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ARAŞTIRMA YAZISI / RESEARCH ARTICLE

SIÇANLARDA KARBOPLATİN İLE İNDÜKLENEN NEFROTOKSİSİTE ÜZERİNE NİGELLA SATİVA YAĞININ KORUYUCU ETKİLERİ

PROTECTIVE EFFECT OF NIGELLA SATIVA OIL ON CARBOPLATIN INDUCED NEPHROTOXICITY IN RATS

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ÖZ

AMAÇ: Karboplatin yaygın kullanılan bir kemoteröpatik ajandır. Böbrekler kemoteröpatiklerin yan etkilerinden etkilenen önemli organlardır. Bu çalışmanın amacı karboplatin kullanımına bağlı oluşan böbrek hasarına karşı Nigella sativa yağının (NSY) koruyucu etkisini araştırmaktır.

GEREÇ VE YÖNTEM: Tüm hayvanlar (n=24 dişi wistar-albino sıçan) 4 gruba bölündü; birinci grupta 4 ml/kg serum fizyolojik (SF) 1 ve 2.gün uygulandı. İkinci gruba ilk gün 4 ml/kg NSY ve 4 ml/kg SF 2. Gün i.p. verildi. Üçüncü gruba 1.gün 4 ml/kg SF ve 2.gün ise karboplatin 80 mg/kg i.p. uygulandı. Dördüncü gruba 1.gün NSY ve 2. gün ise karboplatin 80 mg/kg i.p. uygulandı. İkinci günün sonunda sıçanlar sakrifiye edildi ve böbrek dokuları nötral formalin ile fikse edildi. Histopatolojik değişiklikler ve apoptotik index (AI) değerlendirildi.

BULGULAR: Apoptotik indexte, karboplatin+SF grubunda kontrol grubuna göre artış görülürken, karboplatin+ NSY grubu ile anlamlı bir fark görülmemiştir. Histopatolojik değerlendirmede ise; Karboplatin+SF grubunda proksimal ve distal tubul epitelinde, glomerular kapiller yumaklarında dejenerasyon, tubuller arasında bulunan vasküler oluşumlarda konjesyon, intraglomerular, periglomerular, tubuller arası ve vasküler oluşumların tunika adventisyasında kollagen lif yoğunluğunda artış, Periyodik Asit Schiff (PAS) reaksiyonu sonucu yer yer basal membran bütünlüğünün bozulduğu görülmüştür. Karboplatin+ NSY verilen grupta ise bazı alanlardaki tubul yapılarında dejeneratif değişikliklerin devam ettiği görülürken glomerul yapılarının daha düzenli olduğu gözlemlenmiştir. Karboplatin+ NSY verilen grupta karboplatin+SF verilen gruba göre sklerotik değişimlerin daha az olduğu gözlendi. PAS reaksiyon sonucu karboplatin+ NSY verilen grupta basal membranların daha düzenli bir yapıda olduğu görüldü.

SONUÇ: Baharat olarak da kullanılan nigella sativanın karboplatin ile indüklenen nefrotoksisite üzerine koruyucu etkileri olabilir.

ANAHTAR KELİMELER: Apoptozis, Böbrek, Karboplatin, Nigella sativa yağı, Sıçan

ABSTRACT

OBJECTIVE: Carboplatin is a commonly used chemotherapeutic agent. Kidneys are an important organ affected by the adverse effects of chemotherapeutic agents. This study aimed to investigate the protective effect of Nigella sativa oil (NSO) against kidney damage due to carboplatin exposure.

MATERIAL AND METHODS: All animals (n=24 female wistar-albino rats) were divided into four groups; 4 ml/kg saline was intraperitoneally (i.p.) administered on day one and two in the first group. 4 ml/kg NSO on day one and 4 ml/kg saline on day two was i.p. administered in the second group.4 ml/kg saline on day one and 80 mg/kg carboplatin on day two was i.p. administered in the third group.4 ml/kg NSO on day one and 80 mg/kg carboplatin on day two was i.p. administered in the fourth group. Rats were sacrificed at the end of day two and renal tissues were fixed in neutral formalin. Histopathologic changes and apoptotic index (AI) were evaluated.

