Original Article

Nigella sativa Prevented Liver and Renal Tissue Damage in Lipopolysaccharide-Treated Rats

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ABSTRACT. Liver and renal dysfunction accompanying with the tissues’ oxidative damage has been reported to occur during inflammation. Nigella sativa has been well known for its antioxidant and anti-inflammatory effects. The aim of this study was to investigate preventive effects of N. sativa on liver and renal tissue damage in lipopolysaccharide (LPS)-treated rats. The rats were divided into five groups: (1) control; (2) LPS (1 mg/kg, IP, for 10 days), (3–5) N. sativa hydroethanolic extract (100, 200, or 400 mg/kg) before LPS. Compared to LPS group, treatment by the extract decreased malondialdehyde, nitric oxide (NO) metabolites, and interleukin-6 while increased thiol content and superoxide dismutase and catalase activities in both renal and liver tissues. N. sativa extract also decreased serum aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase concentration, while it increased serum protein and albumin compared with LPS group. In LPS group, serum blood urea nitrogen and creatinine were higher than control group. The extract reversed the negative effects of LPS. The results demonstrated that the N. sativa prevented liver and renal tissue damage in LPS-treated rats. It is suggested that the effects are due to its antioxidant and anti-inflammatory effects.

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Introduction

Lipopolysaccharide (LPS) is a bacterial endotoxin in the wall of Gram-negative bacteria that can cause sepsis.1-3 Some evidence indicated that LPS administration can be followed by an increased level of some pro-inflamma-
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Inflammatory mediators such as chemokines and cytokines including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, IL-8, IL-12, monocyte chemotractant protein 1, tissue inhibitor of metalloproteinase 1, and interferon γ (IFN-γ). These mediators play an important role in LPS-induced inflammation that causes damage to the organs. LPS also induces a pro-oxidant effect and increases the production of reactive oxygen species (ROS), and subsequently, organ failure occurs because of sepsis and tissue oxidative damage.1,5

Liver inflammation starts and continues with cytokine production and is characterized by an invasion of inflammatory cells which produce cytokines including IL-6 and TNF-a. These cytokines are responsible for progression of liver damages.6,7 Furthermore, LPS has been reported to be able to decrease superoxide dismutase (SOD) activity in liver tissue.8 In addition, LPS can affect liver function parameters such as ascorbic oxidase (AST) and alanine aminotransferase (ALT).9

On the other hand, sepsis-related endotoxemia commonly leads to acute renal failure (ARF), a major medical problem with high morbidity and mortality rate.11-14 While the main mechanism of LPS-induced ARF is almost unknown, ROS production accompanying inflammation, leading to nonprogrammed necrotic cell death (necrosis), and three types of programmed cell death including autophagy, apoptosis, and pyroptosis15-18 might be responsible. Production of ROS might damage most of glomeruli and tubules in the kidney.19

Recently, there have been renewed interests in consuming herbal remedies, such as rosemary, as an antioxidant due to having fewer side effects compared to chemical drugs.20,21 Nigella sativa (N. sativa), also known as black seed or black cumin, usually grows in Eastern Europe, Mediterranean regions, Western Asia, and the Middle East. Its extract or oil has been used as an herbal medicine to maintain good health.22-24 Data from several studies revealed some pharmacological characteristics of N. Sativa such as anti-inflammatory,25-27 antidiabetic,28-30 antimetastatic,31 anxiolytic,32 hepatoprotective,33 immunomodulatory,33 and relaxant properties.34 In addition, it can reduce the nephrotoxic complications of some drugs such as gentamicin,35 cisplatin,36,37 and doxorubicin.38

Renal protective effects of the plant in diabetic patient have also been reported.39 Therefore, according to the evidence that shows the beneficial effects of N. sativa and its non-toxicity effect on renal tissue, in this study, we attempt to determine whether the N. sativa hydroalcoholic extract would prevent from liver and renal tissue damage in LPS-treated rats.

