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The Protective Effects of *Nigella sativa* Oil and *Allium sativum* Extract on Amikacin-induced Nephrotoxicity

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Abstract: Amikacin is a valuable aminoglycoside in serious infections. However, its use is associated with undesirable renal toxicity. This study was designed to check the effect of *Nigella sativa* oil and *Allium sativum* extract in ameliorating amikacin-induced renal damage by using a rat model of nephrotoxicity. Rats received nephrotoxic dose of amikacin and the amelioration of amikacin-induced nephrotoxicity was assessed through microscopic lesion scoring of damaged renal tissue and estimating the changes in biomarkers of tissue damage. *Nigella sativa* oil and *Allium sativum* extract significantly decreased the levels of NO, malondialdehyde and total antioxidant capacity. Furthermore, they increased the level of reduced glutathione. These changes are indicative for lower tissue damage and reduced free radical formation. These results were coinciding with the lower levels of urea, uric acid and creatinine (which were significantly elevated in amikacin treated groups). Semi-quantitative analysis of cellular infiltration, necrosis of tubular cells and tubular cellular damage indicated the protective effect of the used plant materials in reducing renal damage induced by amikacin. By using a rat model, *Nigella sativa* oil and *Allium sativum* extract efficiently ameliorated the renal toxic effect of amikacin. Further studies are required for applications in other animals or human subjects.

Key words: Amikacin, nephrotoxicity, *Nigella sativa, Allium sativum*, oxidative stress, lesion scoring

INTRODUCTION

Amikacin (AMK) is one of aminoglycoside antibiotics acting on gram negative and some gram positive bacteria by inhibition of protein synthesis. Amikacin is characterized by its broader antimicrobial spectrum and its resistance to destruction by enzymes inactivating other aminoglycosides. Thus, amikacin is of a value in treating fevers and systemic affections in plasmid mediated resistant bacteria. Long term use of amikacin is prohibited due to its potential nephrotoxicity (Dwivedi *et al*., 2009). The amelioration of amikacin-induced nephrotoxicity will allows extended use of the drug, minimizes its side effects and reduces tissue damage. The mechanisms of aminoglycosides toxicity were extensively studied (Lin *et al*., 2011; Lopez-Novoa *et al*., 2011; Quiros *et al*., 2011; Said, 2010). One of the possible mechanisms of amikacin-induced nephrotoxicity is the production of oxygen free radical and increasing the oxidative damage of cells.

Nigella sativa (NS), an annual herbaceous plant of the Ranunculaceae, have been used traditionally for centuries in the Middle East, Northern Africa, Far East and Asia for the treatment of various diseases, where it is known as the black seed (Al-Ghamdi, 2001; Aljabre *et al*., 2005). NS, its extract or some of its components showed marked antioxidant (Ali and Blunden, 2003), anticancer (Worthen *et al*., 1998), hepatoprotective (Abuelgasim *et al*., 2008), anti-ulcergenics (Rifat-uz-Zaman *et al*., 2004), antipyretic analgesic and anti-inflammatory (Al-Ghamdi, 2001), diuretic and hypotensive effect (Zaoui *et al*., 2000), hypoglycemic (El-Dakhakhny *et al*., 2002), protective effect on pancreatic β-cells (Mansi, 2006), improves fertility (Bashandy, 2007), improves chicken performance (Al-Beitawi and El-Ghousein, 2008) and has immune modulatory properties (Salem, 2005).

Garlic or its extract (GE) (Dillon *et al*., 2003; Imai *et al*., 1994; Jeong *et al*., 2011; Poldma *et al*., 2011) and garlic compounds (Chung, 2006) showed antioxidant properties The use of garlic gave protection against drug-induced cardiotoxicity (Alkreathy *et al*., 2010; Das and Poudel, 2006; Mukherjee *et al*., 2003), nervous system disorders (Chauhan and Sandoval, 2007; Gupta *et al*., 2009)

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drug-induced neurotoxicity (Perez-Severiano *et al*., 2004), modulation of immunity (Ghazanfari *et al*., 2006; Nya and Austin, 2011), improved feed conversion in poultry (Fadlalla *et al*., 2010), hypoglycemic agent (Sukandar *et al*., 2010), antimicrobial (Bachrach *et al*., 2011), in gastric ulcers associated with *Helicobacter pylori* (Adeniyi *et al*., 2006), anticancer (Huang *et al*., 2011), antifungal (Davis, 2005), antiprotozoal (Wabwoba *et al*., 2010) and anthelmintic actions (Ayaz *et al*., 2008; Singh *et al*., 2009).