RESULTS: While an increase was observed in the apoptotic index of carboplatin+saline group compared to the control group, no significant differences were found in the carboplatin+saline and carboplatin+NSO group. In the histopathological evaluation, degeneration in the proximal and distal tubular epithelium and glomerular capillary glomus bodies, congestion in the vascular formations between the tubules, increase in collagen fiber density in the tunica adventitia of intraglomerular, preglomerular, intertubular and vascular formations, and sporadic basal disintegration due to Periodic Acid Schiff (PAS) reaction were observed in the carboplatin+saline group. In the carboplatin+NSO group, degenerative changes in some areas of tubular structures continued while it was observed that glomerular structures were more regular. It was observed that sclerotic change was fewer in the carboplatin+NSO group than in the carboplatin+saline group. It drew attention that basal membranes were more regular in the carboplatin-nigella sativa oil group as a result of PAS reaction.

CONCLUSIONS: NSO, is used as a spice, may have a protective effect on carboplatin induced nephrotoxicity.

KEYWORDS: Apoptosis, Carboplatin, Kidney, Nigella sativa oil, Rat

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INTRODUCTION

Carboplatin is used as a 2nd generation platinum-group medication for gynecologic etc. cancers (1, 2, 3). Carboplatin is wholly removed from the body through kidneys after administration (4). The biggest factor that limits the use of chemotherapeutic agents in cancer treatment is their dose-related toxic adverse effects. The use of this cytotoxic medication poses a particular risk in the dysfunction of kidneys which play a role in drug metabolism and elimination (5, 6).

It is known that these drugs cause tubular and glomerular dysfunctions, tubular obstruction ,tubulo-interstitial damage and nephrotoxicity (7). Carboplatin binds to DNA in tumor tissue to create a lesion, showing its cytotoxic effect (8).

The biggest adverse effect of chemotherapeutics used for the tumor cells is the formation of free radicals in the tissue and that large molecules like DNA in cells cause irreversible changes (9, 10).

Nigella sativa (NS) is a substance which is generally used as an herb and has been considered in alternative medical treatment recently (11). Oil of Nigella sativa (NSO) which has a black seed and the thymoquinone (TQ) within are used in medical applications (11, 12, 13).

Its antioxidant feature which prevents oxidative damage has been proved in several studies (11, 12, 14, 15, 16). This study aimed to histopathologically investigate the protective effects of NSO administered i.p. before the carboplatin administration against kidney damage caused by carboplatin which is used especially for ovarian, head-neck and childhood cancers.

MATERIALS AND METHODS

Nigella sativa oil was obtained form the legal manufacturer. Nigella sativa seeds were provided from Burdur, Turkey. Nigella seeds were pressed by chrome-nickel cold press oil machines (any solvent or heating) and %30 oil was obtained. After the filtration process, NSO was applied. The Nigella sativa oil contained % 24.55 Thymoquinone according to chemical analysis by Gas Chromatography (29).

ETHICS COMMITTEE

This study was approved by the Kobay Local Animal Ethics Committee, Ankara, Turkey with Ethical approval no: 2018/298 .

Experimental procedure : All animals (24 female, wistar-albino rats (250-300 grams each) were obtained from Kobay Ltd. & Co. (Ankara, Turkey) and were divided into four groups. 4 ml/kg saline was administered intraperitoneally (i.p.) on days one and two in the first group (n=6). 4 ml/kg NSO on day one and 4 ml/kg saline on day two was i.p. administered in the second group (n=6). 4 ml/kg saline on day one and 80 mg/kg carboplatin on day two was i.p. administered in the third group (n=6). 4 ml/kg NSO on day one and 80 mg/kg carboplatin on day two was i.p. administered in the fourth group (n=6). Rats were sacrificed at the end of day two. Renal tissues were taken away and were fixed in 10% neutral formalin for 72 hours. After tissue processing, all kidney tissues were made paraffin block and 4 µm-thick slices were taken from each. Hematoxylin-eosin, Masson's trichrome and Periodic acid-Schiff (PAS) stains were performed for histopathological evaluation. TUNEL was used for staining and the apoptotic index (AI) was evaluated.

Hematoxylin-Eosin (H-E) Staining Protocol: The paraffin slices (4 µm-thick) were placed in an oven overnight at 37-62°C and rinsed with xylene to achieve (3x20 minutes) deparaffinization. They were rinsed with descending ethyl alcohol series (10') for rehydration. Next, they were stained with Harris hematoxylin stain solution and eosin (x10 minutes) for each. After dehydration, all slides were covered with Entellan®. All kidney figures were captured and were evaluated in Leica Q Win 3 software.

