female, but not in male rats ( $P < 0.05$ ) [Figures 1 and 2].

### Kidney tissue damage score and kidney weight

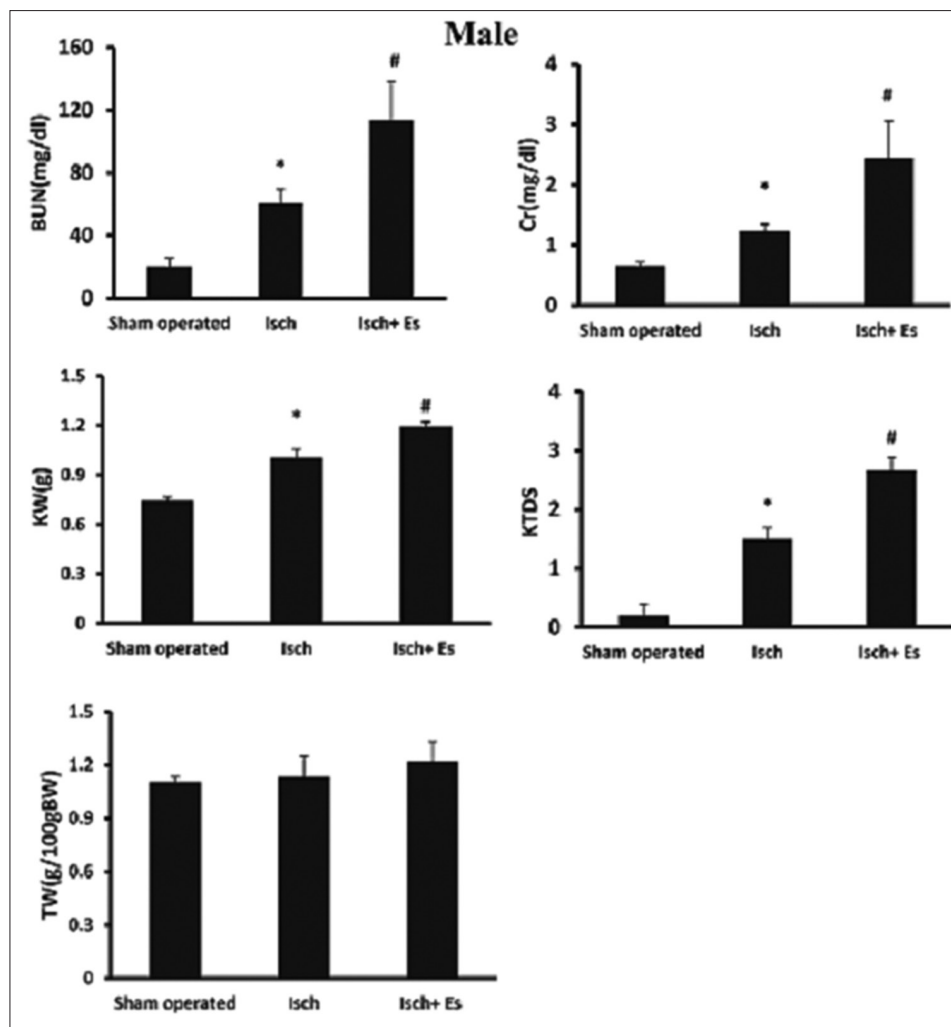
Renal ischemia-reperfusion increased kidney weight in both genders ( $P < 0.05$ ) and estradiol intensified kidney weight in male ( $P < 0.05$ ), but not in female rats. In addition, estradiol ameliorated KTDS induced by RIR in female, but not in male rats ( $P < 0.05$ ) [Figures 1-3].

### Testis and uterus weights

Administration of estradiol increased uterus weight ( $P < 0.05$ ). However, it did not alter the testis weight [Figures 1 and 2].