Based on the various applications and uses of traditional medicine in combating the drug-induced toxicities, NS and GE may have the capability of ameliorating amikacin-induced renal toxicity. Therefore, the present study aimed to examine the renal protective effect of NS and GE against AMK induced renal toxicity.

MATERIALS AND METHODS

Drugs and chemicals: AMK was obtained from Amoun Company for pharmaceutical and chemical industries. NS oil was provided from a commercial source in Cairo, Egypt. The company produced NS oil by cold pressing of fresh seeds without using chemicals. Tomex© (SEKEM, Egypt) 300 mg tablets were used as a source of dried garlic extract produced during the year 2011.

Experimental design: Twenty five adult Wistar-albino rats weighting approximately 180-200 g were used in this experimental study. All experiments in this study were performed in accordance with the guidelines for animal research from the National Institutes of Health and were approved by our local animal ethics committee. Rats were divided randomly into four equal groups including five animals each: (1) Negative control group: The rats received a daily intraperitoneal (i.p.) injection of 0.5 mL isotonic saline for 7 days. (2) AMK control group: 1.2 g kg^{-1} AMK was injected i.p., to rats as a single dose (Parlakpinar *et al*., 2006). (3) NS group: 1.2 g kg^{-1} AMK was injected i.p., to rats as a single dose. Two doses of NS oil (0.5 mL) were given orally at the day of injection and one day before injection of AMK. (4) GE group: 1.2 g kg^{-1} AMK was injected i.p., to rats as a single dose. Two doses of GE (300 mg) were given orally at the day of injection and one day before injection of AMK. The rats were sacrificed on the eighth day; blood samples were collected and let to clot at room temperature and centrifuged. Fresh blood as well as serum samples was subjected to biochemical analyses.

Biochemical analysis

Determination of BUN: BUN level was measured using Urea Enzymatic Colorimetric Kit (Biosystems, Barcelona, Spain) as described before by Young (1997).

Determination of serum creatinine level: Creatinine level was determined using Creatinine Colorimetric Kit (Human, Germany) according to the method of Bartels and Bohmer (1971).

Determination of serum uric acid: Uric acid level was determined using uric acid colorimetric Kit (Human, Germany) according to the method of Fossati *et al*. (1980).

Determination of oxidative stress biomarkers: (GSH, Nitric oxide assay and total antioxidant capacity) were estimated by a commercial kit.

Lipoperoxidation: Lipoperoxidation was measured by using a commercial kit to determine the amount of Malondialdehyde (MDA).

Histopathological examinations: Kidneys of rats were used for histopathological evaluation. Kidney sections were fixed in 10% neutral buffered formaldehyde solution, dehydrated in graded alcohol and embedded in paraffin. Paraffin embedded specimens were cut into 5 μm thickness and stained Hematoxylin-Eosin for light microscopic examination. All sections of kidney samples were examined for mononuclear cell infiltration, necrosis of renal tubular cells and tubular cell degeneration. For each slide, minimum of 20 fields (20×)/slide were examined, evaluated and an average score was obtained. The severity of changes is scored according to the following scale: no change (0), mild, <10% tubular damage (1), moderate changes affecting 10-25% of tubules (2), severe damage affecting 25-50% of tubules (3) and extensive damage affecting >50% of tubules (4).

Statistical analysis: All data are presented as Means±SD. Results were evaluated by a one-way ANOVA and significant differences defined with Tukey post-tests. Differences are considered significant when $p<0.05$.

RESULTS

The effect of *Nigella sativa* oil and *Allium sativum* extract on lipid peroxidation: The AMK group showed significant increase in MDA activity compared with saline group. Furthermore, NS and GE groups showed significant decrease in MDA levels compared with AMK group ($p<0.001$). The AMK group showed the highest value, while NS group showed the lowest value of serum MDA. Interestingly, the level of MDA in NS and GE groups showed significant decrease in comparison with the saline (control nontreated) group (Table 1).

