Original Article

Nigella sativa protects against ischaemia/reperfusion injury in rat kidneys

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Abstract

Background: Renal ischaemia followed by reperfusion leads to acute renal failure in both native kidneys and renal allografts, which is a complex pathophysiological process involving hypoxia and free radical (FR) damage. The oil of Nigella sativa (NSO) has been subjected to considerable pharmacological investigations that have revealed its antioxidant activity in different conditions. But there is no previously reported study about its effect on ischaemia/reperfusion (I/R) injury of kidneys. The aim of this study was to investigate the possible effects of NSO in I/R-induced renal injury in rats.

Methods: Thirty healthy male Wistar albino rats were randomly assigned to one of the following groups: control, sham, I/R, NSO+I/R, I/R+NSO and NSO. I/R, NSO+I/R and I/R+NSO rats were subjected to bilateral renal ischaemia followed by reperfusion and then all the rats were killed and kidney function tests, serum and tissue oxidants and antioxidants were determined and histopathological examinations were performed.

Results: Pre- and post-treatment with NSO produced reduction in serum levels of blood urea nitrogen (BUN) and creatinine caused by I/R and significantly improved serum enzymatic activities of superoxide dismutase (SOD) and glutathion peroxidase (GSH-Px) and also tissue enzymatic activities of catalase (CAT), SOD and GSH-Px. NSO treatment resulted in lower total oxidant status (TOS) and higher total antioxidant capacity (TAC) levels and also significant reduction in serum and tissue malondialdehyde (MDA), nitric oxide (NO) and protein carbonyl content (PCC) that were increased by renal I/R injury. The kidneys of untreated ischaemic rats had a higher histopathological score, while treatment with NSO nearly preserved the normal morphology of the kidney.

Conclusions: In view of previous observations and our data, with the potent FR scavenger and antioxidant properties, NSO seems to be a highly promising agent for protecting tissues from oxidative damage and preventing organ damage due to renal I/R.

Keywords ischaemia/reperfusion injury; kidney; Nigella sativa oil; rat model

Introduction

Ischaemia/reperfusion (I/R) injury in the kidney is a complex pathophysiological process that occurs in the context of cardiac arrest with recovery, transplantation, heminephrectomy and vascular surgery which is a common cause of renal cell death, renal failure, delayed graft function [1,2] and renal graft rejection [3,4]. The mechanisms underlying I/R damage to kidneys are likely multifactorial and interdependent, involving hypoxia, free radical (FR) damage and inflammatory responses [5].

The seeds or oil of Nigella sativa (NSO), commonly known as black seed or black cumin, have been used as a natural remedy for a number of diseases and conditions such as asthma, cough, bronchitis, headache, eczema, fever, dizziness and influenza [6]. NS contains > 30 w/w of a fixed oil, and 0.40–0.45 w/w of a volatile oil. The volatile oil has been shown to contain 18.4–24% thymoquinone (TQ) and 46% monoterpenes such as p-cymene and a-pinene [7]. Recently, clinical and experimental studies have demonstrated many therapeutic effects of NS extracts, including immunomodulative [8], antiinflammatory [9], antitumour [10], antidiabetic [11–13] and antiulcerogenic [14] effects. Furthermore, modern toxicological studies have demonstrated that crude extracts of the seeds and some of its active constituents (volatile oil, TQ) might have a protective effect against nephrotoxicity and hepatotoxicity induced by either disease or chemicals [6]. However, the renoprotective effect of this plant in the I/R model remains unknown. The aim of this study was, therefore, to investigate the effect of NSO in I/R-induced renal injury in rats.

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Subjects and methods

Experimental protocol

The experimental protocol for this study was approved by the Animal Care and Use Committee of Fatih University School of Medicine.

Animals

Thirty healthy male Wistar albino rats, weighing 210–260 g, were used in the experiments. They were housed in macrolon cages under standard laboratory conditions (light period: 7:00 a.m. to 7:00 p.m., temperature: 21 ± 2°C and relative humidity: 55%). The animals were given standard rat pellets and tap water ad libitum.

