Effect of Alcoholic Extract of *Nigella Sativa* on Cisplatin-induced Toxicity in Rat

Mousa-Al-Reza Hadjzadeh,[1,2] Zakieh Keshavarzi,[1,3] Seyed Abbas Tabatabaee Yazdi,[4] Mohsen Ghasem Shirazi,[1,3] Ziba Rajaei,[1,2] Abolfazl Khajavi Rad[1]

[1]Department of Physiology, Mashhad University of Medical Sciences, Mashhad, Iran
[2]Neuroscience Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[3]Physiology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[4]Department of Pathology, Qaem Hospital, Mashhad University of Medical Sciences, Mashhad, Iran

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Introduction. The aim of this study was to test whether *Nigella sativa* (NS) seeds can reduce cisplatin-induced toxicity.
Materials and Methods. Thirty rats were divided into 3 groups to receive distilled water (control group), cisplatin (3 mg/kg per body weight for 3 days), and cisplatin and alcoholic extract of NS (100 mg/kg per body weight). Biochemical and histopathologic parameters were compared between the three groups on days 14 and 42 of the study.
Results. Blood urea nitrogen increased in the cisplatin and NS groups on days 14 and 42 compared to day 0 ($P < .001$). It was significantly in the cisplatin than in the control group on day 14 ($P < .001$). Serum creatinine had a similar profile in the cisplatin and NS groups as blood urea nitrogen. Serum triglyceride increased in the cisplatin and NS groups on day 14, but it decreased on day 42 ($P < .05$). Urine glucose concentration decreased in the cisplatin group on days 14 and 42 compared to day 0 ($P < .001$), and the same trend was seen in the NS group ($P < .001$). Histology of the kidneys exposed to cisplatin showed significant kidney injury, but the rats treated with NS showed a relatively well-preserved architecture.
Conclusions. Cisplatin-induced nephrotoxicity was confirmed in our study. Nigella sativa seeds had nonsignificant effects on biochemical parameters, although the histopathologic properties of the kidneys relatively recovered after NS use.

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INTRODUCTION

The complex nature of critical illnesses often necessitates the use of multiple therapeutic agents, many of which may individually or in combination have the potential to cause kidney injury. The use of nephrotoxic drugs has been implicated as a causative factor in up to 25% of all cases of severe acute kidney failure (AKF) in critically ill patients.[1] Acute tubular necrosis is the most common form of kidney injury from nephrotoxins exposure, although other types of kidney failure might be observed.[1]

Cisplatin, a chemotherapeutic drug, is widely used for treatment of several kinds of human disease. Administration of cisplatin is a common cause of AKF, which is a life-threatening illness that continues to have a high mortality rate of 50% to 80% in an intensive care unit setting.[2] Thus, a better understanding of the pathogenesis of cisplatin-induced AKF is needed to allow interventions that would prevent the need for hemodialysis, shorten the course of AKF, and improve survival in cancer

patients treated with cisplatin.[2]

Among the promising medicinal plants, *Nigella sativa* (NS), is an amazing herb with a rich historical and religious background for remedies of several diseases. The black cumin, NS, has been used as a natural healing aid for thousands of years by various cultures and civilizations around the world, as well as a supplement to help maintaining good health. It is most famous for the saying of the holy prophet Muhammad, "Hold on to use of the black seeds, for it has a remedy for every illness except death." The word "hold on to" indicates a long-term use.[3]

Nigella sativa seed, which is classified in the family of *Ranunculaceae*, has been shown to contain fixed oil and volatile oil. The volatile oil has been shown to contain 18.4% to 24% thymoquinone and 46% monoterpenes such as pcymene and a-piene.[4] Recently, clinical and animal studies have shown that the extracts of the black seeds have many therapeutic effects such as bronchodilative, immunomodilative, antibacterial, hypotensive, antidiabetic, hepatoprotective, gastroprotective, antilithiatic, antihistaminic, antioxidative, and neuroprotective charateristics.[5-13] Thymoquinone was isolated as the principal active ingredient from the volatile oil of NS.[14] Thymoquinone has been shown to attenuate eicosanoid generation, cisplatin nephrotoxicity, tetrachloride hepatotoxicity, rheumatoid arthritis, and gastric mucosal damage.[15-19]

Considering Nigella sativa seeds as a safe, widely available, and affordable product, the aim of the present study was to examine the protective effect of NS seeds in cisplatin -induced toxicity in rats.

