Nanoselenium attenuates renal ischemia-reperfusion injury in rats

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Abstract

Using selenium nanoparticles has received attention in recent years because of their therapeutic benefits due to their anticancer, antioxidant, anti-inflammatory, and anti-diabetic effects. This research was conducted to evaluate the possible protective impact of nano-selenium (Nano-Se) on renal ischemia/reperfusion (I/R) injury using an animal model. Using clamping left renal pedicle within 45 min, I/R injury was induced. The animals were randomly divided into nine groups of control, nano-Se (0.25, 0.5, and 1 mg/kg) alone, I/R control, and I/R rats administrated with nano-Se. Thirty days aftertreatment, animals were sacrificed to be assessed biochemically and histopathologically. Nano-Se in I/R groups significantly decreased serum creatinine, urea levels, renal histological damage, and increased antioxidant status. Also, our findings demonstrated that the administration of nano-Se caused a significant increase in the expression of epidermal growth factor (EGF) in kidney tissue of I/R rats. Therefore, nano-Se possesses renoprotective effects, and this effect might be attributable to its antioxidant and free radical scavenger effects. These renoprotective effects may depend on the increased EGF expression level in kidney tissue and improved the structure of the kidney tissue. Thus, our research provided biochemical and histological data supporting the potential clinical use of nano-Se for the treatment of certain kidney disorders.

Introduction

Renal ischemia-reperfusion (I/R) injury is commonly observed in clinical practice and results in high morbidity and mortality. Improving the capability of organs to deal with ischemic injury is of great importance. Kidney I/R injury is defined by the restricted blood supply to the kidney leading to restricted blood flow and re-oxygenation. It also is characterized by tubular necrosis and apoptosis, energy metabolism abnormality, inflammation, and oxidative stress [1, 2]. It can cause structural and functional injury affecting renal tubules through a direct induction death of tubular cells resulting in triggering damaged reactions [3]. Many antioxidant and anti-inflammatory compounds attenuate renal injury caused by I/R injury [4–7].

Selenium (Se) is an essential trace element with pleiotropic effectiveness for human health, including antioxidants [8, 9], anti-inflammatory [10], and anti-cancer [11] effects, as well as preventing cancer initiation, growth, and metastasis with no toxicity [12–15]. Nanoparticles (NPs) have been extensively applied as medications for treating many disorders and improving human health. Selenium nanoparticles (nano-Se) are used as innovative sources of Se with favorable in vivo bioavailability with a lower rate of selenium toxic effects [16]. SeNPs has an LD50 rate of 113 mg Se/Kg, whereas it is 15 mg Se/Kg for sodium selenite [17]. It has been reported that nano-Se has antibacterial [18, 19], anticancer [20, 21], antioxidant [22], anti-inflammatory [12] activities. This research was conducted to clarify whether an administration of nano-Se can be beneficial in attenuating renal I/R injury in a rat model.

Materials And Methods

Chemicals

Nano-Se in the size range of 20–60 nm was purchased from Pishgaman and morphology was near-spherical. Zellbio Company (Germany) supplied the antioxidant enzyme kit. The epidermal growth factor (EGF) immunohistochemical (IHC) kit was supplied by Dako (US). Other chemicals were of an analytical grade.

Animals

Fifty-four Wistar rats (200–230 g) were obtained from the Pasteur Institute Iran and kept in the animal house (temperature: 25 ± 1 ˚C; humidity: 55 ± 5%) where food and water were accessible. All experimental procedures were done based on the Guidelines for the Care and Use of Laboratory Animals (National Institute of Health, No. : 85 – 23, revised: 1996) and confirmed by the Animal Ethics Committee of the University (IR.IAU.SRB.REC.1398.137). Humane endpoints were used according to the NC3Rs guidelines for all animals in the study.

Renal I/R induction

Following 12 h fasting, animals were subjected to surgery using ketamine hydrochloride and xylazine (100 and 5 mg/kg, i.p., respectively). We sterilized their abdomens using povidone-iodine. The abdominal area of the sham group was closed with no more procedures. Following a midline incision, the ischemia was induced through bilateral renal pedicle clamping within 45 min by smooth vascular clamps. Then, the clamps were removed and the kidneys were observed to find blood flow restoration. Their abdomens were closed in two layers. The rats were injected with 50 ml/kg of warm saline instilled into their abdominal cavities thorough the surgery and they were allowed to recover.

Experimental design

Animals were randomly assigned to nine groups of 6 rats:

Group I (healthy control): Rats receiving distilled water (DW); group II (sham-operated): Rats undergoing a sham operation and receiving DW; groups III-V (experimental healthy): Healthy rats that were administrated with nano-Se at doses of 0.25, 0.5, and 1 mg/kg b.w. group VI (I/R control): I/R rats that were administrated with DW; and groups VII-IX (experimental I/R): I/R rats receiving nano-Se at 0.25, 0.5, and 1 mg/kg b.w.