Study design

The rats were randomly assigned to one of the following six experimental groups, each consisting of six animals:

Group I (control): the animals in this group served as controls and received vehicle (inert oil) via a gastric tube for 7 consecutive days.

Group II (sham): the animals in this group were sham operated with the exposure of both the renal pedicles, but were not subjected to any I/R.

Group III (I/R): the animals in this group were exposed to bilateral I/R. They were subjected to 45 min of bilateral renal pedicle occlusion followed by 24 h of reperfusion.

Group IV (NSO + I/R): animals received NSO (0.3 mL via a gastric tube for 7 consecutive days prior to surgery) and the rest of procedure was same as in Group III.

Group V (NSO): animals received NSO (0.3 mL via a gastric tube for 7 consecutive days), but were not subjected to I/R.

Group VI (I/R + NSO): animals in this group were exposed to bilateral I/R and they received NSO right after ischaemia (0.6 mL via a gastric tube).

I/R, NSO + I/R and I/R + NSO rats were anaesthetized with intramuscular (i.m.) injection of xylazine (10 mg/kg) and ketamine (70 mg/kg), the abdominal region was shaved, and the animal was placed on a heated table to maintain constant temperature. The abdominal area was prepared with Betadine, and sterile drapes were applied. A midline incision was made and ischaemia was induced by bilateral renal pedicle clamping for 45 min with smooth vascular clamps under sterile conditions. After the clamps were removed, the kidney was inspected for restoration of blood flow. The abdomen was then closed in two layers and the animals were allowed to recover. Twenty-four hours after reperfusion the animals were reanaesthetized with xylazine (10 mg/kg) and ketamine (70 mg/kg), and the abdominal wall was reopened. Having collected blood by cardiac puncture, blood samples were immediately centrifuged and plasma samples were stored at −70°C until assayed. After bilateral nephrectomies were carried out, the left kidney was stored at −70°C until biochemical analysis, whereas the right kidney was stored in 10% formalin for histological examination.

NSO was obtained by crude extraction of NS seeds without using any solvent.

Renal histology

The kidneys, fixed in a 10% neutral buffered formalin solution, were embedded in paraffin and were used for histopathological examination. Four micrometre (µm) thick sections were cut, deparaffinized, hydrated and stained with haematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) staining methods. The renal sections were examined in a blind fashion for tubular cell swelling, cellular vacuolization, pyknotic nuclei, medullary congestion and moderate to severe necrosis. Tubular necrosis was scored semiquantitatively using a scoring system ranging from 0 to 4 by a pathologist who examined at least 20 separate fields (×400) in the outer medulla, which is the most sensitive zone for ischaemic injury. Tubular necrosis was defined as tubular dilatation, sloughing of tubular epithelial cells, intratubular cast formation and naked tubular basement membrane. The scoring system was as follows: 0, no damage; 1, patchy isolated unicellular necrosis; 2, tubular necrosis <25%; 3, tubular necrosis between 25 and 50% and 4, tubular necrosis >50%.

Assessment of renal function

Blood urea nitrogen (BUN) and creatinine were assessed by a dimension clinical chemistry system (Dade Behring-RXL MAX, Newark, DE, USA). Cystatin C concentrations were measured by immunonephelometry on a Behring Nephelometer II (Dade Behring BN 100).

Biochemical analyses

After weighing the tissues, homogenized in five volumes of ice-cold Tris–HCl buffer (50 mM, pH 7.4), homogenization (homogenizer: IKA Ultra-Turrax T 25 Basic, Germany) was carried out for 2 min at 13 000 rpm. All procedures were performed at 4°C. Homogenate, supernatant and extracted samples were prepared and the following determinations were made on the samples using commercial chemicals supplied by Sigma (St Louis, MO, USA). Protein measurements were made in the samples according to the method explained elsewhere [15].

Estimation of antioxidant enzymes

Total (Cu–Zn and Mn) SOD (EC 1.15.1.1) activity was determined according to the method of Sun et al. [16]. The SOD activity is expressed as U/mg protein.

Glutathione peroxidase (GSH-Px) activity was measured by the method of Paglia and Valentine [17]. Activity is expressed as U/g protein.

