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Role of Nigella sativa oil and Alpha lipoic acid on Amikacin induced hepatorenal toxicity in rabbits

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ABSTRACT

Amikacin (AK) has long been used as an effective antibiotic to prevent Gram-negative infections. Despite its numerous benefits, Amikacin has considerable hepato-renal toxic adverse effects. The potential preventive benefits of NSO, "Nigella sativa oil" and/or ALA, "Alpha lipoic acid" were investigated in rabbits with Amikacin-induced acute hepato-to- renal injury. Rabbits were treated with AK (100mg/kg BW/day I/M injection) alone or with NSO (2ml/kg BW orally) and /or ALA (50 mg/kg BW/day) daily for seven days. Serum biochemical parameters well as tissue oxidative stress indicators were measured to evaluatehepato-renal damage. Rabbits administered AK showed substantial changes in serum biochemical hepato-renal damage indicators with reduction in tissue antioxidant biomarkers and rise in hepato-renal oxidant. NSO plus ALA treatment potentially alleviated the AK induced changes in blood and tissue biochemistry. The current study found that NSO and/or ALA could ameliorate the harmful effects of AK via free radicals scavenging plus their antioxidants effects.

INTRODUCTION

Amikacin (AK) isthe most broad-spectrum antibiotic among amino glycosides used in veterinary that obtained by acetylating of natural kanamycin. It is more preferred due to its potent bactericidal action with its synergistic effect with other antibiotics like Lactam **(Poulikakos and Falagas 2013).** AK produces its bactericidal effect by blocking protein synthesis while binding to bacterial 30 S riboso-

mal subunits of RNA **(Krause et al. 2016)**. Therefore, AK in rabbits is the drug of choice against gram-negative bacteria. **The un-desirable reversible nephrotoxicity side effect of AK however with lowest dose may end up with acute renal failure (Hadjipour 2011; Wargo and Edwards 2014)**. This nephrotoxic effects is due to inability to metabolizeamikacin with its sedimentation in the proximal convoluted tubules and glomeruli that slows down glomerular filtration. Additionally, increased ROS (reactive oxygen species) production, generation of vasoconstrictors and platelet-activating factor secretion have been seen and linked with **ototoxicity, hepatotoxicity, and nephrotoxicity (Ozer et al. 2009; Lopez-Novoa et al. 2011; Wargo and Edwards 2014).**

Natural scavenging system can be induced by antioxidant usage that results in ROS reduction and hence oxidative stress prevention. This potentially could be corrected the renal failure may be inhibited **(Sing et al. 2006).One of the Ranunculaceaefamilies, Nigella Sativa is sometimes referred to as "black seed or black cumin ".** Nigella Sativa oil (NSO) **has widespread pharmacological effects including anti-inflammatory, pain-relieving and antioxidant capabilities (Lutterodt et al. 2010; Farooqui et al. 2017; Mohammed 2019). Along with these actions, it possesses** stimulant, carminative, antipyretic, diuretic, antibacterial, ant diabetic and antifungal activities. In addition, numerous studies have demonstrated the anticancer, hepatoprotective, nephroprotective, and gastro protective properties of NS **(Elseweidy et al. 2018; Hosseinian et al. 2018; Abo Saleh et al. 2019)**. NSO combines vitamins, pigments, fatty acids, and volatile compounds. It contains thymoquinone, thymol, and thymoquinone as the main active components that lead to its antioxidant activity **that** scavenging liberated ROS during several illnesses **(Kanter et al. 2005; Barakat et al. 2010).**

Alpha lipoic acid, ALA protects against drugs toxicity and liver disease because of its crucial role in activation of mitochondrial dehydrogenases. In eukaryotic cells, ALA is reduced intodihydrolipoic acid (DHLA), which has the potential ROS scavenge effect **(Şehirli**

et al. 2008; Gomes and Negrato 2014; Hamzawy et al. 2014).Additionally, ascorbic acid, alpha-tocopherol, and intracellular glutathione are natural endogenous antioxidants that activated by ALA **(Maczurek et al. 2008; Tanaka et al. 2015**).

The existing research was performed to investigate the Amikacinhepato-renal toxicity in rabbits and explore the ameliorating role of dietary supplementation of **Nigella Sativa (NS) and** alpha-lipoic acid (ALA) by evaluating renal and liver function parameters in serum and oxidative status of kidney and liver tissue.