Periodic Acid-Schiff (PAS) Staining Protocol: The slices (4 µm-thick) were sectioned from the paraffin blocks. Having deparaffinized in a vacuum oven at 37°C overnight and then rinsed with xylene and descending ethyl alcohol series, the slices were rinsed with distilled water and soaked in 0.5% periodic acid solution for 10 minutes. After being rinsed with distilled water and soaked in Schiff reactive solution for 20 minutes, they

were washed with sodium metabisulphite for 2x5 minutes. Next, the slices were washed with tap water and stained with Harris hematoxylin for 10-15 minutes. The slices were rinsed with increased alcohol series and xylene and covered with Entellan. They were evaluated in Leica DM 4000B (Germany) computer-aided image analysis system.

Masson's Trichrome Staining Protocol: After deparaffinization, Atom Scientific BIOSTAIN Masson's Trichrome Stain Kit – Methylene Blue (Code: RRSK20-100) was applied on the kidney slices (4 µm-thick),. The slices were stained with Weigert's Iron Hematoxylin prepared by mixing Hematoxylin Weigert's A and Hematoxylin Weigert's B solutions in equal amounts for 20 minutes. After being washed with 1% acid alcohol solution, they were stained in Ponceau-Fuchsin solution for 5 minutes and washed with deionized water. The slices were soaked in 1% phosphotungstic acid solution for 10 minutes and stained with 2.5% acetic acid-2% Methylene Blue solution for 5 minutes. Rinsed with ascending alcohol series and xylene, they were covered with Entellan.

MATERIALS AND METHODS

***TUNEL Method*:** To identify apoptosis Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) method was applied. For this, Millipore ApopTag Peroxidase in Situ Apoptosis Detection kit (ApopTag Peroxidase in Situ Apoptosis Detection kit, Lot: 2603388, Merck Millipore) was used. The slices (4 µm-thick) were washed with PBS for 5 minutes after deparaffinization with xylene and descending alcohol series.

They were incubated with 20 µg/ml proteinase K for 15 minutes. Following washing with PBS for 2 minutes they were soaked in 3% hydrogen peroxide to block the indigenous peroxidase activity. Equilibration Buffer was dripped on the slices washed with PBS for 2x5 minutes and the slices were kept at room temperature.

They were then kept in a humid environment at 37°C for 1 hour. Next, the tissues were kept in Stop/Wash tampon for 10 minutes and washed with PBS. They were incubated with Anti-Digoxin Peroxidase solution at room temperature for 30 minutes. Washed with PBS, the tissues were incubated with chromogen DAB (Cat: DABC-004, Lot: HD25395, Spring Bioscience).

Then, they were washed with distilled water for 3x1 minutes and soaked in Methylene Green for 10 minutes. Washed with distilled water again, the tissues were rinsed with ascending alcohol series and xylene and covered with Entellan. Figures captured from the slices in Leica DM 4000B (Germany) computer-aided image analysis system were evaluated in Leica Q Win 3 software.

Cells which have undergone apoptosis and with TUNEL positive stain in random 10 areas in different slices of each group were counted in x40 magnification, and the apoptotic cell distributions were determined with the statistic.

STATISTICAL ANALYSIS

Statistical analysis were performed in IBM SPSS Statistics 20. Kruskal-Wallis non-parametric variance analysis was used. Post-hoc comparisons was performed with Bonferroni correction Mann-Whitney U to identify the differences between groups. Values at $p<0.05$ were accepted to be statistically significant.

RESULTS

Hematoxylin-Eosin: In small and large magnifications in the control group, it was seen that the glomerules, proximal and distal tubules in the cortex were normal **(Figure 1A)**.

In the NSO-saline group, glomerulus, proximal and distal tubules in the cortex were observed to be similar to the control group in small and large magnifications and no degenerative change was observed **(Figure 1B)**.

Degenerative changes in epithelial cells of proximal and distal tubules in the cortex, the disintegration of some epithelial cells from the tubular basal membrane, distinct congestion in intertubular vascular formations and degenerative changes in some glomerular capillaries, were noticed in the carboplatin-saline group **(Figure 1C)**. In the carboplatin-NSO group, degenerative changes in some tubular epithelial cells, congestion in vascular formations conges-

tion and inflammatory cellular reactions continued like in the carboplatin-saline group, but degenerative changes in glomerulus and tubular structures in some areas were not observed. It was also noticed that the were in a more regular histological structure compared to the carboplatin group **(Figure 1D)**.