Treatment continued for 30 consecutive days. The nano-Se concentration was measured according to a study by Dkhil et al [16]. After the experimental procedures, animals were sacrificed followed by immediate blood and renal sample collection and freezing at -70°C until analysis. Kidney tissue specimens were divided into two parts: one part was considered for determination of stress oxidative parameters, while the other was fixed immediately for histological studies.

Assessment of biochemical parameters

After the experimental period, blood sampling from the heart and serum was done. Serum creatinine and urea levels were applied as an index for renal (glomerular) function [23]. The kidney samples were weighed for 100 mg and homogenized using phosphate buffer (2 mL). We then centrifuged the kidney homogenate (5000 rpm/ 20 min/ 4°C) and transferred the supernatant to eppendorf to maintain at -80°C. The catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX) activities as well as the malonaldehyde (MDA) level were determined from renal homogenate using commercial kits following the manufacturer's instructions.

Histological evaluation

The kidneys were kept in phosphate-buffered formalin (10%), followed by chopping into small sections, embedding in paraffin, cutting (3-μm sections), and staining by hematoxylin and eosin. Histopathological alterations were assessed regarding tubular necrosis, tubular degeneration, and inflammatory cell infiltration [24].

Tissue injuries were graded as follows: 0 = not at all, 1 = 0–25%, 2 = 26–45%, 3 = 46–75%, and 4 = 76–100% measured by an observer blind to the treatments.

Immunohistochemical staining

The blocks (5 μm) were sent to the IHC laboratory and stained with EGF antibodies as follows: after deparaffinization of the kidney tissues, they were incubated for 10 min in a methanol solution containing hydrogen peroxidase for inactivating endogenous peroxidase activity and washed in phosphate-buffered saline (PBS). Next, incubation of the kidney tissues was done for 10 min in an antigen retrieval solution for eluting the antigens and washing in PBS. We added a protein block solution for preventing nonspecific binding followed by washing in PBS. Following incubation, using the primary and secondary antibodies (EGF, Abcam, USA), 3.3'diaminobenzidine chromogen was applied and counterstained with hematoxylin. The IHC data were interpreted through a light microscope (Olympus, Germany): 10 fields were selected in a random manner and 100 epithelial cells were calculated at 400× magnification. The rate of nuclear immunoreactivity was stated as %: 0 = not at all, 1 = 1–35%, 2 = 37–65%, and 3 = 66–100% [25].

RT-PCR analysis

The part of kidney tissue (~ 10 g each) was snap-frozen in liquid nitrogen, and stored at -80°C. Total RNA extraction was done with an GeneJET RNA Purification Kit (Thermo Scientific, #K0731) based on the manufacturer’s guideline. A rotor-stator homogenizer was used to homogenize the kidney tissue until the specimen was uniformly homogeneous. The RNA concentration was assessed by a NanoDrop (DeNovix DS-11 FX). A total RNA specimen (2 μg/sample), oligo deoxythymidine primer (20 pmol), deoxynucleotide triphosphate mix (5 mM each, 2 μl), and reverse transcriptase (Omniscript Reverse Transcriptase, Qiagen, 1 μl) were applied in a 20-μl scale for generating cDNA. The primers were used for gene expression, including *EGFR* forward, 5'-GACAGCTATGAGATGGAGGAA – 3' and reverse, 5'-GAGTCACCCCTAAATGCCA-3' and β-actin gene (used as reference gene) forward, 5'-TCCTTCCTGGGCATGGAGT-3' and reverse, 5'-

AAAGCCATGCCAATCTCATC-3' [26]. Then, 10 µl of SYBR Green PCR Master Mix (Applied Biosytems), cDNA (4µl), and primers (2 pmol each) were employed for real-time PCR with a total volume of 20 µl. The reaction was conducted at 95° C/ 15 sec and 60°C, 1 min for 40 cycles and then denaturation was done at 95°C for 10 min. PCRs were carried out in triplicate for each specimen. The cDNA levels were measured through the approved curve of cycle thresholds. The data related to each cDNA were within the related standard curve and the data were normalized to β-actin cDNA.

Statistical analysis

Values are introduced as mean ± SEM. Data analysis was done by SPSS-23 through a one-way analysis of variance (ANOVA) and the Tukey test at $P < 0.05$.