Catalase (CAT) activity was determined according to Aebi’s method [18]. Results are expressed as k/g protein. All samples were assayed in duplicate.
Estimation of tissue protein carbonyl content (PCC)

The carbonyl contents were determined spectrophotometrically (Cintra 10 E, Austria) based on the reaction of the carbonyl group with 2,4-dinitrophenyldrazine to form 2,4-dinitrophenyldrazone [19]. The results were given as nanomoles of carbonyl per milligram of protein.

Estimation of malondialdehyde levels

The malondialdehyde (MDA) levels were determined by the method based on the reaction of MDA with thiobarbituric acid [20]. Results were expressed as nmol/g wet tissue.

Estimation of the NO level

NO was measured after the conversion of nitrate to nitrite by copperized cadmium granules by a spectrophotometer at 545 nm. A standard curve was established with a set of serial dilutions (10–8–10–3 mol/L) of sodium nitrite. The resulting equation was then used to calculate the unknown sample concentrations. Results were expressed as nmol/g wet tissue.

Estimation of total antioxidant capacity (TAC) and total oxidant status (TOS) of serum

TAC of serum was determined using a novel automated measurement method, developed by Erel [21]. The results are expressed as mmol Trolox equiv./L.

TOS of serum was determined using a novel automated measurement method, developed by Erel [22]. The results are expressed in terms of micromolar hydrogen peroxide equivalent per litre (µmol H2O2 equiv./L).

Statistical analysis

Statistical analyses for biochemical and pathological parameters were carried out by analysis of variance (ANOVA) followed by appropriate post hoc tests including multiple comparison tests (LSD). The Kruskal–Wallis one-way ANOVA by ranks was used for a simultaneous statistical test of the pathologic score for the ischaemia and ischaemia plus Nigella groups. When the null hypothesis could be rejected, comparisons between the two groups were made with the Mann–Whitney non-parametric test for independent samples. Data were expressed as means ± standard deviation (SD). All analyses were made using the SPSS statistical software package and P value of <0.05 was accepted as statistically significant.

Results

As illustrated in Table 1, animals that underwent renal ischaemia exhibited significant increases in serum BUN, creatinine and cystatin C levels compared to normal and sham animals, suggesting a significant decrease of glomerular function caused by renal I/R. Pre- and post-treatment of rats with NSO produced reduction in the serum levels of BUN, creatinine and cystatin C that was statistically significant in serum BUN and creatinine levels (P < 0.01 and P < 0.05, respectively). Rats treated with NSO prior to surgery had significantly lower serum levels of BUN and creatinine than rats that received NSO right after I/R (P < 0.05).

Renal I/R significantly decreased serum enzymatic activity of SOD and GSH-Px. This reduction was significantly improved by pre- and post-treatment with NSO (P < 0.05 and P < 0.01, respectively) (Table 2).

Ischaemia and reperfusion also caused significant decreases in tissue enzymatic activities of CAT, SOD and GSH-Px when compared with the control group that was significantly increased by NSO pre- and post-treatment (P < 0.05) (Table 3).

Renal I/R injury produced a significant increase in serum and tissue MDA, NO and PCC as compared to control and sham-operated animals. Treatment with NSO resulted in a significant reduction in these markers (P < 0.05, P < 0.01 and P < 0.05 for serum levels, respectively, and P < 0.001 for tissue levels for pre-treatment with NSO) (P < 0.05, P < 0.01 and P < 0.05 for serum levels, respectively, and P < 0.001 for tissue levels for MDA and NO and < 0.05 for PCC, for post-treatment with NSO) (Tables 2 and 3).

In the control group renal tissue sections had a normal morphology (Figure 1A). No morphological damage was observed in the NSO only and sham-operated groups. Histological examination of kidneys exposed to I/R showed the distinctive pattern of ischaemic renal injury, which included widespread degeneration of tubular architecture, loss of brush border, sloughing tubular epithelial cells from the basement membrane, tubular cell necrosis and intratubular cast formation especially in the outer medulla (Figure 1B).

