Effect of *Nigella sativa* against cisplatin induced nephrotoxicity in rats

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Abstract

In this study, the gross composition and mineral content of *Nigella sativa* seed powder (NSP) and fatty acid composition of *Nigella sativa* oil (NSO) were investigated. The ability of NSP, extract (NSE) and NSO in reducing the effects of cisplatin-induced renal toxicity in Sprague-Dawley rats were examined. The obtained results showed that NSP contains high amounts of carbohydrates, protein, and fiber while NSO has higher amounts of linoleicacid, oleic acid, and myristic acid. Rats treated with NSP, NSO, and NSE exhibitedreducedserum levels of urea, creatinine, and potassium, and a significant increase of Na, Na/K, vitamin D, nutritional markers, and antioxidant enzymes compared to the cisplatin-induced renal toxicity group receiving no *Nigella sativa* seed treatment. This study determined that all powder, oil, and extracts of *N. sativa* contain potent bioactive components that may aid in treatment against cisplatin-induced renal toxicity in rats.

Introduction

Cisplatin is clinically proven as a potent treatment for various types of cancers, including sarcomas, testicular cancers, and cancers of soft tissue, lung, bones, muscles, and blood vessels. Unfortunately, clinical use of cisplatin is limited because it accumulates in renal tubules, causing acute kidney injury and, thus, harming normal tissues (Arany and Safirstein, 2003). Recent studies on the molecular mechanisms of chemotherapy-induced toxicity have revealed that cisplatin is the most frequent cause of nephrotoxicity when administered in higher doses. However, nephrotoxicity is multifactorial and involves numerous signaling pathways. Cisplatin act as oxidant stress promotes apoptosis of proximal tubule renal cells through mitochondrial dependent and independent pathways *in vitro* and *in vivo*. Cisplatin induces renal damage by generating reactive oxygen species, which cause mitochondrial dysfunction, DNA damage, and necrotic cell death as determined via both *in vitro* and *in vivo* studies (Maimaitiyiming *et al.,* 2013; Sahu *et al.,* 2014).

Nigella sativa (black seed) is an annual flowering plant that is used as a flavorful food additive and has long been used in folk medicine all over the world. *Nigella sativa* seeds may help prevent and treat respiratory diseases, inflammation, high blood pressure, and cell damage (El-Gharieb *et al.,* 2010). *Nigella sativa* seeds are more than 30% fixed oil and 0.40-0.45 w/w volatile oil. The volatile oil of *Nigella sativa* contains 18-24% thymoquinone and 46% monoterpenes. Nigella seed extract (NSE), the primary active ingredient of which isthymoquinone, has been traditionally used to promote respiratory, stomach, and intestinal health (Arslan *et al.,* 2005). *Nigella sativa* oil (NSO) has been traditionally used as a natural remedy for many diseases and ailments. The most potent bioactive components of NSO arethymoquinone, thymol, dithymoquinone, and thymo hydroquinone, which are used for treating epilepsy and allergies and supporting kidney function, liver function, the circulatory system, and the immune system (Kanter *et al.,* 2006).

The aim of this study was to investigate the gross composition and mineral content of *Nigella sativa* seed powder (NSP) and fatty acid composition of *NSO* in addition to investigate if *N. sativa* seed powder, extract, and/or oil could reduce cisplatin-induced renal toxicity in Sprague-Dawleyrats.

Materials and Methods

Chemicals

All reagents and materials were of analytical grade. Chemicals for biodignostic tests were obtained from BioMeriuex Kits (Alkan Co.). NSO was purchased from Silver Seas Co. Cisplatin was obtained from Sigma Chemical Company (St. Louis, MO, USA).