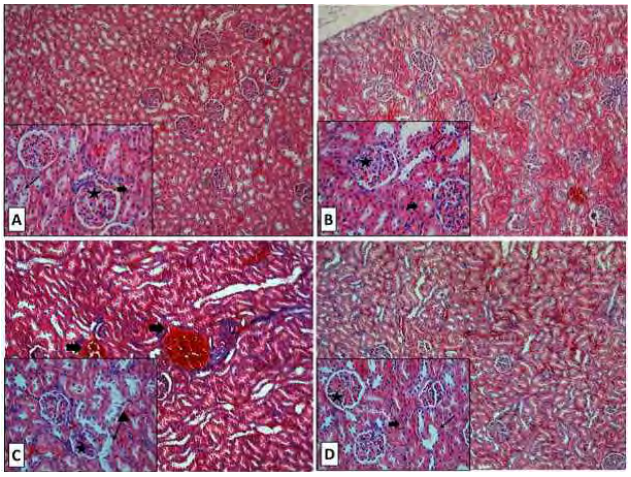

Figure 1 : Control group (A): normal glomerule in renal cortex (star), proximal (thick arrow) and distal tubules (thin arrow) (Hematoxylin-Eosin, x100, x400); Nigella sativa group (B): glomerule observed with the normal histological structure in cortex (star], proximal (thick arrow] and distal tubules (thin arrow] (Hematoxylin-Eosin, x100, x400]; Carboplatin group (C]: degenerated glomerule in cortex (star], tubules (thin arrow], tubular epithelial cells disintegrated from basal membrane (triangle], congestion (arrow] (Hematoxylin-Eosin, x100, x400]; Nigella sativa-carboplatin group (D]: glomerule observed in cortex compared to carboplatin group (star], proximal (thick arrow) and distal tubules (thin arrow), preserved morphology (Hematoxylin-Eosin, x100, x400)

Masson's Trichrome: Collagen fiber structure of normal distribution and density in the glomerular basal membrane, around veins and between tubules was observed **(Figure 2A)**.

Collagen fiber density and distribution in the NSO group were similar to the ones in the control group **(Figure 2B)**.

It was observed in the carboplatin-saline group that intraglomerular, periglomerular, between collecting duct collagen fiber density increased, and a sclerotic appearance occurred in these areas, collagen fiber density increased in the tunica adventitia of vascular formations **(Figure 2C)**.

It was observed that the NSO-carboplatin group had a collagen fiber density in an amount between those of the control and carboplatin groups and the sclerotic changes were fewer in capboplatin+NSO group compared with carboplatin group. It was noted that the normal structure was preserved in certain areas **(Figure 2D)**.

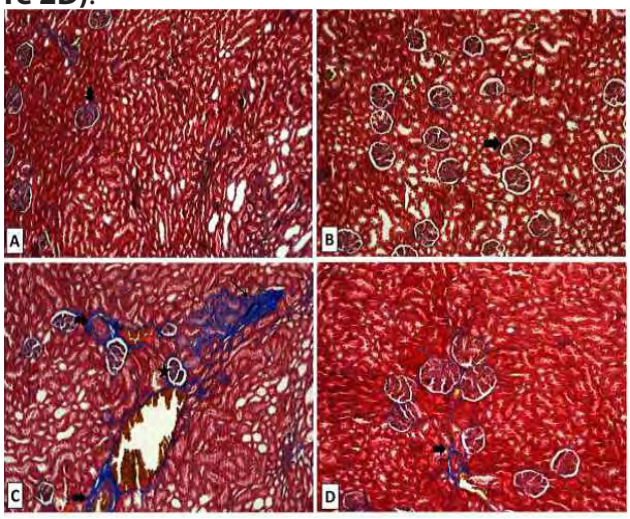

Figure 2 : Control group (A): normally distributed collagen fiber structure in glomerular (thick arrow), distal and proximal tubules (thin arrow); Nigella sativa group (B): normally distributed collagen fiber structure in glomerular (thick arrow) and distal and proximal tubules (thin arrow); Carboplatin group (C): intra glomerular, periglomerular (star), in-vein tunica adventitia (thick arrow) collagen fiber density and intertubular sclerotic space (think arrow); Nigella sativa-carboplatin group (D): lower collagen fiber density compared to carboplatin group (arrow) (A,B,C,D: Masson's Trichrome, x100)

Periodic Acid-Schiff (PAS): In the control group, it was seen that the basal membranes of proximal and distal tubules in the cortex were normal **(Figure 3A)**.