Table 1. Effect of Nigella sativa oil on serum levels of BUN, creatinine and cystatin C in rats exposed to I/R

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Sham</th>
<th>I/R</th>
<th>NSO</th>
<th>NSO+I/R</th>
<th>I/R+NSO</th>
</tr>
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<tr>
<td>BUN (mg/dL)</td>
<td>16.33 ± 1.97</td>
<td>17 ± 2.19</td>
<td>71 ± 14*</td>
<td>16.67 ± 1.03</td>
<td>55 ± 10.04b,d</td>
<td>62.73 ± 11.08a,e</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.68 ± 0.19</td>
<td>0.55 ± 0.08</td>
<td>1.77 ± 0.47*</td>
<td>0.52 ± 0.13</td>
<td>1.27 ± 0.4eb,d</td>
<td>1.45 ± 0.2a,e</td>
</tr>
<tr>
<td>Cystatin C (mg/L)</td>
<td>0.16 ± 0.01</td>
<td>0.18 ± 0.02</td>
<td>0.25 ± 0.04*</td>
<td>0.15 ± 0.01</td>
<td>0.22 ± 0.06c</td>
<td>0.23 ± 0.04c</td>
</tr>
</tbody>
</table>

The results are mean ± SD for each group.
*P < 0.01 as compared to the control, sham and NSO groups.
†P < 0.001 as compared to the control, sham and NSO groups.
‡P < 0.05 as compared to the control, sham and NSO groups.
§P < 0.05 as compared to the I/R group.
µP < 0.05 as compared to the I/R group.

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Renal sections obtained from rats pre- and post-treated with NSO demonstrated marked reduction of the histological changes were graded and the kidneys of untreated ischaemic rats had a score of 3.67 and 4.21, respectively. Pre-treatment with NSO before and after I/R injury resulted in higher TAC and lower TOS levels than I/R and I/R groups. 

As illustrated in Table 4 TAC was lower, while TOS was higher in the I/R group than control, sham and NSO groups. The decrease in SOD levels is also in harmony with the previous findings [27,28]. Dobashi et al. [27] demonstrated mRNA levels of CAT significantly decreased after I/R. Moreover, administration of exogenous SOD was reported to minimize renal damages DNA, thereby inhibiting transcription and repair [27,28].}

**Table 2.** Effect of *Nigella sativa* oil on serum superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities, malondialdehyde (MDA) and nitric oxide (NO) levels and protein carbonyl content (PCC)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Sham</th>
<th>I/R</th>
<th>NSO</th>
<th>NSO+I/R</th>
<th>I/R+NSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (U/mg protein)</td>
<td>4.25 ± 0.28</td>
<td>3.12 ± 0.19</td>
<td>2.72 ± 0.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.20 ± 0.24</td>
<td>3.26 ± 0.64&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.22 ± 0.41&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>GSH-Px (U/g protein)</td>
<td>2202.75 ± 156.73</td>
<td>1879.85 ± 92.74</td>
<td>1800.5 ± 125.89&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2053.78 ± 147.38</td>
<td>1967.53 ± 103.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1952.2 ± 214.7&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>MDA (nmol/g protein)</td>
<td>0.17 ± 0.09</td>
<td>0.33 ± 0.03</td>
<td>0.4 ± 0.06&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.15 ± 0.02</td>
<td>0.33 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.34 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>NO (nmol/g wet tissue)</td>
<td>43.97 ± 3.18</td>
<td>63.79 ± 5.41</td>
<td>65.22 ± 4.28&lt;sup&gt;c&lt;/sup&gt;</td>
<td>56.14 ± 7.08</td>
<td>55.25 ± 5.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>52.32 ± 4.46&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>PCC (nmol/mg protein)</td>
<td>675.33 ± 55.11</td>
<td>796.81 ± 66.35</td>
<td>836.15 ± 67.84&lt;sup&gt;f&lt;/sup&gt;</td>
<td>701.19 ± 71.04</td>
<td>789.68 ± 104.93&lt;sup&gt;c&lt;/sup&gt;</td>
<td>792.34 ± 98.91&lt;sup&gt;c&lt;/sup&gt;</td>
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The results are mean ± SD for each group. 
I/R: Ischaemia/reperfusion, NSO: *Nigella sativa* oil. 
<sup>a</sup>P < 0.001 as compared to the control group. 
<sup>b</sup>P < 0.05 as compared to the sham, NSO, NSO+I/R and I/R+NSO groups. 
<sup>c</sup>P < 0.01 as compared to the control group. 
<sup>d</sup>P < 0.001 as compared to the control, sham, NSO+I/R and I/R+NSO groups. 
<sup>e</sup>P < 0.001 as compared to the NSO+I/R and I/R+NSO groups. 
<sup>f</sup>P < 0.01 as compared to the NSO group. 