MATERIAL AND METHODS

Animal Experiment

Thirty albino male rats (weight, 270 g to 320 g) were procured from animal house of Mashhad University of Medical Sciences. The animals were acclimatized under room temperature and humidity with a regular light-dark cycle. All experiments in this study were performed in accordance with the guidelines for the care and use of laboratory animals, and the study was approved by Mashhad University of Medical Sciences.

Study Design

The animals were divided into 3 experimental groups, each consisting of 10 rats, and received following treatments: distilled water, 0.75 mL, intraperitoneally for 3 alternate days (control group); cisplatin, 3 mg/kg, intraperitoneally for 3 alternate days (cisplatin group); and cisplatin, 3 mg/kg per body weight, intraperitoneally for 3 alternate days, and the alcoholic extract of NS, 100 mg/kg per body weight, added in drinking water daily, after 2 weeks (NS group).

Sample Collection and Biochemical Assays

Urine and serum samples were collected on days 0, 14, and 42 after injection of cisplatin. Blood samples were collected for determination of serum creatinine, blood urea nitrogen (BUN), and triglyceride levels. Spot urine glucose was also measured. The levels of these parameters were determined by an Olympus AU600 auto-analyzer (Olympus Corp, Tokyo, Japan) according to the manufacturer's instruction.

Histologic Examination

At the end of the experiment (42 days), the animals were decapitated and the kidneys were excised, trimmed of the connective tissues, rinsed with saline to eliminate blood contamination, and preserved in 10% formalin solution for histologic examination. After preparation and staining with hematoxylin-eosin, the specimens were examined by a pathologist who was unaware of details of animal groups with light microscopy.

Statistical Analyses

Continuous values were expressed as mean ± standard error of mean. Comparisons of the data between the three groups were done by 1-way analysis of variance, followed by the Tukey multiple comparison test, using 5% level of significance. The statistical package used was the SPSS software (Statistical Package for the Social Sciences, version 15.0, SPSS Inc, Chicago, Ill, USA).

RESULTS

Kidney Function Biomarkers

As shown in Figures 1 and 2, Serum creatinine and BUN concentrations increased in the cisplatin group on days 14 and 42 compared to day 0 ($P < .001$). In addition, both of these markers significantly increased in this group compared to the control group on day 14 ($P < .001$). In the

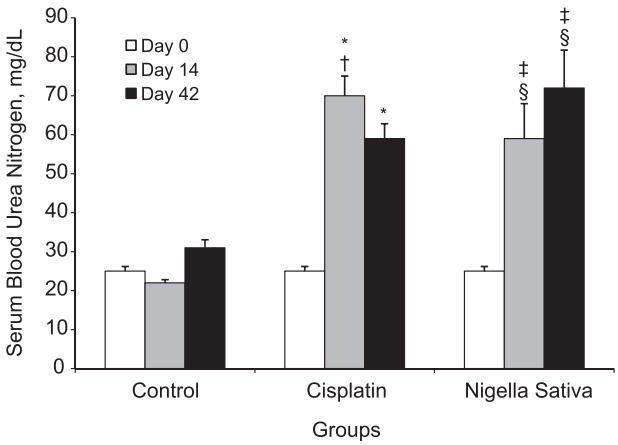

Figure 1. Comparison of serum blood urea nitrogen level in the three groups of rats.
*P < .001 as compared to day 0.
†P < .001 as compared to the control group.
‡P < .001 as compared to day 0.
§P < .001 as compared to the control group.