It was also observed that, proximal and distal tubular structures in the cortex were normal and the integrity of basal membranes was preserved in the nigella sativa-saline group **(Figure 3B)**.

In the carboplatin-saline group, it was noticed with PAS staining that there was partial disintegration of glomerular and tubular basal membrane **(Figure 3C)**. Integration of the glomerular basal membrane was better in the

nigella sativa-carboplatin group than in the carboplatin-saline group, but in some area similar, pathological changes continued like in the carboplatin group **(Figure 3D)**.

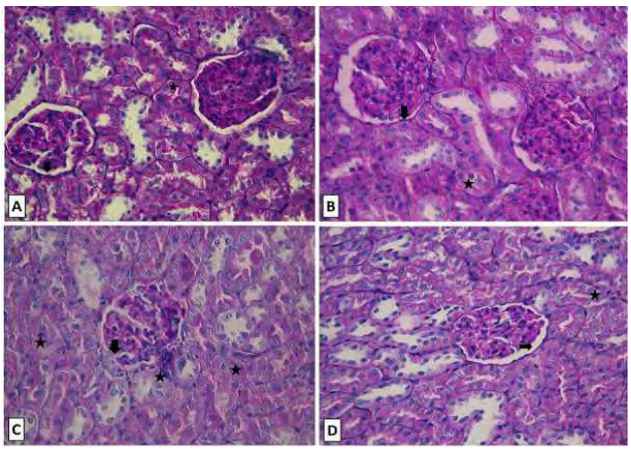

Figure 3 : Control group (A) Glomerular (thick arrow) and tubular (star) basal membrane observed in a normal histologic structure in cortex; Nigella sativa group (B): glomerular (thick arrow) and tubular (star) basal membrane with normal integrity in the cortex; Carboplatin group (C): disintegrated glomerular (arrow) and tubular (star) basal membrane in the cortex; Nigella sativa-carboplatin group (D): more preserved glomerular (thick arrow) and tubular (star) basal membrane compared to carboplatin group (A,B,C,D: PAS, x400)

Apoptosis: No statistically significant difference was found between the number of TUNEL-positive renal tissue cells in the control and NSO groups (p=1.000) Number of TUNEL-positive cells in the control group was significantly lower than the numbers of the carboplatin-saline and carboplatin-NSO groups (p<0.000). When comparing the carboplatin-saline and carboplatin-NSO groups, mean values for the number of TUNEL-positive renal tissue cells were lower in the carboplatin-NSO group whereas no statistically significant difference was found between the two groups (p=0.062) **(Figure 4, Figure 5)**.

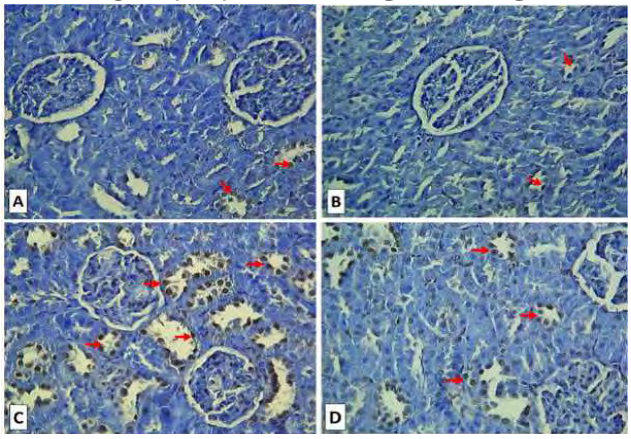

Figure 4:TUNEL-positive renal tissue cells (arrow) in control group (A), Nigella sativa group (B), Carboplatin group (C), Nigella sativa-carboplatin group (D) (DAB & hematoxylin; x400)

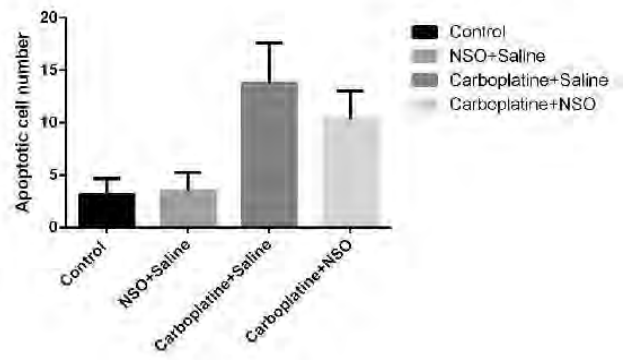

Figure 5: Number of TUNNEL (+) cells in the groups (p<0.005).