Results

Effects of nano-Se on biochemical parameters

The serum levels of creatinine and urea showed a significant increase in the I/R control animals ($p < 0.001$). Also, treatment with nano-Se (0.5 and 1 mg/kg) significantly lowered their levels in the I/R rats dose-dependently ($p < 0.001$). Nonetheless, normal rats treated only with nano-Se showed no significant changes (Table 1). The CAT, GPX, and SOD activities in the kidney tissue of I/R control animals showed a significant decrease compared with the normal controls ($p < 0.001$). Administration of nano-Se (0.5 and 1 mg/kg) resulted in a significant enhancement in the activities of these enzymes than the I/R control rats ($p < 0.001$). No significant changes were observed in normal rats treated only with nano-Se. The MDA levels in the renal tissue of I/R control animals were significantly more than the normal controls ($p < 0.001$). Administration of the rats with nano-Se (0.5 and 1 mg/kg) led to a significant reduction in the MDA concentratins than the I/R control animals ($p < 0.001$) (Table 2).

Effects of nano-Se on histopathological indices

Histopathological findings revealed no tubular degeneration, necrosis, or inflammation in normal control rats, sham-operated group, and those receiving nano-Se alone. Severe renal tubules necrosis and inflammatory cell infiltration were found in I/R control rats. Our results showed that the treatment with nano-Se (0.5 and 1 mg/kg) resulted in a significant decrease in tubular necrosis and inflammation in I/R rats (Fig. 1 and Table 3).

Effects of nano-Se on EGF expression

Our results showed that EGF expression significantly elevated in I/R rats in comparison with the normal control animals, while the treatment with nano-Se (0.5 and 1 mg/kg) significantly reduced EGF expression in the I/R rats. No significant changes were observed in normal rats treated only with nano-Se (Fig. 2 and Table 4).

Effect of nano-Se on EGFR expression

EGFR expression increased significantly in the renal homogenate tissue of I/R control group in comparison with the normal control group ($P < 0.001$). However, nano-Se (0.5 and 1 mg/kg) caused a significant reduction in the EGFR expression in the I/R rats than the I/R control rats ($P < 0.001$) (Fig. 3).

Discussion

Our results showed that the administration of nano-Se produced beneficial effects in the animal model of I/R injury in terms of reduced renal damage and improved renal function. These renoprotective effects may depend on increased antioxidant status and decreased MDA levels as well as increased expression EGF in kidney tissue and improved architecture kidney tissue.

Through the I/R induction, after blockage of the aorta or renal pedicle, blood flow restoration to ischemic tissue can exacerbate the injury of the kidneys [27]. The renal I/R injury affects different mediators, such as inflammation, oxidative stress, and activation of adhesion molecules, leading to inflammation, renal tubular damage, endothelial dysfunction, and apoptosis [28, 29]. Currently, anti-apoptotic and anti-oxidative stress agents can inhibit a decrease in renal function and tubular damage. We found that the kidney protection by nano-Se was assessed as a possible therapeutic agent regarding renal I/R injury.

The I/R rats were found with a remarkable elevation in the serum creatinine and urea concentrations as well as a substantial enhancement in renal damage score evidenced by histopathological tests than the control group, which is consistent with other findings [30, 31]. I/R injury leads to the lack of cytoskeletal integrity, cell polarity, as well as collapsing the proximal tubule brush border. After severe damage, viable and nonviable cells can be desquamated and leave the regions, at which the only barrier is the basement membrane separating the filtrate and the peritubular interstitium [32]. Our data indicated that the administration of nano-Se significantly decreased the serum creatinine and urea levels and attenuated renal tissue damage in the I/R-exposed rats. In agreement with the present results, it has been reported that nano-Se was effective in reducing the effects of diabetic neuropathy [33]. Kumar et al [34] reported that nano-Se was effective to lower the BUN, creatinine, fibronectin, and collagen concentrations and elevate the albumin concentration in diabetic rats. Histological data confirmed these protective effects by SeNPs. Also, Kojouri and Sharifi [35] showed that nano-Se significantly improved serum BUN and creatinine alterations after intense exercise in donkey and claimed that the SeNPs effectiveness can be associated with the Se incorporation into proteins, like selenocysteine as well as its preventive effect on tissue oxidative damages.

Our data indicated that the CAT, GPX, and SOD activities reduced, while MDA concentration increased in I/R rats. In agreement with our data, it is reported that stimulating oxidative stress and deteriorating the systemic reactions cause remote organ dysfunction due to I/R [36]. Oxidative stress is a crucial mechanism of I/R-induced renal injury [37, 38]. CAT can decompose hydrogen peroxide leading to the protection of the tissues against hydroxyl free radicals [39]. SOD and GPX are able to convert superoxide