Materials and Methods

Animals

Forty male Wistar rats weighing 250 ± 10 g from the Central Animal House of Mashhad University of Medical Sciences were used for this study. They were kept in a room with standard temperature (22°C ± 2°C) and condition of 12 h light/dark cycle. The animals were randomized into five groups:

1. Control: the rats in this group received 1 mL/kg saline by injection into the peritoneum (i.p.) instead of N. sativa extract and also 1 mL/kg (i.p.) saline instead of LPS
2. LPS: the animals in this group took 1 mL/kg saline (i.p.) instead of N. sativa and 1 mg/kg LPS (i.p.)
3. LPS-NS100: the rats were administered with 100 mg/kg N. sativa and 1 mg/kg LPS (i.p.)
4. LPS-NS200: in this group, the rats got 200 mg/kg N. sativa (i.p.) and 1 mg/kg LPS (i.p.)
5. LPS-NS400: the animals received 400 mg/kg N. sativa (i.p.) and 1 mg/kg LPS (i.p.).

LPS and the extract were daily administered during two weeks. The extract was injected 30 min before LPS.

In the final stage, urethane was injected to induce deep anesthesia. Then, the kidneys and livers were removed to evaluate renal and liver tissues oxidative damage including malondialdehyde (MDA), thiol, catalase CAT) and SOD, and serum total protein and albumin, renal function parameters such as blood urea nitrogen (BUN) and creatinine, and liver enzymes including AST, ALT, and alkaline phosphatase (ALP).
The Animal Care and Use Committee of Mashhad University of Medical Sciences and also the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals approved this experimental protocol.

**Biochemical measurements**

**Liver and renal tissues oxidative damage criteria**

Protocols for measuring MDA and total thiol concentration have been elucidated in previous documents.\(^{40-42}\) SOD activity was measured by Madesh and Balasurbamanian colorimetric assay\(^{43}\) and CAT activity was assessed by adopting Aebi method based on the determination rate constant of decomposing hydrogen peroxide.\(^{44}\)

**Interleukin-6 and nitric oxide metabolites**

The tissues’ IL-6 content was determined with a specific rat ELISA kit (ebioscience Co, San Diego, CA, USA) according to the manufacturer instructions. The absorbance was measured using a microplate reader (Biotek, USA) and concentration was calculated by comparison curve established in the same measurement. The tissue nitric oxide (NO) metabolites (NO\(_2\)/NO\(_3\)) were measured according to the Griess reagent method. In brief, after adding 100 µL supernatant to the Griess reagent, contents were transferred to a 96-well flat-bottomed microplate and absorbance was read at 520 nm using a microplate reader, and the final values were calculated from standard calibration plots.

**Liver function criteria**

AST, ALT, ALP, total protein, and albumin were measured in the serum. The commercial kits (Pars Azmoon Company, Tehran, Iran) and the protocol provided by the company were used.

**Renal function criteria**

Serum BUN and creatinine levels were measured by the commercial kits (Pars Azmoon Company, Tehran, Iran). The protocol provided by the company was used.

**Statistical Analysis**

All data were given as mean ± standard error of the mean. Data was evaluated by analysis of variance (one-way ANOVA) followed by Tukey–Kramer *post hoc* test using Statistical Package for the Social Sciences (SPSS) version 11.0. The differences were considered statistically significant when \(P < 0.05\).

**Results**

**Liver tissues’ oxidative damage criteria**

The liver MDA concentration of the LPS group was significantly higher than that of the control group (\(P < 0.001\)). It was also shown that both doses, i.e., 200 and 400 mg/kg of the extract had a protective effect against increasing of lipid peroxidation due to LPS treatment which was reflected in a lower concentration of MDA in the liver tissues (\(P < 0.001\)) (Figure 1a). In addition, the highest dose was more effective than that of both medium and the lowest doses (\(P < 0.001\)) (Figure 1a). In addition, the medium dose was more effective than the lowest dose (\(P < 0.001\)) (Figure 1a).