Preparation of *Nigella sativa* forms

Nigella sativa seeds were purchased from a local herbal shop in Riyadh, Saudi Arabia. The seeds (250 g) were cleaned, dried in a hot air oven, and mechanically grinded to obtain *Nigella sativa* powder (NSP). One hundred grams of NSP was extracted three times with 300 mL of 96% ethanol. The extracts were filtered, and the solvent was evaporated using a rotary, resulting in a blackish-brown liquid concentrate of NSE as expected (Hadjzadeh *et al.,* 2012). NSE was emulsified in water and administered to rats using a stomach tube.

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Key words: *Nigella sativa*, Renalfunction, Rats.

Conflict of interest: the author declares no potential conflict of interest.

Funding: none.

Received for publication: 18 December 2017.
Revision received: 29 March 2018.
Accepted for publication: 10 April 2018.

Italian Journal of Food Safety 2018; 7:7242
doi:10.4081/ijfs.2018.7242

Chemical composition of Nigella sativa powder

Moisture, protein, lipids, ash, and crude fiber content were determined using analytical methods as recommended by the AOAC (2000). Carbohydrate content was estimated by difference. Ca, Fe, Zn, Mg, K and Na levels were assessed using an Atomic absorption spectrophotometer as recommended by the AOAC (2000).

Animals and experimental design

The experiment was performed with a total of 50 male Sprague-Dawley rats weighing 165±10 g. Rats were provided from an experimental animal center in Prince Sultan Military Medical City, Riyadh. Rats were housed with a 12h dark/light cycle schedule, placed as groups in wire cages under normal laboratory conditions, and fed a standard diet during a one-week adaption period. Rats were given free access to water and standard diet. Ethical guidelines were maintained during animal handling and permission was obtained from the concerned Department. After one week of acclimatization, rats were divided into 5 subgroups (n=10). The negative control (NC) group was given a single I/P injection of saline. The other treatment groups were injected with a single I/P dose (8 mg/kg body weight) of cisplatin (1 mg/mL) dissolved in saline to induce nephrotoxicity. Nephrotoxicity in rats was

confirmed by observing elevated creatinine, urea nitrogen, and uric acid. The nephrotoxic rats were then divided into four subgroups: a positive control (PC) group that was administered distilled water; the NSP treatment group that was administered 3 g/kg/day of nigella sativa powder; the NSO treatment group that received 2 g/kg/day of nigella sativa oil; and the NCE group which was administered 0.5 g/kg/day of nigella sativa extract. All treatments given to nephrotoxic rats were administered daily via stomach tube for 60 days. Twenty-four hours after the last dose of the experiment, rats were sacrificed to obtain blood and kidney samples.

Biochemical analyses

Commercially available kits were used to estimate blood plasma levels of blood urea nitrogen (BUN), uric acid, creatinine, total protein, albumin, and globulin. Commercially available kits were also used to assess activity of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), and malondialdhyde (MDA) in the kidneys.

Parameter calculations

Body weight gain (BWG), body weight gain percent (BWG%), food intake (FI), feed efficiency ratio (FER), protein intake (PI), protein efficiency ratio (FER), and albumin/globulin ratio (A/G) were calculated.

Statistical analysis

Collected data were presented as mean±SD and statistically analyzed using one-way analysis of variance (ANOVA) according to Artimage and Berry (1987).