to peroxide followed by H2O and O2 that inhibit ROS generation and the chain reaction of lipid peroxidation (LPO) [40]. MDA as the final product of LPO reflects the sensitivity of LPO and indirectly represents the degree of cell damage [41]. Consistent with reported results [42], our data indicated that the administration of nano-Se significantly increased CAT, GPX, and SOD activities and reduced the MDA concentration in the kidney of I/R-exposed rats. These findings suggest that nano-Se elevated the antioxidant status of the kidney for counteracting oxidative stress due to I/R. Many reports indicated that the effective role of SeNPs supplementation in animals exposed to oxidative stress [43–45] or toxic environments [46]. It is found that nano-Se significantly increased plasma GPX activity in mice; however, showed a lower toxic effect than selenite. We demonstrated that the nanoscale administration of Se, as an antioxidant, can be done with a lower risk of toxic effect [47–49]. Also, SeNPs inhibited oxidative stress via the prevention of GSH depletion. Nano-Se possibly elevated the activity of antioxidant selenoenzyme GPX thioredoxin reductase (TrxR) leading to up-regulating other antioxidant enzyme systems and preventing oxidative stress to body tissues [15, 50, 51].

I/R rats' kidneys were found with morphological alterations, including extensive degeneration of tubular architecture, tubular cell necrosis, and inflammation. It has been reported that I/R induced shedding of the brush border and tubular epithelial cells from the basement membrane, tubular cell necrosis, and intratubular cast generation, particularly in the outer medulla [52]. Our results showed that administration with nano-Se led markedly reduced the histological characteristics of kidney damage, including focal and mild tubular degeneration and necrosis. Nano-Se possibly protects the tubular epithelium from reperfusion damage. In addition, nano-Se alone is not effective in kidney morphology.

Our results showed that the administration of nano-Se significantly increased the expression of EGF in kidney tissue of I/R rats. EGF (derived from pre-pro-EGF), as a 53-amino acid protein, is involved in the proliferative reaction of tubular regeneration of the kidney. It is produced in the medullary thick ascending limb (mTAL) areas of Henle as well as the distal convoluted tubule [53, 54]. Delayed recovery after I/R-related kidney injury can be due to damages to and necrosis of kidney cells generating EGF in the mTAL tubules. Other reparative or survival growth factors produced in the distal nephron, such as EGF, IGF-1, and HGF can exhibit paracrine impacts for protecting the sensitive proximal tubule against damage and promoting proliferation and repairment of surviving proximal tubules cells by distal-proximal cell-to-cell cross-talk mechanisms [55, 56]. It is claimed that the administration of some growth factors (HGF, IGF-1, and EGF) accelerates normalizing the kidney dysfunction in animal models of acute kidney injury [57, 58]. EGF has been shown to oppose apoptosis resulting from oxidative injury in the kidney [59]. Down-regulating the EGF expression has been announced in rat kidney tissues after treatment with tobramycin and cisplatin resulting in acute tubular necrosis in the proximal convoluted and straight tubules and regenerative hyperplasia [60, 61]. The reduced EGF, which characterizes chronic kidney disease in humans [62] and mice possibly represent the absolute and irretrievable loss of the tubular cells leading to producing and secreting EGF. EGFR plays an important role in renal biology from growth to homeostasis and damage repair [63]. EGFR activation in proximal tubular cells is involved in the recovery phase following acute kidney damage and phospho-EGFR is the most important upregulated receptor tyrosine kinase against renal I/R injury [64]. Because of the EGFR signaling involvement in nephrogenesis and the

mitogenic potential of adult proximal tubule cells, its role in renal repair following acute injury has been studied. There was an elevation in EGFR phosphorylation in the renal proximal tubules in several experimental models of acute kidney injury, such as I/R, aminoglycoside toxicity, and folic acid treatment [63–65].

The renal I/R injury may act by dysfunctions in regional blood flow, inflammation and caspase activation, endothelial and epithelial cell impairment, free radical generation, apoptosis, and necrosis [66, 67]. It is suggested that biogenic nano-Se has immune-stimulatory effects in breast cancer-induced mice. Treatment with nano-Se significantly increased the levels of cellular immunomodulatory components (such as IL-12, IFN-γ, and IL-2) while the levels of TGF-ß decreased in breast cancer-induced mice [68].

Conclusion

Based on the results obtained from the present research, nano-Se possesses renoprotective effects, and this effect might be attributable to its antioxidant and free radical scavenging effects. Accordingly, our research provided biochemical and histological data supporting the potential clinical use of nano-Se for the treatment of certain kidney disorders.

Declarations

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Author contribution to study

Farzaneh Sadeghmanesh: Visualization, Investigation

Akram Eidi: Conceptualization, Methodology, Writing-Reviewing and Editing, Formal analysis

Pejman Mortazavi: Conceptualization, Methodology, Writing- Reviewing and Editing

Shahrbanoo Oryan: Conceptualization, Methodology

All authors have read and approved the final version of the article and agree with the order of presentation of the authors

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Ethics approval

The Ethics Committee principles, and the Guide for the Care and Use of Laboratory Animals by the National Institute of Health (No. 85-23, revised in 1996) were considered and confirmed by the Animal Ethics Committee of the University (IR.IAU.SRB.REC.1398.137). Humane endpoints were used according to the NC3Rs guidelines for all animals in the study. Consent for publication Not applicable.