LPS administration also attenuated the liver tissues’ thiol contents (\(P < 0.001\)). Both 200 and 400 mg/kg of *N. sativa* extract improved the thiol contents of the liver tissues (\(P < 0.001\) for both doses compared to LPS group); however, the lowest dose was not effective (Figure 1b). The results also showed that the both medium and highest doses of the extract were more effective than that lowest dose (\(P < 0.001\) for both) (Figure 1b).

A comparison of SOD activity in the liver tissues of LPS and control groups showed a significant difference between the two groups (\(P < 0.001\)). Both medium and highest doses of the extract were effective to enhance SOD activity in the liver tissues compared to the LPS group (\(P < 0.001\)). However, 100 mg/kg of the extract did not show a significant effect on SOD activity in the liver tissues compared to the LPS group (Figure 1c). Compared to 100 mg/kg, both 200 and 400 mg/kg of the extract were more effective to increase SOD activity (\(P < 0.001\)). In addition, the highest dose
was more effective than the medium dose \((P < 0.001)\) (Figure 1c).

It was also observed, and the CAT activity in the liver tissues of LPS group was significantly lower than that of the control group \((P < 0.001)\). The findings also showed that the highest \((P < 0.001)\) dose of the extract increased the CAT activity in the liver tissues compared to the LPS (Figure 1d). CAT activities in the liver tissues of LPS-NS 200 and LPS-400 groups were higher than that of LPS-NS 100 \((P < 0.05\) and \(P < 0.001\), respectively). In addition, the highest dose was more effective than that of the medium dose \((P < 0.001)\) (Figure 1d).

Liver function criteria

The results of NS extract on liver function criteria of the LPS-treated rats were shown in Figure 2. In LPS group, serum AST concentration of the rats was higher than that of the control ones \((P < 0.001)\). Treatment of the animals by both two higher doses including 200 and 400 mg/kg of the extract attenuated the
Figure 2. Liver function tests: AST (a), ALT (b), ALK-P(c), albumin (d) and total protein (e).
Data are presented as mean ± standard error of the mean (n = 8). The rats in control group received saline while in LPS group 1 mg/kg of LPS was injected. In LPS-NS 100, LPS-NS 200, and LPS-NS 400 groups, the animals received 100, 200, and 400 mg/kg of *Nigella sativa* extract, respectively, before LPS.

*P <0.05 and **P <0.01, vs. control group, *P <0.05, **P <0.01 and ***P <0.001 vs. LPS group, *P <0.05 and ###P <0.001 vs. LPS-NS 100 group, $P <0.01 and $$$P <0.001 vs. LPS-NS 200 group.
serum concentration of AST (P <0.001 for both); however, 100 mg/kg was not effective (Figure 2a). The results also showed that the two higher doses, i.e., 200 and 400 mg/kg of the extract were more effective than the lowest dose (P <0.001) (Figure 2a).

LPS also increased serum ALT compared to the control group (P <0.001). All three doses of the extract prevented from increasing of ALT concentration due to LPS administration (P <0.05 for 100 mg/kg, P <0.01 for 200 mg/kg, and P <0.001 for 400 mg/kg). The results also showed that ALT-lowering effect of 400 mg/kg of the plant extract was significantly more than that of both 100 and 200 mg/kg (P <0.01 – P <0.001) (Figure 2b).

Furthermore, the impairing effects of LPS on liver function were confirmed when it was seen that the serum ALP concentration in LPS-exposed rats was significantly higher than that of the control ones (P <0.001). Pretreatment by 100 mg/kg (P <0.001), 200 mg/kg (P <0.001), and 400 mg/kg (P <0.001) of the extract attenuated the serum ALP concentration compared to the LPS group (Figure 2c).

The serum concentrations of total protein and albumin were also compared between the groups. The results showed that LPS injection lowered both total protein and albumin compared to the control group (P <0.02, Figure 2d and e). Both the two higher doses of Vitamin C including 100 and 400 mg/kg increased albumin concentration compared to the LPS group (P <0.05 for both, Figure 2d). The lowest dose of the extract was not able to increase albumin concentration compared to LPS group (Figure 2d).