Table 1: Oxidant and antioxidant biomarkers in nephrotoxic rat induced by amikacin and treated with NS and GE

	Treatment			
Group	GSH (mg dL^{-1})	Total antioxidant (mM L^{-1})	NO (μmol L^{-1})	MDA (nmol mL^{-1})
Control nontreated	32.16±2.97^{a}	0.09±0.01^{b}	3.90±1.3^{a}	50.98±31.7^{c}
AMK	24.69±3.95	0.21±0.03***	4.91±0.8*	762±4.6**
NS	31.50±3.2^{a}	0.12±0.02^{a}	3.80±1.5^{a}	26.23±2.7^{c***}
GE	32.20±2.26^{a}	0.13±0.02^{a}	3.80±1.8^{a}	34.72±6^{c**}

Significance: Comparison with AMK group (within column) is expressed as $^{a}p<0.05$, $^{b}p<0.01$ and $^{c}p<0.001$, Comparison with saline group (within column) is expressed as $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$

Table 2: Renal function tests in nephrotoxic rat induced by amikacin and treated with NS and GE

	Treatment		
Group	Urea	Uric acid	Creatinine
Control nontreated	32.0±4.7^{c}	3.00±0.35^{c}	0.78±0.09^{c}
AMK	45.4±7.3***	4.05±0.3**	1.80±0.09***
NS	24.0±6.4^{c}	3.40±0.7^{b}	0.50±0.08^{c***}
GE	26.6±2.9^{c}	3.00±0.25^{c}	0.70±0.06^{c**}

Significance: Comparison with AMK group (within column) is expressed as $^{a}p<0.05$, $^{b}p<0.01$ and $^{c}p<0.001$, Comparison with saline group (within column) is expressed as $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$

Table 3: Semiquantitative analysis of cellular infiltration, necrosis of tubular cells and tubular cellular damage in nephrotoxic rat induced by amikacin and treated with NS and GE

	Treatment		
Group	CI	N	TD
Control nontreated	0.90^{a}	0.2^{c}	0.8^{a}
AMK	1.84***	1.0***	2.5***
NS	0.10^{c***}	0.2^{c}	1.6
GE	0.90^{a}	0.1^{c}	1.1^{a}

CI: Cellular infiltration, N: Necrosis of tubular cells, TD: Tubular cellular damage, Significance: Comparison with AMK group (within column) is expressed as $^{a}p<0.05$, $^{b}p<0.01$ and $^{c}p<0.001$, Comparison with saline group (within column) is expressed as $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$

The effect of *Nigella sativa* oil and *Allium sativum* extract on antioxidant activity: The effects of amikacin on biomarkers of oxidative stress are presented in Table 1. The level of GSH was significantly decreased in AMK group ($p<0.05$) while NO level was significantly increased compared with the saline group ($p<0.05$). The AMK group showed the highest value, while NS group showed the lowest value of serum NO. NS and GE groups did not show statistically significant change compared with saline group.

A significant increase in the total antioxidant capacity was noticed in AMK group compared with the saline group. NS and GE groups did not show statistically significant change compared with the saline group and were significantly lower than AMK group ($p<0.05$).

The effect of *Nigella sativa* oil and *Allium sativum* extract on serum urea, uric acid and creatinine: The effects of aminoglycoside antibiotics on urea, uric acid and creatinine are presented in Table 2. The AMK group showed the highest value while NS group showed the lowest value of serum urea. The level of urea in AMK group was significantly higher than the saline group. NS and GE groups did not show statistically significant change compared with the saline group and were significantly lower than AMK group ($p<0.001$).

The level of uric acid in AMK group was significantly higher than the saline group ($p<0.01$). The AMK group showed the highest value, while GE group showed the lowest value of serum uric acid. NS and GE groups did not show statistically significant change compared with the saline group and were significantly lower than AMK group.

The level of creatinine in AMK group was significantly higher than the saline group ($p<0.001$). The AMK group showed the highest value, while NS group showed the lowest value of serum creatinine. Interestingly, NS and GE groups showed statistically significant decrease in the creatinine level compared with the saline group ($p<0.001$ and $p<0.01$, respectively) and were significantly lower than AMK group ($p<0.001$).

Microscopic lesion scoring: Semiquantitative analysis of cellular infiltration, necrosis of tubular cells and tubular cellular damage was adopted to assess the lesions severity using stained sections of kidneys. Histopathologic examination of kidneys showed marked damage in AMK treated rats. AMK group showed the highest scores of the measured parameters that were significantly different from the saline group. The scores of histopathological investigations were summarized in Table 3. AMK group showed the highest necrotic changes eliciting a score of 1 (approximately 10% of the examined tubules showed necrotic changes). Furthermore, the tubular cellular damage was involving about 40% of the examined tubules (lesion score of 2.5). NS and GE groups were more or less similar to the saline group and were significantly different from the AMK group.