Competing of Interests

The authors declare that there is no conflict of interest**.**

Data availability

All data in this study are available from the corresponding author upon request.

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Tables

Table 1. Effect of nano-Se on serum parameters in the normal and I/R rats

Groups	Creatinine (mg/dL)	Urea (mg/dL)
Group I [Normal control]	0.56 ±0.016	55.17 ± 1.33
Group II [Sham-operated]	0.56 ± 0.019	50.50 ± 0.89
Group II [Normal + nano-Se (0.25 mg/kg)]	0.57 ± 0.019	51.83 ± 1.64
Group III [Normal + nano-Se (0.5 mg/kg)]	0.54 ± 0.008	51.67 ± 1.82
Group IV [Normal + nano-Se (1 mg/kg)]	0.56 ± 0.012	50.33 ± 0.84
Group V [I/R control]	0.84 ± 0.015 ***	73.33 ± 2.17 ***
Group VI [I/R + nano-Se (0.25 mg/kg)]	0.78 ± 0.011 ***	68.17 ± 1.78 ***
Group VII [I/R + nano-Se (0.5 mg/kg)]	0.68 ± 0.015 ***+++	62.83 ± 1.68 *+++
Group VIII [I/R + nano-Se (1 mg/kg)]	0.64 ± 0.014 *+++	59.33 ± 2.06 +++

Values are expressed as mean ± SEM for six rats.

* p<0.05, *** p<0.001 significantly different from the normal control group.

+++ P<0.001 significantly different from the I/R control group.

Table 2. Effect of nano-Se on antioxidant enzyme activities and MDA levels in the kidney tissue of normal and I/R rats

Groups	CAT (U/mg protein)	SOD (U/mg protein)	GPX (U/mg protein)	MDA (nmol/mg protein)
Group I [Normal control]	13.03 ± 0.28	18.37 ± 0.32	42.33 ± 1.05	0.34 ± 0.013
Group II [Sham-operated]	13.17 ± 0.47	18.53 ± 0.41	42.50 ± 0.76	0.36 ± 0.016
Group II [Normal + nano-Se (0.25 mg/kg)]	14.02 ± 0.29	19.00 ± 0.18	43.17 ± 0.95	0.34 ± 0.016
Group III [Normal + nano-Se (0.5 mg/kg)]	14.38 ± 0.22	18.87 ± 0.26	44.83 ± 0.54	0.32 ± 0.007
Group IV [Normal + nano-Se (1 mg/kg)]	14.52 ± 0.24	18.57 ± 0.39	45.00 ± 1.23	0.32 ± 0.011
Group V [I/R control]	7.62 ± 0.23 ***	9.80 ± 0.29 ***	23.17 ± 1.07 ***	0.64 ± 0.013 ***
Group VI [I/R + nano-Se (0.25 mg/kg)]	8.43 ± 0.34 ***	10.97 ± 0.23 ***	24.50 ± 0.76 ***	0.60 ± 0.027 ***
Group VII [I/R + nano-Se (0.5 mg/kg)]	10.25 ± 0.21 ***+++	12.68 ± 0.32 ***+++	28.83 ± 1.60 ***+	0.55 ± 0.015 ***++
Group VIII [I/R + nano-Se (1 mg/kg)]	10.63 ± 0.22 ***+++	12.56 ± 0.27 ***+++	32.83 ± 1.19 ***+++	0.50 ± 0.011 ***+++

Values are expressed as mean ± SEM for six rats. *** p<0.001 significantly different from the normal control group. + P<0.05, ++ P<0.01, +++ P<0.001 significantly different from the I/R control group.

Table 3. Effect of nano-Se on histopathological indices in the kidney tissue of normal and I/R rats

Groups		Tubular necrosis	Tubular degeneration	Inflammatory cell infiltration
Group I	[Normal control]	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Group II	[Sham-operated]	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Group II	[Normal + nano-Se (0.25 mg/kg)]	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Group III	[Normal + nano-Se (0.5 mg/kg)]	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Group IV	[Normal + nano-Se (1 mg/kg)]	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Group V	[I/R control]	2.17 ± 0.17 ***+++	3.33 ± 0.21 ***	1.50 ± 0.22 ***
Group VI	[I/R + nano-Se (0.25 mg/kg)]	1.33 ± 0.21 ***+++	2.83 ± 0.17 ***	1.33 ± 0.21 ***
Group VII	[I/R + nano-Se (0.5 mg/kg)]	1.17 ± 0.17 ***+++	1.33 ± 0.21 ***+++	1.17 ± 0.17 ***
Group VIII	[I/R + nano-Se (1 mg/kg)]	0.17 ± 0.16 +++	0.96 ± 0.18 ***+++	0.67 ± 0.21 *++

Values are expressed as mean ± SEM for six rats. *** p<0.001 significantly different from the normal control group. ++ P<0.01, +++ P<0.001 significantly different from the I/R control group.