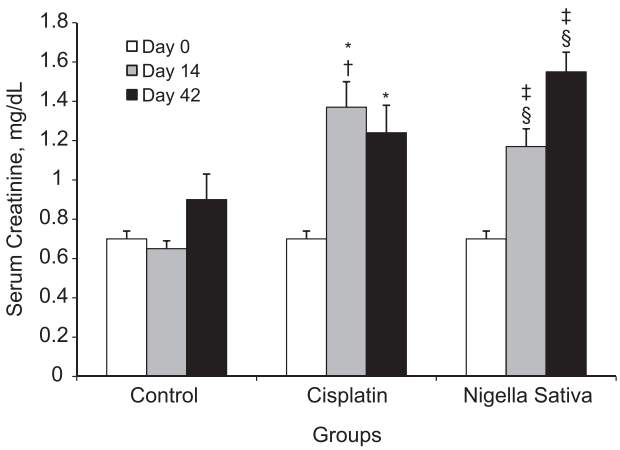

Figure 2. Comparison of serum creatinine levels in the three groups of rats.
*P < .001 as compared to day 0.
†P < .001 as compared to the control group.
‡P < .001 as compared to day 0.
§P < .05 as compared to the control group.

NS group, increased serum creatinine and BUN concentrations on days 14 and 42 were observed when compared to day 0 (P < .001). The BUN concentrations in this group were higher than those in the control group on days 14 and 42 (P < .001), and the same was observed for serum creatinine concentration (P < .05).

Serum Triglyceride Concentration

As shown in Figure 3, serum triglyceride concentration increased in the cisplatin group on day 14 compared to baseline (P < .05), but it significantly decreased on day 42 as compared with

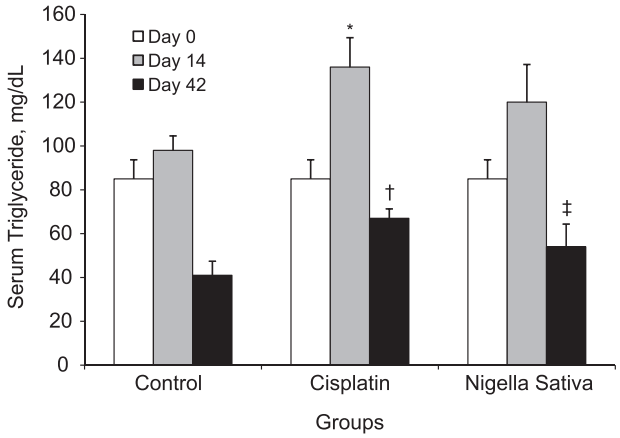

Figure 3. Comparison of serum triglyceride levels in the three groups of rats.
*P < .05 as compared to day 0.
†P < .01 as compared to day 14.
‡P < .05 as compared to day 14.

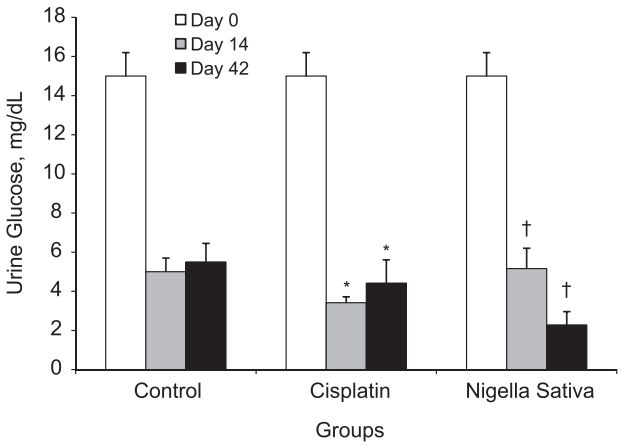

Figure 4. Comparison of urine glucose levels in the three groups of rats.
*P < .001 as compared to day 0.
†P < .001 as compared to day 0.

day 14 (P < .01). Serum triglyceride concentration in the NS group significantly decreased on day 42 compared to days 14 (P < .05).