Materials and methods:
Drugs and Chemicals:
Amikacin (AK) was purchased (500 mg/2 ml vial) from the Amoun's pharmaceutical company in Cairo, Egypt. AK was injected daily via I/M for a week in dose of hundred mg/kg body weight. 450 mg capsules of Baraka Nigella Sativa oil, NSO, were purchased from "Pharco pharmacy" in Alex, Egypt. 2ml/kg BW of NSO must be taken orally for seven days as the recommended dose. EVA Pharma, Egypt, was the source of the alpha lipoic acid (ALA). The daily oral ALA consumption recommendation is 50 mg/kg BW. All the biochemical kits for analysis were obtained from bio diagnostic Co. Cairo, Egypt.

Experimental design:
In the present study, twenty-five healthy NewZeland rabbits weighing 1600± 200g, 60 days old were raised in metal sets, where basal diet and water were given *ad libitum* **(NRC 1977)**throughthe experiment. Rabbits were allocated in five groups at random (five each). After one week of acclimation, normal saline was administered to group 1 (negative control). For seven days, rabbits in Group 2 (AK) received 100 mg/kg BW of AK viaI/Minjection as **(Mohammed et al. 2014)**. Group 3 (AK+NSO)and group 4 (AK+ALA) were given the previous dose of AK in addition to 2 ml/kg BW of NSO and ALA dose at 50 mg/kg BW by stomach tube, respectively according **to Saleem et al. (2012) and Tahira et al. (2012)** for seven successive days. Group 5 (AK+NSO+ALA)was given AK, NSO, ALA at the same doses as previously explained once

daily for seven days. The study was accepted by the institutional committee for the carefulness and usage of laboratory animals at the Animal Health Research Institute. Ethical committee authorized the sample collection by a licensed veterinarian who utilized approved sample collection methods and restrained the animals as little as possible (ARC-AHRI).

Biochemical tests:

Blood samples were collected a day post administration from the ear vein of all animals to obtain serum. Kidneys and livers were rapidly gathered from all rabbits after slaughtering. Methods defined by **Larsen (1972); Whitehead et al. (1991) and Coulombe and Favreau (1963) were used** for serum creatinine, urea, and uric acid levels estimation, respectively. Serum aspartate andalanine aminotransferase (AST and ALT) enzymes and total bilirubin were assessed **(Reitman and Frankel 1957; Schattmann 1952).** Malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) ranks of kidney and liver tissues were analyzed **(Mihara and Uchiyama (1978); Beutler et al. (1963); Aebi (1984) and Nishikimi et al. (1972).**

Statistical analysis:

The statistical program SPSS version 16.0, one-way analysis of variance (ANOVA) was charity to evaluate all data **(SPSS, Inc, Chicago, IL. 2007)**. Statistics showed that the difference was significant at P≤0.05. The gathered data was presented by way of mean ± SE.

RESULTS

Table 1. Mean ±SE value of some kidney function parameters in different rabbit groups

Groups	Creatinine (mg/dL)	Urea (mg/dL)	Uric acid (mg/dL)
Negative control	0. 76±0.11d	24.50±2.71 de	2.37±1.07c
AK	3.58±0.82a	43.30±4.67a	4. 31±0.73 a
(AK+NSO)	2.12±0.60b	36.60±3.72 c	3. 22±0.74 b
(AK+ ALA)	2.78±0.42b	40.98±4.70b	3.53 ±0.72b
(AK+NSO+ALA)	0. 83±0.10cd	25.60±2.56d	2.36±0.81c

The different subscripts (abcd) in each column were significantly (P≤0.05)

Data analysis showed significant (P≤0.05) risein serum levels of creatinine, urea and uric acidin AK treated group than negative control group. NSO or ALA induced reduction in the values of creatinine, urea and uric acid compared to AK group without reach to values of negative control group. Nevertheless, both NSO and ALA administration partially restored the normal concentrations of renal parameters in comparison to negative control group (table1).