Table 4. Effect of nanoselenium on EGF expression levels in normal and I/R rats

Groups		Level of EGF expression
Group I	[Normal control]	1.00 ± 0.00
Group II	[Sham-operated]	1.00 ± 0.00
Group II	[Normal + nano-Se (0.25 mg/kg)]	1.00 ± 0.00
Group III	[Normal + nano-Se (0.5 mg/kg)]	1.00 ± 0.00
Group IV	[Normal + nano-Se (1 mg/kg)]	1.00 ± 0.00
Group V	[I/R control]	2.83 ± 0.17 ***
Group VI	[I/R + nano-Se (0.25 mg/kg)]	2.67 ± 0.21 ***
Group VII	[I/R + nano-Se (0.5 mg/kg)]	1.67 ± 0.21 *+++
Group VIII	[I/R + nano-Se (1 mg/kg)]	1.33 ± 0.21 +++

Values are expressed as mean ± SEM for six rats.

* p<0.05, *** p<0.001 significantly different from the normal control group.

+++ P<0.001 significantly different from the I/R control group.

Figures

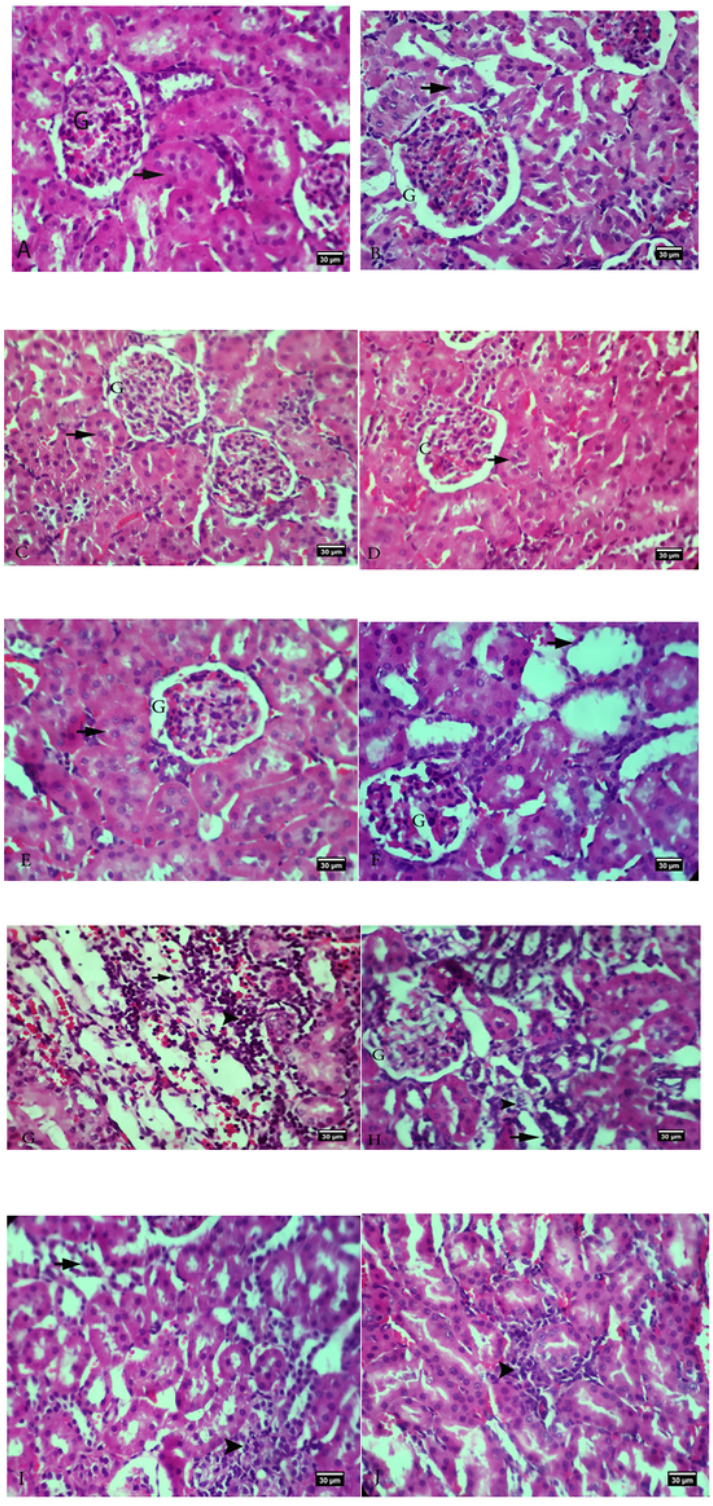

Figure 1