In addition, the total protein concentration in the rats treated by the highest dose of the extract was higher than that of LPS group (P <0.05, Figure 2e). Neither 100 nor 200 mg/kg of the extract was not able to increase total protein concentration compared to LPS group (Figure 2e).

Liver tissues nitric oxide metabolites and interleukin-6

The results also showed that LPS increased no metabolites in the liver tissues compared to the control group (P <0.001). In all three extract treated groups including LPS-NS 100, LPS-NS 200, and LPS-NS 400, the liver tissues’ NO metabolites were lower than that of LPS group (P <0.001 for all) (Figure 3a).

Liver tissues’ IL-6 concentration was also compared between the groups. The results showed that LPS administration increased IL-6 concentrations in the liver tissues compared to the control group (P <0.01). Interestingly, all three doses of the plant extract reversed the
effects of LPS which was presented by a lower level of IL-6 in the liver tissues of LPS-NS 100, LPS-NS 200, and LPS-NS 400 groups compared to the LPS group ($P < 0.01 - P < 0.001$) (Figure 3b).

**Renal tissues’ oxidative damage criteria**

The results also showed that LPS injection affected on renal tissues oxidative damage criteria. The results showed that renal tissue MDA in the LPS group was significantly higher than that of the control group ($P < 0.001$). Only 400 mg/kg of the plant extract was able to attenuate renal tissue MDA compared to LPS group ($P < 0.05$). However, there was no significant difference between LPS-NS 100, LPS-NS 200, and LPS groups in the renal tissue MDA concentration (Figure 4a).

LPS administration attenuated the renal tissues thiol concentrations ($P < 0.001$). The highest dose of *N. sativa* extract administration improved the thiol contents of the renal tissues ($P < 0.05$) compared to LPS group; however, the lowest and the medium doses were not able to increase renal tissues thiol content (Figure 4b). The results also showed that the highest dose of the extract was more effective than that lowest dose ($P < 0.05$) (Figure 4b).

A comparison of SOD activity in the renal tissues showed a significant decrease in LPS

![Figure 4. Renal tissue MDA (A), thiol (B), SOD (C) and CAT (D). Data are presented as mean ± standard error of the mean (n = 8). The rats in control group received saline while in LPS group 1 mg/kg of LPS was injected. In LPS-NS 100, LPS-NS 200 and LPS-NS 400 groups, the animals received 100, 200 and 400 mg/kg of *Nigella sativa* extract respectively before LPS, ***$P < 0.001$, vs. control group, $+ P < 0.05$, $++ P < 0.01$ and $+++ P < 0.001$ vs. LPS group, $\# P < 0.05$ and ###$P < 0.001$ vs. LPS–NS 100 group.](http://www.sjkdt.org)
group compared to the control group \((P < 0.001)\). Only the highest dose of the plant extract was effective to enhance SOD activity in the renal tissues compared to LPS group \((P < 0.001)\). However, 100 and 200 mg/kg of the extract did not show a significant effect on SOD activity in the renal tissues compared to the LPS group (Figure 4c). Compared to 100 mg/kg, 400 mg/kg of the extract was more effective to increase SOD activity \((P < 0.001)\) (Figure 4c).

It was also seen that the CAT activity in the renal tissues of LPS group was significantly lower than that of control group \((P < 0.001)\). The findings also showed that both the medium and the highest doses of the extract increased the CAT activity in the renal tissues compared to the LPS \((P < 0.01\) and \(P < 0.001\), respectively) (Figure 4d). No significant difference was observed in the renal tissues CAT between the treated rats with 100 mg/kg of the extract and the LPS group. CAT activities in the renal tissues of both LPS-NS 200 and LPS-400 groups were higher than that of LPS-NS 100 \((P < 0.01\) and \(P < 0.001\), respectively) (Figure 4d).

**Renal function criteria**

The results of the effects of LPS and the plant extract on renal function criteria including BUN and creatinine were shown in Figure 5.