DISCUSSION

Carboplatin is a platin-group chemotherapeutic commonly used in the treatment of ovarian, lung, head-neck, vs. cancers (17). It is a drug largely removed from the body through kidneys and with 0-25% nephrotoxicity according to previous studies (18, 19). Kidneys are the most functional organs in drug metabolism and elimination due to the adverse effects of these drugs used for the elimination of tumor cells, (7). More studies have been now conducted on antioxidants to prevent tissue damage caused by chemotherapeutics due to oxidative stress. In several recent studies, NSO's antioxidant feature has been put forward and it has been demonstrated that it causes a decrease in free oxidative stress markers at tissue level (15, 20, 21, 22, 23, 24). This study examined the protective effects of NSO administered before carboplatin administration on kidneys.

In summary, i.p. exposure of carboplatin caused histopathological changes and damage in renal tissue, induced apoptosis, as well as NSO having positive impacts on the histopathological damage and causing a decrease (even if not statistically significant) in apoptosis.

According to the apoptosis results of our study, although there was not a statistically significant difference between the carboplatin-NSO and carboplatin groups, apoptosis was found to be lower in the NSO group. Elsherbiny et al. (2017) examined the protective effects of TQ, an NS derivative, in different doses against renal damage induced by sodium nitrite in rats. In the oral administration of 25 mg/day and 50 mg/day for 3 months, an increase was observed in the sodium nitrate group in apoptotic markers caspase-3, caspase-8, and caspase-9 while a decre-

ase was seen in TQ groups in caspase-3, 8, and 9 in proportion to dose increment (24). In our study, the fact that NSO (4mg/kg) was i.p. administered pre-protectively and in a shorter period with a lower dose caused a decrease (even if not statistically significant) in the number of apoptotic cells which is in agreement with this study. The shorter duration and lower dose of the administration might have been less effective in decreased apoptosis. In the study conducted to examine NSO's effectiveness against renal ischemia-reperfusion damage, Havakhah et al. (2014) observed that administration of 150 mg/kg and 300 mg/kg intravenous (i.v.) Nigella sativa hydro-alcoholic extract (NSE) before ischemia and during reperfusion was not effective against DNA damage in the pre-ischemia group but caused distinct decreased DNA damage in the reperfusion group (21). Preventive effect on DNA damage in acute and high-dose exposure has partially compatible results with our study.

Al-Gayyar et al. (2016) observed a decrease in apoptosis marker caspase-3 due to (2, 5, 5, 10 ml/kg) oral NSO exposure in similar doses to and higher doses than our study in parallel with dose increment against chronic sodium nitrate exposure for 12 weeks (22).

Histopathologically, it was noted in the carboplatin and non-NSO group that there were degenerative changes in proximal and distal tubular epithelial cells in the cortex, distinctive congestion in intertubular vascular formations and degenerative changes in certain glomerular capillaries; intraglomerular, periglomerular and inter-collecting duct collagen fiber density increased and a sclerotic appearance occurred, collagen fiber density increased in the tunica adventitia of vascular formations, and tubular basal membrane disintegrated partially. It was observed after only NSO administration without carboplatin that degenerative changes in partial tubular epithelial cells, congested vascular formations and inflammatory cellular reactions continued, glomerules exhibited a more regular histological structure, collagen fiber density was normal, sclerotic changes were fewer, and glomerular basal membrane integrity was preserved better. In line with our study, Farooqui et al. (2017) orally administered 2 ml/kg NSO and 1.5 mg/kg TQ separately for 14 days against cisplatin exposure (25). It was observed that the cisplatin caused a decrease in brush border membrane enzymes and NSO and TQ administration eliminated the decrease. Glomerular congestion, edema in renal tubules, and interstitial hemorrhage had been observed in renal tissues of the cisplatin group. In NSO and TQ groups, glomerular congestion decreased, and no tubular edema and interstitial hemorrhage were observed. Although the administration was for a shorter period and in a different method in our study, comparable results were achieved.

Elsherbiny et al. (2017) reported that fewer tubular degeneration and recovery in basal membrane damage were achieved when 25 mg/day and 50 mg/day TQ administered orally for 3 months against sodium nitrite exposure (24).