Renal histopathology in all experimental groups. A, Normal control group, B, sham-operated group, C-E experimental normal groups receiving nano-selenium (nano-Se) at 0.25, 0.5, and 1 mg/kg body weight with normal renal structure with normal glomeruli (G) and normal renal tubules (arrow) ; F-G, ischemia/reperfusion (I/R) control rats with severe renal tubules necrosis (arrow) and inflammatory cells infiltration(arrowhead); H-J, experimental I/R groups receiving nano-Se at 0.25, 0.5, and 1 mg/kg body weight with moderate to mild tubular necrosis (arrow) and inflammation(arrowhead) (H&E).

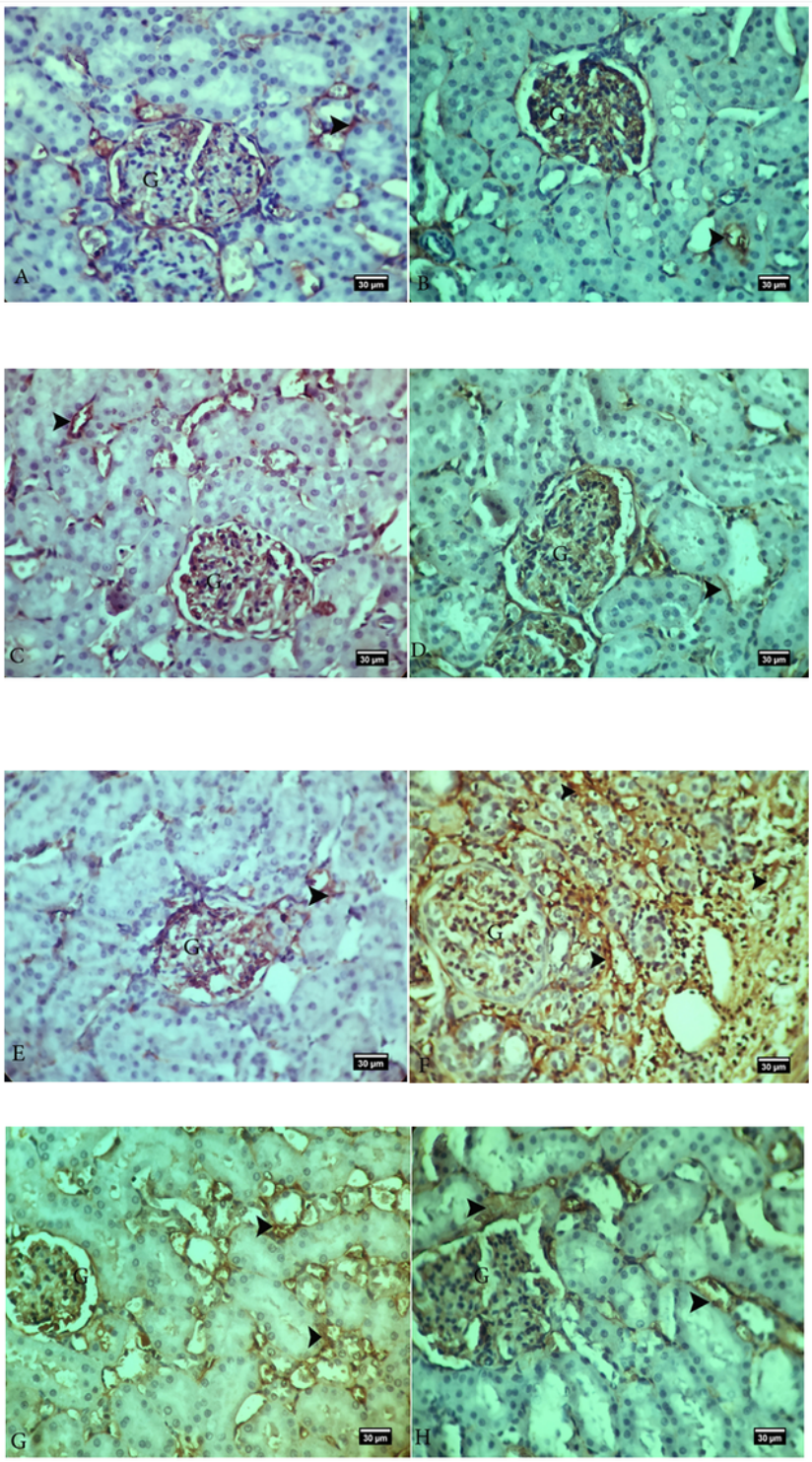

Figure 2

EGF expression in renal tissue in all experimental groups. A, normal control group; B sham-operated group; C-E experimental normal groups receiving nano-selenium (nano-Se) at 0.25, 0.5, and 1 mg/kg body weight with normal renal structure with normal glomeruli (G) and mild expression of EGF in glomeruli and renal tubules (arrowhead); F, ischemia/reperfusion (I/R) control rats with a severe expression of EGF in