Results and Discussion

Table 1 showed that NSP contain high amount of carbohydrate, protein and fiber and low amount of fiber, fat and ash. Our results were somewhat in agreement with the values reported by Nergiz and Ötleş (1993). The obtained mineral content of NSP indicated major differences in the amounts of calcium, iron, sodium and potassium compared to results by Nergiz and Ötleş (1993) who reported 188±1.5, 57.5±0.5, 85.3±16.07 and 1180±10.0, respectively. Lower levels of potassium, iron, and sodium and higher levels of calcium *were estimated* in NSP as compared to Nergiz and Ötleş (1993). However, El-Tahir and Bakeet, (2006) observed the presence of 0.5-1% calcium, 0.6% phosphorus, 0.6% potassium, and 0.1% sodium in NSP. The difference in observed NSP composition is likely related to different crop varieties, regional environmental conditions, growing techniques, and fertilizer application. From these results, *NSP have* high carbohydrate, protein, calcium, and potassium content. The predominant fatty acids were linoleic, oleic, myristic, and palmitic acids, as the estimated percent values were 55.88±4.27, 23.89±2.57, 18.05±1.02, 10.95±1.82, and 3.90±0.41, respectively. There were also trace amounts of arachidic, linolenic and palmitoleic acids (0.30% ±0.04, 0.25% ±0.03, and 0.1% ±0.01, respectively). NSO had a higher percentage of unsaturated fatty acids (81.50% ±1.96) than saturated fatty acids (16.11% ±2.35). *Similar fatty* acid values were reported by Nickavar *et al.,* (2003) and Atta (2006). The fixed oil of *Nigella* seeds is rich in linoleic, oleic, and palmitic acids. The dominating fatty acid is linoleic acid, which is an essential fatty acid and accounts for more than 50% of total fatty acids in NSO. Linoleic and oleic acids represented the predominant unsaturated fatty acids and represented more than 80% of the total fatty acids. There was a high linoleic/oleic acid ratio, so Nigella oil should not be used in frying. Also, Bourgou *et al.,* 2010reported that linoleic acid was the major fatty acid (58.09%) in NSO, and was followed by oleic acid (19.21%) and palmitic acid (14.77%). Differences in Nigella seed oil fatty acid composition between studies are likely due to various factors, such as genetic variation and different oil processing methods.

In this study, *Nigella sativa* treatment, either in powder, oil, or extract form, induced a significant decrease of nutritional markers (*i.e.*, BWG, BWG%, FER, and PER) compared to the NC and a significant increase of nutritional markers compared to the PC. There were no significant differences between the *Nigella sativa* treatment groups (Table 2).

Table 1. Proximate, mineral and fatty acids composition of *Nigella sativa*.

Proximate composition (%) of NSP	
Protein	11.33±1.05
Fat	0.81±0.03
Fiber	1.75±0.10
Ash	0.65±0.04
Moisture	12.66±1.05
Carbohydrate	72.80±1.54
Minerals (mg per 100 g) of NSP	
Ca	201.08±1.83
Fe	18.88±0.51
Zinc	1.99±0.65
Mg	4.33±0.12
K	215.75±10.03
Na	37.27±2.12
Fatty acidcomposition (%) of NSO	
Myristic acid (C14:0)	18.05±1.02
Palmitic acid (C16:0)	10.95±1.82
Palmitoleic acid (C16:1)	0.1±0.01
Stearic acid (C18:0)	3.90±0.41
Oleic acid (C18:1)	23.89±2.57
Linoleic acid (C18:2)	55.88±4.27
Linolenic acid (C18:3)	0.25±0.03
Arachidic acid (C20:0)	0.30±0.04
Saturated fatty acids	16.11±2.35
Unsaturated fatty acids	81.50±5.96

Table 2. Nutritional markers in experimental rat groups.

	NC	PC	NSP	NSO	NSE
BWG	95.66±5.11^{a}	38.42±3.77^{d}	68.95±6.41^{b}	74.84±6.96^{b}	71.27±6.89^{bc}
BWG%	57.97±5.61^{a}	22.65±2.67^{d}	39.86±3.66^{bc}	42.77±4.31^{b}	40.96±4.1^{b}
FI	18.71±1.11^{a}	15.75±1.03^{b}	17.88±1.13^{a}	18.33±1.41^{a}	18.65±1.35^{a}
FER	0.085±0.003^{a}	0.040±0.002^{c}	0.064±0.001^{b}	0.068±0.002^{b}	0.063±0.001^{b}
PE	3.74±0.21^{a}	3.15±0.25^{ab}	3.57±0.38^{a}	3.66±0.26^{a}	3.73±0.24^{a}
PER	0.426±0.03^{a}	0.203±0.01^{c}	0.321±0.02^{b}	0.340±0.01^{b}	0.318±0.01^{b}

NC: normal control; PC: positive control; NSP: Nigella sativa powder; NSO: Nigella sativa oil; NSE: Nigella sativa extract. ^{a,b,c,d}Mean values in each raw having different superscript are significantly different at P<0.05.