**Table 3.** Effect of *Nigella sativa* oil on tissue catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities, malondialdehyde (MDA) and nitric oxide (NO) levels and protein carbonyl content (PCC)

<table>
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<tr>
<th></th>
<th>Control</th>
<th>Sham</th>
<th>I/R</th>
<th>NSO</th>
<th>NSO+I/R</th>
<th>I/R+NSO</th>
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<tbody>
<tr>
<td>CAT (k/g protein)</td>
<td>0.55 ± 0.11</td>
<td>0.38 ± 0.89</td>
<td>0.23 ± 0.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.43 ± 0.08</td>
<td>0.34 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.35 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>0.07 ± 0.12</td>
<td>0.03 ± 0.13</td>
<td>0.04 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.05 ± 0.01</td>
<td>0.06 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.06 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GSH-Px (U/g protein)</td>
<td>0.25 ± 0.46</td>
<td>0.14 ± 0.02</td>
<td>0.09 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.16 ± 0.46</td>
<td>0.13 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.14 ± 0.27&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MDA (nmol/g protein)</td>
<td>2.88 ± 0.27</td>
<td>3.7 ± 0.47</td>
<td>4.78 ± 0.46&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.44 ± 0.26</td>
<td>2.98 ± 0.48&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.74 ± 0.35&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>NO (nmol/g wet tissue)</td>
<td>0.13 ± 0.32</td>
<td>0.16 ± 0.68</td>
<td>0.29 ± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.18 ± 0.03</td>
<td>0.17 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.15 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PCC (nmol/mg protein)</td>
<td>0.61 ± 0.2</td>
<td>1.11 ± 0.2</td>
<td>2.1 ± 0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.1 ± 0.21</td>
<td>0.74 ± 0.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.2 ± 1.5&lt;sup&gt;b&lt;/sup&gt;</td>
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</table>

The results are mean ± SD for each group. 
I/R: ischaemia/reperfusion, NSO: *Nigella sativa* oil. 
<sup>a</sup>P < 0.001 as compared to the control, sham and NSO groups. 
<sup>b</sup>P < 0.05 as compared to the I/R group. 
<sup>c</sup>P < 0.001 as compared to the control group. 
<sup>d</sup>P < 0.001 as compared to the I/R group. 

**Discussion**

The results of the present study demonstrate that pre- and post-treatment with NSO has protective effects on I/R-induced renal damage. The impaired renal functions indicating acute renal failure and histopathological changes are inhibited by NSO. Moreover I/R-induced elevations in MDA, NO and PCC are inhibited and antioxidant enzyme status is improved by NSO treatment. It is worth noting that administration of NSO right after ischaemia was as effective as pre-treatment with NSO. These findings are relevant for their clinical and pathophysiological implications.

Although many factors are involved in I/R-induced renal damage, reactive oxygen species (ROS) play a major role in the pathogenesis of reperfusion injury. ROS are capable of reacting with proteins, lipids and nucleic acids leading to lipid peroxidation in biological membranes, which in turn impacts enzymatic processes such as ion pump activity and damages DNA, thereby inhibiting transcription and repair [23,24].

The cell natural protective system against the devastat- ing actions of ROS includes the protective enzymes SOD, CAT and the antioxidant molecule, GSH. Superoxide radicals formed by I/R injury are converted into H2O2, either spontaneously (in pH 4.8) or by dismutation with the SOD enzyme (especially, in neutral and alkaline pH). H2O2 is then converted to H2O by either CAT or GSH-Px. In our study CAT, SOD and GSH-Px activities were found to be significantly decreased in the I/R group when compared to the control group. This is in accordance with previous studies that reported renal injury to be linked with depletion or reduction in the glutathione content [25,26]. The decrease in SOD levels is also in harmony with the previous findings [27,28]. Dobashi et al. [27] demonstrated mRNA levels of CAT significantly decreased after I/R. Moreover, administration of exogenous SOD was reported to minimize renal...
Fig. 1. Light photomicrographs of the rats’ kidney sections (H&E ×400). Kidney sections from the control group with normal renal morphology (A), the I/R group shows the distinctive pattern of ischaemic renal injury (B) and the rats treated with NSO prior to and right after the I/R process shows relatively well-preserved architecture with focal tubular necrosis (C and D).