Histopathological changes included vacuolar degeneration of renal tubular cells with rarefied cytoplasm taking reticular appearance with occasional pyknotic nuclei (Fig. 1a). Massive necrosis was observed in epithelial cells of the proximal tubules (Fig. 1b). Furthermore, there was cystic dilatation with slight atrophy of renal tubular epithelial cells (Fig. 1c). In

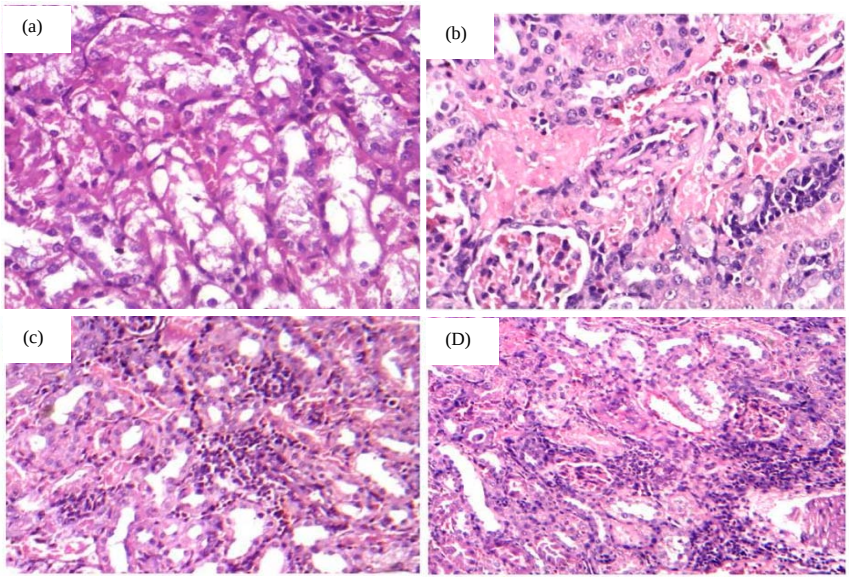

Fig. 1 (a-d): Histopathological view of renal sections in AMK group showing (a) Vacuolar degeneration of renal tubular cells where the cytoplasm became rarefied taking the reticular appearance with occasional pyknotic nuclei. (b) Massive necrosis of renal tubular epithelium together with mild mononuclear inter-tubular cell infiltrations. (c) Necrosis of renal tubules, intertubular infiltration of mononuclear cells along with cystic dilatation with slight atrophy of renal tubular epithelial cells. (d) Perivascular, periglomerular and inter-tubular mono nuclear cell infiltration (H and E x200)

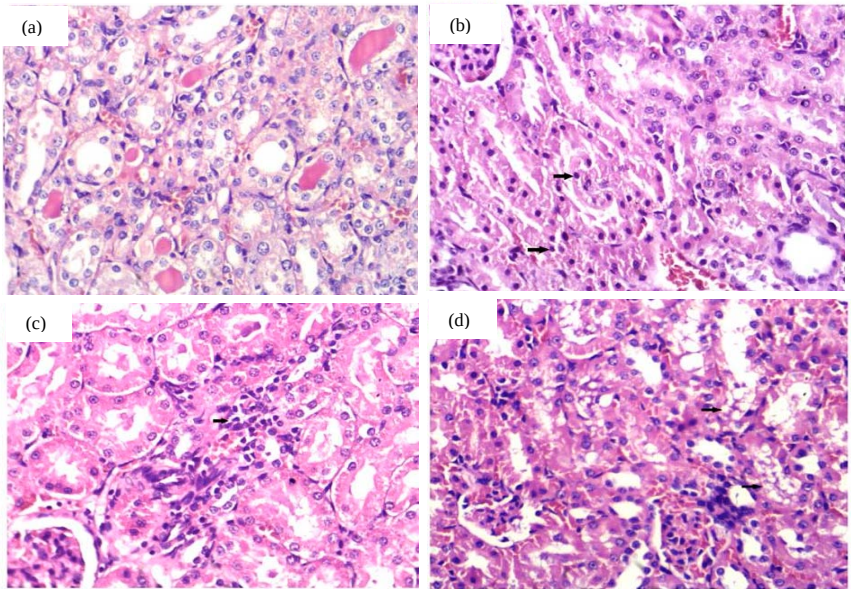

Fig. 2 (a-d): (a) Histopathological view of renal sections in AMK group showing renal cast. (b) Histopathological view of renal sections in NS group showing pyknotic nuclei in some renal cells. (c) Histopathological view of renal sections in NS group showing slight intertubular cell infiltration. (d) Histopathological view of renal sections in GE group showing slight vacuolation (arrows, H and E x200)

addition, severe inflammatory infiltrate in the form of mononuclear cells either intertubular, periglomerular or perivascular cellular infiltration (Fig. 1d). The lumens of tubules were filled with degenerate and desquamated epithelial cells in the form hyaline cast (Fig. 2a). In NS and GE groups, the morphological changes which were observed in AMK group were disappeared as tubular cell necrosis, severe inflammatory cell infiltration and renal cast. The observed changes in these groups were nuclear pyknosis (Fig. 2b), slight intertubular cell infiltration (Fig. 2c) and slight vacuolation (Fig. 2d).