## DISCUSSION

The present study was performed to compare the protective effect of estradiol against RIR-induced renal injury in male and female rats. The results obtained



**Figure 1:** Serum creatinine (Cr) and blood urea nitrogen (BUN) levels, testis weight (TW) and kidney weight (g)/100 g bodyweight (KW), kidney tissue damage score (KTDS) in the male groups. Abbreviations of Isch and Isch + Es were applied for ischemia and ischemia + estradiol. \*and# indicates significant differences between the sham-operated and Isch groups, and between the Isch and Isch + estradiol groups, respectively

show that RIR induces renal dysfunction in both male and female rats; characterized by increasing serum BUN and Cr levels. These results were in agreement with the findings of other studies.<sup>[2,3,26]</sup> RIR-induced renal failure increases oxidative stress, inflammation, and apoptosis by increasing the malondialdehyde, interleukines, and tumor necrosis factor-alpha levels, respectively.<sup>[27]</sup> Estrogen may protect renal and cardiovascular systems by affecting glomerular mesangial cells in the kidney and smooth muscle cells in the vasculature<sup>[28]</sup> and improve renal and vascular damage induced by renal ischemia in atherosclerotic female mice through antiinflammatory cytokine, interleukine-6.<sup>[29]</sup> Furthermore, administration of estradiol decreased albuminuria, glomerulosclerosis and tubulointerstitial in spontaneously hypertensive stroke-prone female rats.<sup>[30]</sup> In addition, the protective effects of estrogen were exhibited via nitric oxide synthase system,<sup>[16]</sup> antioxidant,<sup>[31]</sup> and inflammatory<sup>[32]</sup> properties. It is possible that the estrogen ameliorates renal blood flow

diminished by ischemia due to activation of endothelial nitric oxide synthase and nitric oxide production.<sup>[16]</sup> Estrogen protects kidney against RIR through suppression of endothelin 1 overproduction.<sup>[17]</sup> Also, it is possible that the presence of progesterone in the female gender plays a role in ameliorating RIR injury. It has been reported that progesterone has neuroprotective effects<sup>[33]</sup> and in combination with estradiol improves the brain ischemia-induced injury<sup>[34,35]</sup> and ameliorates bone loss in female rats.<sup>[36]</sup> In the present study, administration of estradiol not only did not ameliorate kidney dysfunction in male gender, but also intensified it. It is demonstrated that administration of estradiol (10  $\mu\text{g}/\text{kg}$ ) attenuated renal damage induced by bilateral RIR, while this was not the case at the doses of 20 and 100  $\mu\text{g}/\text{kg}$ .<sup>[16]</sup> In another study, high mortality rate in male pretreated with estradiol was reported, while the course of ischemia-induced renal failure improved.<sup>[7]</sup> It seems that estradiol administration led to some unusual results<sup>[37]</sup> with an unknown