Table 4. Effect of *Nigella sativa* oil on serum total antioxidant capacity (TAC) and total oxidant status (TOS)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Sham</th>
<th>I/R</th>
<th>NSO</th>
<th>NSO+I/R</th>
<th>I/R+NSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAC (mmol Trolox equiv./L)</td>
<td>3.6 ± 0.38</td>
<td>2.53 ± 0.26</td>
<td>1.4 ± 0.09(^a)</td>
<td>3.17 ± 0.16</td>
<td>2.91 ± 0.12(^b)</td>
<td>3.14 ± 0.25(^b)</td>
</tr>
<tr>
<td>TOS (µmol H(_2)O(_2) equiv./L)</td>
<td>17.84 ± 1.18</td>
<td>21.91 ± 1.12</td>
<td>24.86 ± 1.05(^a)</td>
<td>15.97 ± 1.67</td>
<td>21.44 ± 1.57(^b)</td>
<td>20.34 ± 1.22(^b)</td>
</tr>
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</table>

The results are mean ± SD for each group.
I/R: ischaemia/reperfusion, NSO: *Nigella sativa* oil.
\(^a\)P < 0.001 as compared to the control, sham and NSO groups.
\(^b\)P < 0.001 as compared to the I/R group.

injury [3,29]. The decrease in renal CAT, SOD and GSH-Px activities is probably the result of the inactivation by ROS produced by I/R.

The present study demonstrated that renal I/R resulted in raised MDA and PCC in renal tissue. These data are in good agreement with the work of Irmak *et al.* [30] who found high lipid peroxidation after renal IR injury in rats. The increase in NO seen during renal I/R may play a biphasic role in subsequent injury. NO is known for its beneficial effects at physiological concentrations, whereas at high concentrations it may yield large amounts of the peroxynitrite radical (ONO\(_{2}^-\)) from its reaction with superoxide (O\(_2^-\)); up to 100-fold for each 10-fold increment in O\(_2^-\) [9]. In addition, ONOO\(^-\) oxidizes sulfhydryl groups and produces hydroxyl radicals (OH\(^-\)). All three radicals are able to induce membrane lipid peroxidation. High oxidative stress rates may also deplete GSH reserves, further reducing the endogenous antioxidant protective mechanisms.
Although determination of either oxidants or antioxidant components alone may give information about oxidative stress, determination of oxidants along with antioxidants is more useful in this context [31]. Thus, oxidants and antioxidant capacity may be measured simultaneously to assess oxidative stress more exactly. Our study evaluated TAC and TOS in rats with renal I/R injury. To the best of our knowledge, this is the first study to evaluate the association between TAC, TOS, renal I/R injury and also the effect of NSO. We observed that serum TOS levels were significantly higher and TAC levels were significantly lower in rats with I/R as compared to controls. This increase may be due to overproduction or decreased excretion of oxidant substances. In addition, NSO pre-treatment resulted in lower TOS and higher TAC levels that were statistically significant.

NSO is an effective FR scavenger showing antioxidant activity and protecting against the damage caused by FRs. Therefore, the oil is useful in diseases in which FRs are involved, e.g. anoxia and ischaemia of different organs [7,9,32–34]. In the present study, NSO had a marked protective action against I/R-induced renal injury. These findings are considerable as there is no previously reported study on the effect of NSO on I/R-induced renal injury. The renoprotective effect of NSO could be attributed to the improvement of the antioxidant status of the animals [35,36] or the presence of FR scavenging substances such as TQ [37]. The latter authors also showed that NSO given as TQ [37]. The latter authors also showed that NSO given

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