In LPS group, serum BUN concentration was higher than that of the control ones \((P < 0.001)\). Treatment of the animals by all three doses including 100, 200, and 400 mg/kg of the extract attenuated the serum concentration of BUN \((P < 0.001\) for all) (Figure 5a). There also showed that the two highest dose of the plant extract was more effective than the lowest dose \((P < 0.001)\) (Figure 5a).

Furthermore, the impairing effects of LPS on renal function were confirmed when it was seen that the serum creatinine concentration in LPS-exposed rats was significantly higher than that of the control ones \((P < 0.05)\). Co-treatment by 200 mg/kg \((P < 0.05)\) and 400 mg/kg \((P < 0.01)\) of the extract attenuated the serum creatinine concentration compared to the LPS group; however, 100 mg/kg was not effective to decrease creatinine concentration in the serum of LPS-NS 100 group compared to the LPS group (Figure 5b).

**Renal tissues nitric oxide metabolites and interleukin-6**

In a similar manner to the liver tissues, LPS administration increased NO metabolites in the renal tissues compared to the control group \((P < 0.001)\). In all three extract treated groups including LPS-NS 100, LPS-NS 200, and LPS-NS 400 groups, serum nitric oxide metabolites decreased compared to LPS group (Figure 5c).

The results of the effects of LPS and the plant extract on renal function criteria were shown in Figure 5.

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**Figure 5. Serum blood urea nitrogen (a) and creatinine (b).**

Data are presented as mean ± standard error of the mean \((n = 8)\). The rats in control group received saline while in LPS group 1 mg/kg of LPS was injected. In LPS-NS 100, LPS-NS 200, and LPS-NS 400 groups, the animals received 100, 200, and 400 mg/kg of Nigella sativa extract, respectively before LPS.

\*\(P < 0.05\) and \***\(P < 0.001\), vs. control group, \^\(P < 0.05\), \^\(^\^\)\(P < 0.01\) and \^\(^\^^\)\(P < 0.001\) vs. LPS group, \###\(P < 0.001\) vs. LPS–NS 100 group.
NS 400, the renal tissues’ NO metabolites were lower than that of LPS group (P < 0.001 for all). The results also showed that, in the renal tissues of both LPS-NS 200 and LPS-NS 400 groups, the NO metabolites were lower than that in LPS-NS 100 group (P < 0.05 and P < 0.001) (Figure 6a).

An increased level of IL-6 concentration was observed in the renal tissues of LPS compared to control group (P < 0.01). Interestingly, all three doses of the plant extract reversed the effects of LPS which was presented by a lower level of IL-6 in the renal tissues of both kidney and liver which confirms that LPS administration induced an inflammation status in these organs. In animal models of liver toxicity, LPS injection has also been able to increase some cytokines including IL-6 in the serum and the liver tissues. In the current study, liver and renal tissues’ inflammation induced by LPS was accompanied with increased levels of serum ALT, AST, and ALP as markers of liver function parameters and BUN and creatinine as renal function parameters which confirms that inflammation affected the functions of these organs. In agreement with these results in another study, LPS injection induced a hepatitis status in mice and increased ALT and AST in the liver tissues. In the present study, renal and liver dysfunc-

Discussion

The present study was designed to determine the effect of N. sativa on LPS-induced renal and liver functions impairments. MDA, total Thiol, and NO metabolites’ concentration and SOD and CAT activities as markers of tissues oxidative damages and IL-6 as a marker of inflammation in renal and liver tissues were also assessed. LPS has been repeatedly admi-

Figure 6. Renal tissue NO metabolites (a) and IL-6 (b).
Data are presented as a mean ± standard error of the mean (n = 8). The rats in control group received saline, while in LPS group, 1 mg/kg of LPS was injected. In LPS-NS 100, LPS-NS 200, and LPS-NS 400 groups, the animals received 100, 200, and 400 mg/kg of Nigella sativa extract, respectively before LPS.