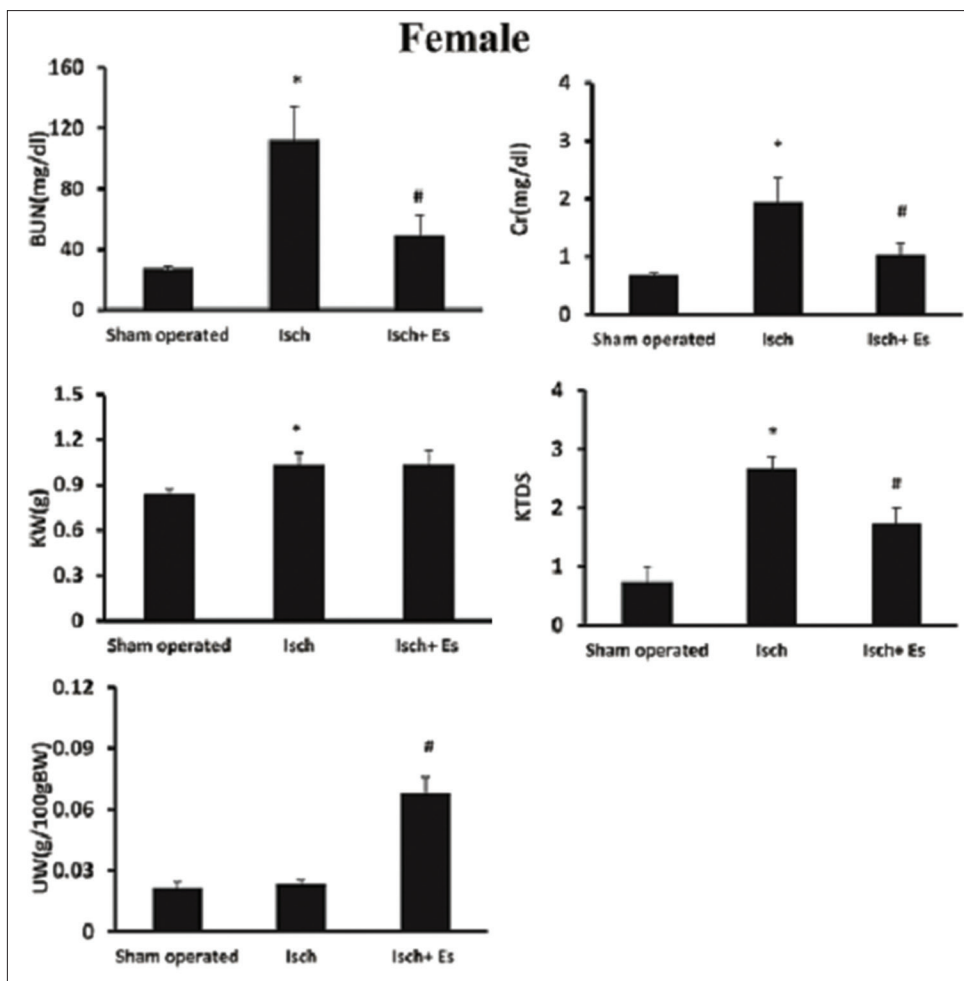


Figure 2: Serum levels of creatinine (Cr) and blood urea nitrogen (BUN) levels, uterus weight (UW) and kidney weight (g)/100 g body weight (KW), kidney tissue damage score (KTDS) in female groups. Abbreviations of Isch and Isch + Es were assigned to ischemia and ischemia + estradiol. \*and# indicates significant differences between the sham-operated and Isch groups, and between Isch and Isch + estradiol groups, respectively

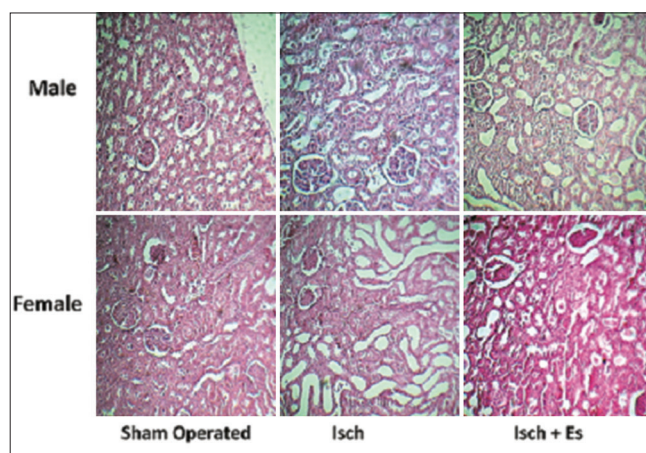


Figure 3: The pathology images (x100) of kidney tissue in the experimental groups. Abbreviations of Isch and Isch + Es were assigned to ischemia and ischemia + estradiol

mechanism. Administration of estradiol also attenuates vascular responses against acetylcholine in women but not men,<sup>[38]</sup> and estrogen supplementation improves

endothelium-related dilation through increasing level of nitrite/nitrate in women, but not in men.<sup>[39]</sup> Our study showed that normalized kidney weight was increased by RIR injury in both genders. This observation was consistent with the findings of other studies,<sup>[2,3,40]</sup> which is probably due to edema.<sup>[40]</sup> Administration of estradiol intensified normalized kidney weight in male gender; confirmed by renal dysfunction and pathological findings. Estradiol also increased normalized uterus weight, confirmed by our recent studies.<sup>[23,25]</sup> It has been demonstrated that estrogen increases uterus weight through increasing the glucose metabolism, activity of RNA polymerase, DNA content, and finally cell proliferation.<sup>[41]</sup>

### CONCLUSIONS

It was concluded that administration of estradiol could attenuate renal injury induced by RIR in female rats; however, as an antioxidant agent, it was not efficient in preventing renal dysfunction induced by RIR in male rats.

## ACKNOWLEDGEMENTS

We appreciate Isfahan University of Medical Sciences for supporting this project.

Received: 01 Oct 14 Accepted: 26 Dec 14

Published: 20 Mar 15

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**Source of Support:** This research was supported by Isfahan University of Medical Sciences, **Conflict of Interest:** None declared.