Previous studies have shown that cisplatin is highly emetogenic, as it has been widely utilized to induce acute nausea and vomiting in preclinical animal models. The increase of nutritional markers in rat groups administrated *Nigella sativa*is likey due to it nutrient content and not due to its antioxidant properties (Takruri and Dameh, 1998). *Nigella sativa* seed powder is a good counteractive agent against the emetogenic effects of cisplatin because it is typically used as a digestive, appetite stimulant, and supplement to supportimmune system function. *Nigella sativa* seeds contain many important phenolics, such as kaempferol, p-coumaroyl acid derivative, and thymol-O-sophoroside. *Nigella sativa* seed extract has significant anti-inflammatory potential because of its antioxidant activity (Kadamand Lele, 2017). Also, there was a significant reduction of body weight in obese patients with fatty liver disease after three months treatment with *Nigella sativa* likely due to its ability to scavenge free radicals and inhibit lipid peroxidation (Heshmati *et al.,* 2015 and Hussain *et al.,* 2017). Thymoquinone is the primary active ingredient of *Nigella sativa*. When combined with polyphenols, compounds that facilitate lipid and carbohydrate metabolism, thymoquinone induces weight loss in diabetic rats (Kaur *et al*., 2017).

Serum urea and creatinine levels are indicators of acute tubular necrosis caused by cisplatin toxicity andnephrotoxicity. The PC group, which was treated with cisplatin alone, exhibited a significant increase in serum levels of urea, uric acid, and creatinine as compared to the NC group (Table 3). Treatment with NSP, NSE and NSO reduced the cisplatin-induced increase of urea, uric acid, and creatinine. Nigella oil and extract were especially effective at reducing the increase observed in the PC groups, but were not able to cause full recovery of normal (NC) urea, uric acid, and creatinine levels. In agreement with previous reports, initial cisplatin-induced renal dysfunction was caused by the inhibition of carnitine synthesis and reabsorption. Cisplatin further causes damage by accumulating in renal parenchymal cells and biotransforming into cysteinyl glycine conjugates and other high thiols via localized enzymes (Yao *et al.,* 2007). In agreement with previous reports, NS was observed to also have potent antioxidant properties and contained flavonoid antioxidants, quercetin, kaempferol, and luteolin (Randhawa and Alghamdi, 2011; Hadjzadeh *et al.,* 2012). Also, NSE has a significant nephroprotective activity for paracetamol-induced nephrotoxicity at various doses (250, 500 and 1000 mg/kg), as confirmed by reduced serum urea and creatinine (Canayakin *et al.,* 2016). Compared to the NC, the PC exhibited a significant reduction of total serum protein and globulin and an increase of albumin and A/G ratio levels. Treatment with NSP reduced levels of total protein and globulin, but there were no significant changes in albumin and A/G ratio. In contrast, treatment with NSO significantly reduced total serum protein, albumin, globulin, and the A/G ratio. Finally, NSE exhibited protein levels similar to the NC. Total protein and albumin measurements may reflect nutritional status and can be used to screen and diagnose kidney disease or liver disease. The NSO group had a significant reduction in total serum protein, albumin, globulin and A/G ratio, however the NSE group exhibited protein levels similar to the NC. Cisplatin binds to cytoplasmic proteins, disrupting their normal physiological function which leads to oxidative damage and cytotoxicity. Administration of NSP and NSO significantly reduced levels of albumin and A/G ratios and increased of globulin as compared to the PC (Karasawa *et al.,* 2013; Sahu *et al*., 2014). The NSO and NSE treatment groups both exhibited nephroprotective effects via lower serum creatinine, blood urea nitrogen, and antioxidant activity as compared to gentamicin control group values (Canayakin *et al.,* 2016). The cisplatin-induced renal toxicity PC group exhibited a significant decrease of Na, Na/K and vitamin D levels as compared to the NC group. All three NSP, NSO and NSE treatment groups exhibited significant increases of Na, Na/K, and vitamin D and significant decreases of potassium levels compared to the PC in the same table.