Urine Glucose Concentration

As shown in Figure 5, urine glucose concentration decreased in the cisplatin group on days 14 and 42 compared to day 0 (P < .001), and the same trend was seen in the NS group (P < .001).

Histopathologic Parameters

Histopathologic changes of the kidneys are shown in Figure 5. In the control group, renal tissue sections had a normal morphology. Histologic examination of the kidneys exposed to cisplatin

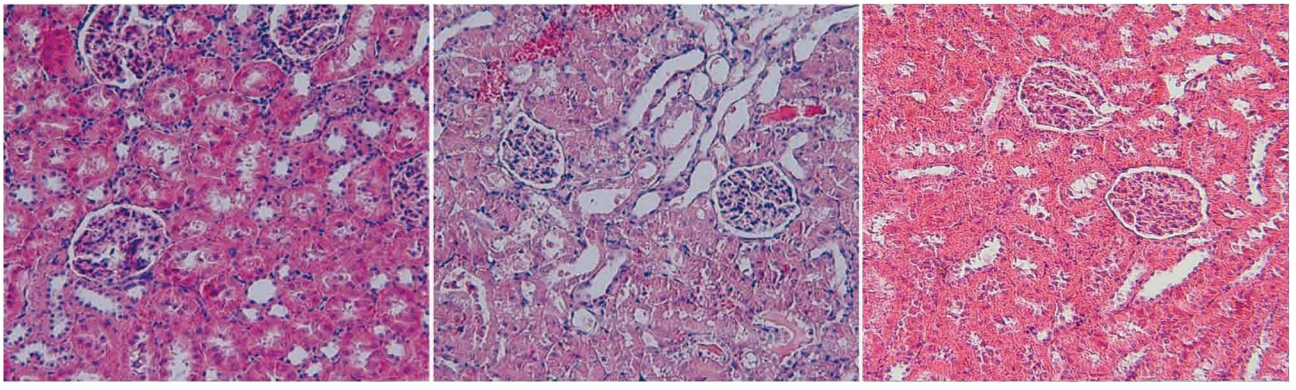

Figure 5. Light photomicrographies of the rats' kidney sections (hematoxylin-eosin, × 200). **Left,** Kidney sections from the control group with normal renal morphology. **Middle,** The cisplatin group showed the distinctive pattern of renal injury. **Right,** The rats treated with *Nigella sativa* showed relatively well-preserved architecture.

showed a distinctive pattern, which included widespread degeneration of tubular architecture, sloughing tubular epithelial cells from the basement membrane, tubular cell necrosis, and intratubular cast formation, especially in the outer medulla. Renal sections obtained from the rats treated with NS demonstrated a relative reduction of the histologic features of the kidney injury.

DISCUSSION

Nigella sativa and its compounds are used as natural herbal products for healing of many diseases. Many studies have been conducted to investigate the pharmacologic use of NS seeds. Most of them confirmed the analgesic, anti-inflammatory, antioxidant, antimicrobial, and anti-parasitic properties of this plant.[20] Therefore, we hypothesized that NS can reduce nephrotoxicity of medications such as cisplatin. As shown in present histopathologic and biochemical data, cisplatin induced kidney failure which continued until the end of the experiment (day 42). Faubel and colleagues demonstrated that cisplatin-induced AKF is associated with increases in interleukin-1, interleukin-18, and interleukin-6 levels, and neutrophil infiltration in the kidney. However, inhibition of the interleukins or neutrophil infiltration is not sufficient to prevent cisplatin-induced AKF.[21]