In the ischemia-reperfusion study by Havakhah et al. (2014), histological changes were graded in renal tissue following the i.v. administration of 150 and 300 mg NSO, and similarly in our study, NSE exposure before the ischemia caused a distinct decrease in tubular lesions (21). Similar to our study, Elsherbiny and Sherbiny (2014) investigated the effect of TQ (50 mg/kg/day, oral, 3 weeks) which is an NS derivative against renal damage due to Doxorubicin (DOX) (3.5 mg/kg i.p.) which is a chemotherapeutic medication and observed increased Bowman's space volume and distinct degeneration in renal tubules due to DOX exposure and administration of TQ with DOX achieved a near-normal structure in renal tissue (26). Yaman and Balikci (2009) examined plasma urine and creatinine levels, antioxidant marker levels and histological changes after administration of 0.2 ml/kg and 0.4 ml/kg i.p. NS (6 days) against renal damage induced by gentamycin (27). While urine creatinine levels increased, decreased tubular brush border and intertubular hemorrhage, congestion in glomerulus, edema and change in the basal membrane were observed in the gentamycin group, decreased urine creatinine levels and moderate histopathological lesion in renal tubules were observed in the NS group. Al-Gayyar et al. (2016) noted a decrease

in serum urine creatinine level and fibrosis markers due to oral administration of 2.5, 5, 10 ml/kg NSO for 12 weeks against sodium nitrite-induced nephrotoxicity and observed a near-normal histopathological structure in kidneys in the sodium nitrite group (22).

In their study with diabetic-nephropathic patients, Ansari et al. (2017) administered 2.5 mg/day NSO orally. A decrease in serum creatinine and blood urea levels and an increase in glomerular filtration ration were observed in the NSO group (28).

CONCLUSION

NSO, when used pre-protectively, proved to have a protective effect even if it is partial on histopathology of renal tissue. Such a protective effect of NS which is utilized as an herb in a natural diet in the Eastern culture promise hope for its usage as an antioxidant against adverse effects of chemotherapy among cancer patients.

REFERENCES

1. Chen X, Wang J, Fu Z, Zhu B, Wang J, Guan S, Hua Z. Curcumin activates DNA repair pathway in bone marrow to improve carboplatin-induced myelosuppression. ScieNTific RePortS 2017; 7:17724.

2. Tanley SWM, Diederichs K, KroonBatenburg LM. J., Levy C, Schreurs AMM, Helliwell JR.Carboplatin binding to histidine. Acta Cryst.2014; 70: 1135–1142.

3. Rajeswarana A, Trojanb A, Burnanda B, Giannelli M. Efficacy and side effects of cisplatin- and carboplatin-based doublet chemotherapeutic regimens versus non-platinum-based doublet chemotherapeutic regimens as first line treatment of metastatic non-small cell lung carcinoma: A systematic review of randomized controlled trials. Lung Cancer 2008; 59: 1-11.

4. Matsuoka A, Ando Y. Nephropathy in Patients Undergoing Cancer Drug Therapy - Platinum Derivatives (Cisplatin and Carboplatin). Gan To Kagaku Ryoho 2017; 44(3):200-203.

5. Sharbaf FG, Farhangi H, Assadi F. Prevention of Chemotherapy-Induced Nephrotoxicity in Children with Cancer .Int J Prev Med 2017; 8: 76.

6. Kintzel PE, Dorr RT. Anticancer drug renal toxicity and elimination: dosing guidelines for altered renal function. Cancer Treat Rev 1995; 21(1): 33-64.

7. Lameire N, Kruse V, Rottey S. Nephrotoxicity of anticancer drugs an underestimated problem? Acta Clin Belg 2011; 66(5):337-45.

8. Rabik CA, Dolan ME. Molecular mechanisms of resistance and toxicity associated with platinating agents. Cancer Treat Rev 2017; 33; 1:9-23.

9. Fuchs-Tarlovsky V. Role of antioxidants in cancer therapy. Nutrition 2013; 29 (1) 15-21.

10. Langer T, am Zehnhoff-Dinnesen A, Radtke S, Meitert J, Zolk O. Understanding platinum- induced ototoxicity. Trends Pharmacol Sci 2017; 34(8):458-69.

11. Khader M, Eckl PM. Thymoquinone: an emerging natural drug with a wide range of medical applications. Iran J Basic Med Sci.2014;17(12):950-7.