As previously mentioned, the predominant bioactive compounds in *Nigella sativa* are thymoquinone (30-48%), thymohydroquinone, dithymoquinone, *p*-cymene, carvacrol, α-pinene, thymol, quercetin, kaempferol, and quercetin-3. *Nigella sativa* also contains phytochemicals such as fixed and volatile oils, proteins, flavonoids, glycosides, alkaloids (mainly magnoflorine), and saponins that have strong antioxidant properties and palliative effects on inflammation (Ahmad *et al.,* 2013; Farag *et al.,* 2014). The observed decrease of Na, Na/K and vitamin D levels in the cisplatin-induced renal toxicity PC group may be due to suppressed renal tubular reabsorption of water and electrolytes into the blood stream. Black cumin and thymoquinone synergizes the nephroprotective effect against cisplatin-induced acute kidney injury and methotrexate induced nephrotoxcity in rats (Benhelima *et al*., 2016).

Table 3. Serum analyses for renal function indicators in experimental rat groups.

	NC	PC	NSP	NSO	NSE
Urea nitrogen (mg/dL)	45.41±4.51^{e}	129.66±10.11^{a}	85.71±8.77^{b}	70.79±7.81^{c}	65.13±6.13^{cd}
Uric acid (mg/dL)	1.45±0.37^{cd}	4.03±0.96a	2.89±0.41^{b}	2.54±0.29^{b}	1.88±0.33^{c}
Creatinine (mg/dL)	0.69±0.03^{d}	5.10±0.41^{a}	3.21±0.51^{b}	2.96±0.46^{bc}	2.11±0.39^{c}
Total protein	6.95±0.57^{a}	5.61±0.43^{bc}	5.99±0.41^{b}	5.95±0.42^{b}	6.44±0.66^{a}
Albumin (A)	3.29±0.22^{b}	3.53±0.23^{a}	2.89±0.20^{bc}	2.70±0.21^{c}	3.03±0.33^{bc}
Globulin (G)	3.66±0.32^{a}	2.08±0.29^{d}	3.10±0.38^{bc}	3.25±0.27^{bc}	3.41±0.35^{ab}
A/G	0.90±0.12^{bc}	1.70±0.19^{a}	0.93±0.10^{b}	0.83±0.11^{d}	0.89±0.11^{cd}
Na (mol/L)	135.77±30.61^{a}	90.79±11.71^{cd}	107.77±13.21^{b}	96.96±10.13^{bc}	110.77±15.22^{b}
K (mol/L)	3.55±0.61^{bc}	6.17±1.71^{a}	4.75±0.78^{b}	4.61±0.77^{b}	4.15±0.68^{b}
Na/K ratio	38.25±3.78^{a}	14.70±1.70^{d}	22.70±2.19^{c}	21.03±2.31^{c}	26.69±2.66^{b}
Vitamin D (nmol/L)	50.77±6.19^{a}	24.75±3.16^{d}	39.66±4.21^{bc}	40.31±5.10^{b}	43.78±3.66^{b}

NC: normal control, PC: positive control, NSP: Nigella sativa powder, NSO: Nigella sativa oil, NSE: Nigella sativa extract. ^{a,b,c,d}Mean values in each raw having different superscript are significantly different at P<0.05.

Table 4. Renal antioxidant parameters in experimental rat groups.