DISCUSSION

The use of aminoglycosides is complicated with its probable nephrotoxicity. AMK is the widely used aminoglycoside, which is conserved for severe infections with Gram negative bacteria. The evidence of renal damage in AMK group was demonstrated in several studies (Kaynar *et al*., 2007; Ozer *et al*., 2009; Parlakpinar *et al*., 2006). The amelioration of gentamicin-induced nephrotoxicity will be important for getting more benefit of the drug in the fight against dangerous infections. This study was designed to check the protective effect of *Nigella sativa* oil and *Allium sativum* extract on amikacin-induced renal injury. The plant material was given to rats one day before administration of the drug and at the day of AMK injection.

The level of MDA was increased in AMK group. MDA is a marker of lipoperoxidation and indicates free radical formation in AMK-induced renal toxicity. Free oxygen radical can induce lipid peroxidation in tissues leading the production of MDA as indicator of increased lipid peroxidation and tissue damage. Overproduction of oxygen free radicals will increase the level of MDA by destruction of unsaturated fatty acids in the cell membrane. The lipoperoxidation was significantly decreased in groups treated with garlic extract and *Nigella sativa* oil. Interestingly, the values in these groups was significantly lower than the control nontreated group. The protective effect of garlic extract and *Nigella sativa* oil is associated with decrease in peroxidation. Both extracts were capable of preventing the rise of lipoperoxidation due to the nephrotoxic effect of AMK and in general indicates lower level of free radical formation in the presence of NS and GE.

AMK induced higher level of serum NO concentration. However, this level was reduced in NS and GE treated groups. Interestingly, the level of NO was nearly equal to that of the control nontreated group.

It was found that NS and GE can reduce the levels of MDA and NO, thus inducing lower tissue damage (Alkreathy *et al*., 2010).

GSH protects the cell against the toxic effects of hydroxyl radicals and singlet oxygen. The decrease in GSH level is associated with lower activity of glutathione related enzymes. The low levels of GSH will lead to poor control on free radicals, therefore, induces more tissue damage. In this regard, the low level of GSH in AMK group indicates more tissue damage. GSH stores were significantly depleted, indicating the use of GSH as an antioxidant for the detoxifcation of toxic oxygen metabolites. In NS and GE groups, the level of GSH was comparable to the saline group, indicating nonsignificant levels of tissue damage.

In this study, the increased level of creatinine, urea and uric acid in AMK group indicates renal damage. These results are in agreement with the previous reports on amikacin induced renal toxicity (Parlakpinar *et al*., 2004, 2003). In NS and GE groups the levels of urea and uric were significantly lower than that of the AMK group. Thus, both NS and GE protected the kidney from amikacin induced renal damage, as indicated by lower serum urea, uric acid and creatinine levels.

The biochemical findings were further confirmed by histopathological examination. The AMK treated group showed the highest lesions scores as there was severe vacuolation of renal tubular cells, massive tubular necrosis, renal cast and mononuclear cell infiltration, which are coinciding with the highest tissue damage. This could be due to the formation of highly reactive radicals as a consequence of oxidative stress caused by AMK. All these changes were histopathologically reduced in NS and GE groups. The lower cellular infiltration and lower tissue damage in GE and NS groups might be associated with their powerful anti-inflammatory and antioxidant properties (Kim *et al*., 2011; Popov *et al*., 1994; Sajitha *et al*., 2010). These properties were important in their protective effect against renal damage.

In this study, *Nigella sativa* oil and *Allium sativum* extract were administered only one day before injection of AMK. Thus, we checked short term prophylaxis with the tested plants materials. In this respect, the results of present study will be more beneficial for physicians and veterinarian who may advise prompt use of amikacin for various infections. The administration of NS and GE for one day only before giving amikacin was capable of ameliorating the nephrotoxic effect of the drug.

In conclusion, based on pathological and biochemical bases *Nigella sativa* oil and *Allium sativum* extract efficiently ameliorated amikacin induced nephrotoxicity. Clinicians could consider the administration of *Nigella sativa* oil or *Allium sativum* extract during long courses of amikacin therapy.

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