renal tubules (arrowhead); G-I experimental I/R groups receiving nano-Se at 0.25, 0.5, and 1 mg/kg body weight with moderate to a mild expression of EGF in renal tubules (arrowhead) (IHC).

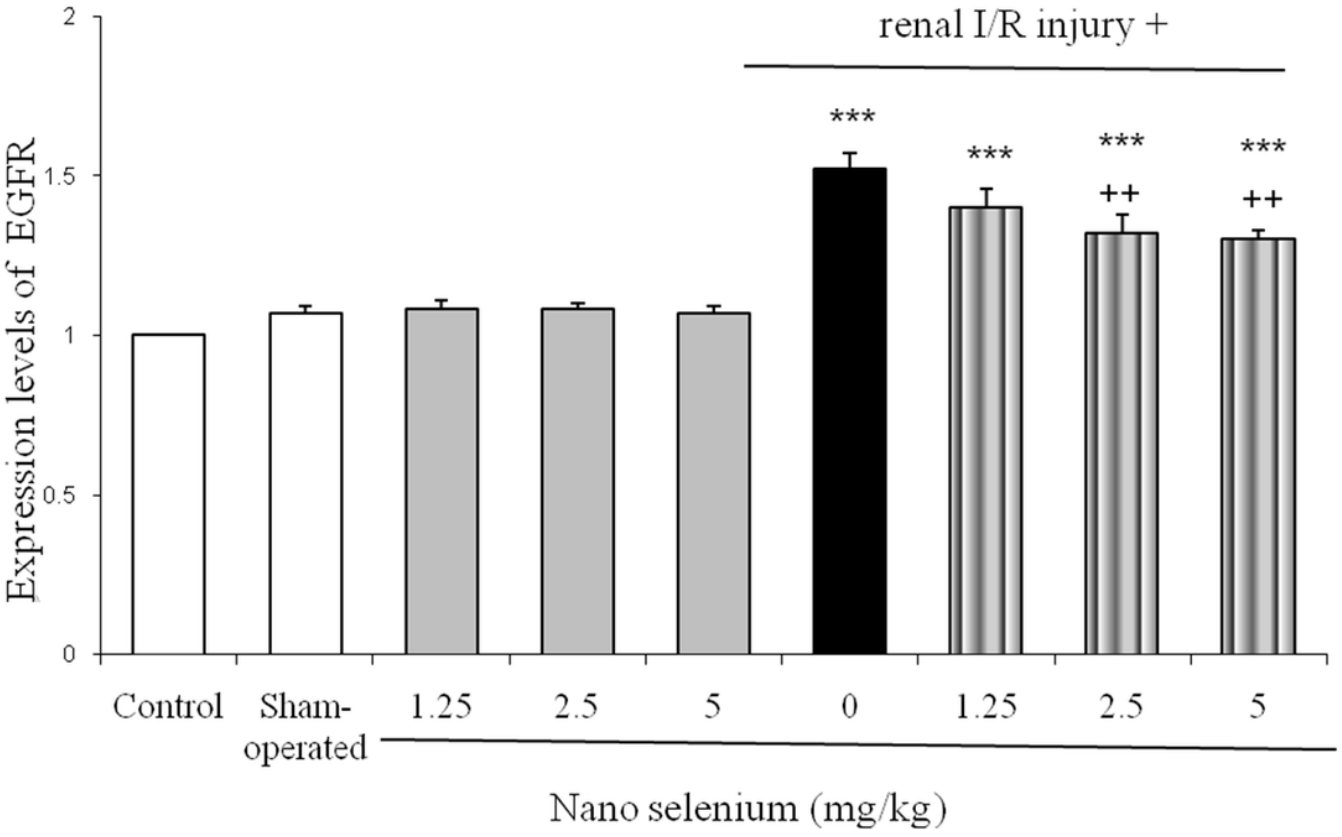

Figure 3

EGFR expression in renal tissue in all experimental groups. Values are presented as mean ± SEM for six rats. *** p<0.001 concentrations the normal control group. ++ P<0.01 concentrations the I/R control group.