**P <0.01 and ***P <0.001, vs. control group, ++P <0.01 and +++P <0.001 vs. LPS group, #P <0.05 and ###P <0.001 vs. LPS–NS 100 group.

nistered in rodents to induce an inflammation model. In the present study, LPS injection was accompanied with a high level of IL-6 in the tissues of both kidney and liver which confirms that LPS administration induced an inflammation status in these organs. In animal models of liver toxicity, LPS injection has also been able to increase some cytokines including IL-6 in the serum and the liver tissues. In the current study, liver and renal tissues’ inflammation induced by LPS was accompanied with increased levels of serum ALT, AST, and ALP as markers of liver function parameters and BUN and creatinine as renal function parameters which confirms that inflammation affected the functions of these organs. In agreement with these results in another study, LPS injection induced a hepatitis status in mice and increased ALT and AST in the liver tissues. In the present study, renal and liver dysfunc-
tions due to LPS administration was accompanied by the tissues’ oxidative damage which presented with a high level of MDA and NO metabolites in the tissues. Total thiol content and SOD and CAT activities in both liver and renal tissues were decreased. These findings are in agreement with the result obtained by Qiao et al who showed that antioxidants including glutathione and CAT levels were depleted, while MDA levels were increased in LPS-induced sepsis. In addition, it has been demonstrated that ROS is involved in the pathogenesis of LPS-induced nephrotoxicity in vivo. In the present study, LPS-treated animals had lower levels of total protein and albumin in their serum which confirms liver and renal dysfunctions. Consistently, LPS has been previously reported that inhibits total protein synthesis by hepatocytes.

High mortality due to renal and liver function impairment implies that it is important to find a cure with the least complications. In recent years, attention has been drawn to various plants and plant-derived compounds for treatment of kidney and liver diseases. For centuries, N. sativa’s seeds and thymoquinone have been used as treatments for various diseases in the Middle and Far East countries. Treatment with N. sativa substantially has been able to decrease kidney and liver oxidative stress-related damage and preserved normal renal and liver morphology. Other beneficial effects of N. sativa on liver and kidney functions have been repeatedly reported in previous studies.

In the current study, treating rats with N. sativa improved renal and liver oxidative damage induced by LPS. In N. sativa-treated rats, MDA and NO metabolites and IL-6 were lower than LPS group and total thiol concentration and SOD and CAT activity were higher than LPS group. These results seem to be consistent with other researches which found N. sativa can be used as a treatment for renal injury due to ROS production. Furthermore, it has been previously observed that N. sativa can be used as a nephroprotective agent against gentamicin nephrotoxicity and cisplatin and doxorubicin-induced nephropathy. In addition, it had no nephrotoxic effect. The activity of two antioxidant-related enzymes was measured as two extra stress oxidative indicators: SOD and CAT enzymes. Our measurement showed a significant reduction of SOD and CAT activities in LPS group and also demonstrated that N. sativa therapy increased the activity levels of these enzymes. These results match with those observed in earlier studies.

Furthermore, N. sativa reversed all the changes induced by diethylnitrosamine, the carcinogen, including severe histopathological lesions and decreased levels of total nitrate/nitrite, total bilirubin, thiobarbituric acid reactive substances, ALT, and ALP and improved the antioxidant elements including CAT.

On the other hand, we observed that N. sativa substantially decreased the amount of both BUN and creatinine, indicating that treatment with N. sativa could preserve renal function. Furthermore, N. sativa decreased serum ALT, AST, and ALP and increased total protein and albumin in LPS-treated rats. These data are in accordance with recent studies that showed N. sativa can decrease BUN and creatinine. In a previous study, N. sativa treatment was able to decrease LPS-induced hepatic enzymes and inhibited an increased level of AST and ALT. Aqueous extract of N. sativa seeds has been able to improve serum total protein and albumin in a hepatic damage model induced by CCl4. Furthermore, N. sativa was able to decrease liver enzymes in hepatorenal toxicity induced by bromobenzene in rats.

**Conclusion**

In this investigation, we studied the effects of N. sativa on LPS-induced renal and liver oxidative damage and inflammation. It reduced stress oxidative indicators and inflammation that was induced by LPS and conserve renal and liver functions.

**Conflict of interest:** None declared.

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