12. Eid AM, Elmarzugi NA, Abu Ayyash LM, Sawafta MN, Daana HI. A Review on the Cosmeceutical and External Applications of Nigella sativa. J Trop Med. 2017; 7092514.

13. Khan MA1, Chen HC, Tania M, Zhang DZ. Anticancer activities of Nigella sativa (black cumin). Afr J Tradit Complement Altern Med. 2011; 8(5):226-32.

14. Hosseinian S, Ebrahimzadeh Bideskan A, Shafei MN, Sadeghnia HR, Soukhtanloo M, Shahraki S, Samadi Noshahr Z, Khajavi Rad A. Nigella sativa extract is a potent therapeutic agent for renal inflammation, apoptosis, and oxidative stress in a rat model of unilateral ureteral obstruction. Phytother Res. 2018;32(11):2290-2298.

15. Darakhshan S, Bidmeshki Pour A, Hosseinzadeh Colagar A, Sisakhtnezhad S. Thymoquinone and its therapeutic potentials. Pharmacol Res 2015; 95-96:138-58.

16. Mollazadeh H, Hosseinzadeh H, 2014. The protective effect of Nigella sativa against liver injury: a review. Iran J Basic Med Sci 2014; 17 (12): 958-966.

17. Ming M, Ma ZL , Xu YT , Sun FY, Cui XH. Carboplatin-based Nanomedicine to Enhance the Anticancer Effect in SK-NEP-1 Wilms Tumor Cells. Iran J Pharm Res 2017;16(4):1305- 1311.

18. Ruggiero A, Ferrara P, Attinà G, Rizzo D, Riccardi R. Renal toxicity and chemotherapy in children with cancer. Br J Clin Pharmacol 2017; 83(12):2605-2614.

19. Jeyabalan N, Hirte HW, Moens F. Treatment of advanced ovarian carcinoma with carboplatin and paclitaxel in a patient with renal failure. Int J Gynecol Cancer 2000; 10(6):463-468.

20. Ali BH, Blunden G. Pharmacological and toxicological properties of Nigella sativa. Phytother Res 2003; 17(4):299-305.

21. Havakhah S, Sadeghnia HR, Hajzadeh MA, Roshan NM, Shafiee S, Hosseinzadeh H, Mohareri N, Rad AK. Effect of Nigella sativa on ischemia-reperfusion induced rat kidney damage. Iran J Basic Med Sci 2014; 17(12):986-92.

22. Al-Gayyar MM, Hassan HM, Alyoussef A, Abbas A, Darweish MM, El-Hawwary AA. Nigella sativa oil attenuates chronic nephrotoxicity induced by oral sodium nitrite: Effects on tissue fibrosis and apoptosis. Redox Re 2016;. 21(2):50-60.

23. Dajani EZ, Shahwan TG, Dajani NE. Overview of the preclinical pharmacological properties of Nigella sativa (black seeds): a complementary drug with historical and clinical significance. J Physiol Pharmacol 2016; 67(6):801-817.

24. Elsherbiny NM, Maysarah NM, El-Sherbiny M, Al-Gayyar MM. Renal protective effects of thymoquinone against sodium nitrite-induced chronic toxicity in rats: Impact on inflammation and apoptosis. Life Sciences 2017; 180:1–8.

25. Farooqui Z, Shahid F, Khan AA, Khan F. Oral administration of Nigella sativa oil and thymoquinone attenuates long term cisplatin treatment induced toxicity and oxidative damage in rat kidney.Biomed Pharmacother 2017; 96:912-923.

26. Elsherbiny NM, El-Sherbiny M. Thymoquinone attenuates Doxorubicin-induced nephrotoxicity in rats: Role of Nrf2 and NOX4. Chem Biol Interact 2014; 5; 223:102-8.

27. Yaman I, Balikci E. Protective effects of nigella sativa against gentamicin-induced nephrotoxicity in rats.Exp Toxicol Pathol 2010; 62(2):183-90.

28. Ansari ZM, Nasiruddin M, Khan RA, Haque SF. Protective role of Nigella sativa in diabetic nephropathy: A randomized clinical trial. Saudi J Kidney Dis Transpl 2017; 28(1):9-14.

29. Erisgin Z, Atasever M, Cetinkaya K, Akarca Dizakar SÖ, Omeroglu S, Sahin H. Protective effects of Nigella sativa oil against carboplatin-induced liver damage in rats. Biomed Pharmacother. 2019 ;110:742-747.