The results of our study indicated that NS seeds did not have any effect on the kidney function indicators or triglyceride levels in cisplatin-treated rats. Although the concentration of serum triglyceride significantly decreased on day 42 compared to day 14 after using of NS, this reduction in triglyceride levels could not be attributed to the effect of black seeds, because there was no significant change in triglyceride levels compared to the cisplatin-treated group on day 42. Zaoui and colleagues showed that oral administration of 2 mg/kg of black seeds for 12 weeks significantly decreased the triglyceride, glucose, and cholesterol levels and the number of leukocytes and platelets, while hematocrit and hemoglobin levels significantly increased.[22] Salim and colleagues demonstrated that NS seeds did not have any effect on the blood parameters.[23] Therefore, there is seems to be no consensus about the effects of NS seeds on biochemical blood factors. Overall, it appears that the effects of NS on blood chemical parameters may be long-term. Although some studies showed the administration of black cumin alone significantly decreased the biochemical parameters of the blood, co-administration of NS with other chemical compounds might have different effects on blood chemical parameters. For example, thymoquinone at doses of 4.8 mg/kg to 50 mg/kg was not able to change serum biochemical parameters after using of carbon tetrachloride,[24] but it could have protective effects against renal toxicity induced by methotrexate and doxorubicin.[25] In another study, oral administration of NS seeds had a protective effect on renal toxicity induced by potassium bromated.[26] In addition, black cumin has potent free radical scavenger and antioxidant properties; it seems to be a highly promising agent for protection of tissues from oxidative damages and organ damage due to kidney failure.[27] Mansour and associates also reported that NS seeds could

produce a marked inhibition in the secretion of leukotrienes, a mediator of mucosal tissue injury and hypoxemia.[28]

As shown in Figures 2 and 3, cisplatin increased the serum BUN and creatinine levels and nigella sativa was not able to decrease these levels. Maliakel and coworkers demonstrated that administration of cisplatin significantly increased the serum creatinine and urea concentration compared to the control group.[29] However, Zaoui and colleagues showed that blood creatinine levels did not decrease even by treatment of NS seeds for 12 weeks.[22] *Nigella sativa* acts in the kidney as a potent scavenger of free radicals to prevent or inhibit the toxic effects of cisplatin on kidney function; however, its effect on the biochemical and histopathologic parameters is not evident by the current data.[27]

Nigella sativa is composed of about 100 pharmacologic active ingredients, one of the most important of which is thymoquinone. It was shown that thymoquinone has antioxidant effect. Oxidative stress can exaggerate kidney toxicity induced by cisplatin. The other ingredients of NS can exert beneficial effects on the renal toxicity induced by cisplatin, as well.[28] Many studies have been conducted, particularly during the past 2 decades, on the effects of NS seeds extract or its active compounds.[30] Administration of NS seeds extract was effective in ameliorating the biochemical and physiological indexes of nephrotoxicity before the administration of the nephrotoxic drug cisplatin.[31] Ali and colleagues reported that thymoquinone attenuated the nephrotoxicity of cisplatin. Also, NS oil produced a dose-dependent amelioration of the biochemical and histological indexes of gentamicin-induced nephrotoxicity.[32]

CONCLUSIONS

Cisplatin-induced nephrotoxicity was confirmed by our study, but administration of alcoholic extract of NS seeds had a little effect on the biochemical factors at this dose. However, histopathologic properties of kidney tissue relatively recovered with NS extract. It seems that NS extract has different effects on the biochemical and histopathologic factors. This difference may be partially due to time-course action on kidney functional improvement. Thus, further investigations on the determination of proper dose and mechanisms of action of NS seeds are required.

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CONFLICT OF INTEREST

None declared.

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Correspondence to:
Keshavarzi Zakieh, PhD
Department of Physiology, Physiology Research center,
University of Medical Sciences, Mashhad, Iran
Tel: +98 511 800 2221
E-mail: zakieh_keshavarzi@yahoo.com

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