	NC	PC	NSP	NSO	NSE
Catalase (µ/mg)	3.88±0.71^{a}	0.55±0.19^{d}	2.61±0.32^{bc}	2.81±0.40^{bc}	2.94±0.38^{b}
SOD (µ/mg)	2.99±0.27^{a}	1.03±0.11^{d}	2.10±0.18^{c}	2.03±0.29^{bc}	2.30±0.33^{ab}
GPX (µ/mg)	1.91±0.22^{a}	0.64±0.13^{d}	0.99±0.19^{c}	1.11±0.31^{bc}	1.39±0.27^{b}
MDA (nmol/g)	6.41±1.45^{d}	13.77±2.17^{a}	10.31±1.96^{b}	9.66±1.41^{b}	8.41±1.03^{bc}

NC: normal control, PC: positive control, NSP: Nigella sativa powder, NSO: Nigella sativa oil, NSE: Nigella sativa extract, SOD: superoxide dismutase, GPX: glutathione peroxidase, MDA: malondialdhyde. ^{a,b,c,d}Mean values in each raw having different superscript are significantly different at P<0.05.

Treatment with NSE increased electrolyte excretion, having and a greater natriuretic effect than a kaluretic effect and a similar uricosuric effect with the control. The Na^+/K^+ ratio can be used to predict the diuretic mechanism due to an increase of the kaliuretic effect, but mostly to a decrease of Na^+ excretion (Toma *et al.,* 2015). This may be attributed to the antioxidant activity of the extracts. Polyphenols and flavonoids have been reported to be effective in reducing the oxidative stress from cisplatin, thus restoring kidney function (Kaur *et al.,* 2017).

As shown in Table 4, the PC group exhibited a significant increase in renal tissue levels of MDA and significant decrease of antioxidant enzymes (catalase, SOD, and GPX) when compared to the NC group. Administration of NSP, NSO and NSE caused significant decreases of MDA and increases of enzymes with antioxidant activity compared to the PC group however did not cause full recovery of these enzymes. Cisplatin generates free radicals such as superoxide and hydroxyl which produce oxidative damage and lipid peroxidation (Santos *et al.,* 2012). Cisplatin induced renal toxicity decreases levels of antioxidant enzymes and non-enzymatic molecules as glutathionein and significantly increases hepatic MDA levels in rats (Kart *et al.,* 2010). Thymohydroquinone and thymoquinone are the most important constituents of NSO that has antioxidant and cytoprotective effect against kidney damage by inhibiting *in vitro* non-enzymatic lipid peroxidation measured as MDA and elevate antioxidant enzymes including SOD, CAT, and GPx in kidney tissues. NSO and NSE have anti-inflammatory properties by inhibiting cyclooxygenase and lipoxygenase and contains kaempferol and quercetin which have strong antioxidant properties (Mahmoud *et al*., 2002; Entok *et al*., 2014). Thus, NSP, NSO, and NSE prevents cisplatin-induced nephrotoxicity via direct antioxidant activity and indirect antioxidant activity from induction of endogenous antioxidant enzymes.

Conclusions

The results presented in our study strongly suggest that *Nigella sativa* is a rich source of many essential nutrients that have a positive effect on body weight and human health. Nigella seed oil have fatty acids with a long shelf life and is suitable for pharmaceutic and nutraceutic application. NSP, NSO, and NSE act as a potent antioxidant to prevent the toxic effects of cisplatin in biochemical parameters in the serum and kidney so have potential to be utilized as an adjunct therapy for nephrotoxicity. In order to prevent life-threatening complications inpatients with nephrotoxicity, it is recommended to supplement their diets with different forms of *Nigella sativa* (powder, oil, or extract). There is a need for further researchers on method of extraction and storage of Nigella seed and clinical trials of long duration administration of seed, extract and oil in patients with renal disease in addition to investigate the active principle that can be used in manufacture of drugs.

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