Table 2. Mean ±SE value of liver function parameters in different rabbit groups

Groups	AST (U/L)	ALT (U/L)	Total bilirubin (g/dl)
Negative control	56.60±5.62d	28.30±2.66d	1.10±0.72d
AK	118.04±10.71a	61.90±7.83a	2.90±0.32 a
(AK+NSO)	72.84±9.55bc	42.11±4.96c	1.53±0.23bc
(AK+ ALA)	80.56±8.66b	50.36±5.74 b	1.65±0.12 b
(AK+NSO+ALA)	59.17±6.74d	30.98±3.863 d	1.31±0.48d

The different subscripts (abcd) in each column were significantly (P≤ 0.05)

AK treated group had substantial (P≤0.05) elevation in the activity of AST and ALT enzymes and total bilirubin level in serum than negative control group. However, these values did not significantly differ in AK+NSO group compared with AK+ALA group while exhibited substantial rise compared to the negative control but lowered value when compared to the AK group. It's notable that these liver function parameters did not significantly differ in AK+NSO+ALA group compared to control group, (table 2).

Table 3. Mean ±SE value of kidney oxidant and antioxidant parameters in different rabbit groups

Groups	MDA (nmol/g)	GSH (mg/g)	SOD (U/g)	CAT (U/g)
Negative control	4.21±0.71c	5.57±0.71a	21±0.83a	0.40±0.05 a
AK	8.31±0.71a	3.58±0.71bc	10±0.71d	0.18±0.07e
(AK+NSO)	4.68±0.71c	5.54±0.74a	17.5±0.72b	0.28±0.07 c
(AK+ ALA)	5.84±0.64b	4.66±0.68ab	13.9±0.71c	0.24±0.05d
(AK+NSO+ALA)	4.76±0.57c	5.88±0.78a	20±0.69a	0.32±0.66b

The different subscripts (abcd) in each column were significantly (P≤ 0.05)

Kidney tissues from the AK group showed significantly higher MDA level and lower kidney tissue antioxidant levels (GSH, SOD and CAT) that confirm the **renal** toxicity. In contrast, AK+NSO group showed an improvement of MDA and GSH levels and appeared within negative control levels however showed increases in SOD and CAT enzymes compared to AK group. Additionally, AK+ ALA group had significant decreases in MDA level and elevation of SOD and CAT levels compared to AK group. Conclusively, AK+NSO+ALA group had ability to maintain the MDA, GSH, SOD levels but lower CAT values to that of negative control group (table 3).

Table 4. Mean ±SE value of liver antioxidant and oxidant parameters in different rabbit groups

Groups	MDA (nmol/g)	GSH (mg/g)	SOD (U/g)	CAT (U/g)
Negative control	2.54±0.75cd	4.9±0.64a	18.6±0.73a	0.198±0.03 a
AK	5.18±1.21a	3.5±0.65cd	9.38±0.75d	0.07±0.07e
(AK+NSO)	3±0.88bc	4.1±0.70ab	16.3±0.69b	0.172±0.07b
(AK+ ALA)	4.34±0.71ab	3.8±0.72c	11.3±0.73c	0.14±0.04d
(AK+NSO+ALA)	2.26±0.81 d	4.62±0.70a	18±0.71a	0.16±0.07c

The different letters (abcd) in each column were significantly (P≤ 0.05)

Liver tissues from the AK group had considerably higher MDA levels and lower antioxidants levels (GSH, SOD, and CAT) which suggest hepatotoxicity. In contrast to negative control group, AK+ NSO group presented non-significant levels of MDA and GSH and significant declines in SOD and CAT activation. The AK+ ALA group exhibited significant MDA elevation and decline in GSH, SOD, and CAT levels. In comparison with AK group, the AK+NSO and AK+NSO+ALA groups both demonstrated significantly decreased level of liver MDA with considerable rise in the estimated antioxidants (table 4).

DISCUSSION

Antibacterial treatment using aminoglycoside antibiotics has long been utilized. Despite their therapeutic benefits, aminoglycosides are harmful to the kidneys and liver (**Yazar et al. 2003 Hadree 2018**). The significant rise in serum urea, uric acid, and creatinine levels and kidney MDA with decrease in kidney GSH, SOD, and CAT in AK rabbit group in our study provided an evidence of renal impairment. Similar effects were recorded by **Kara et al. (2016) Krause et al (2016) and Doğan et al. (2017)**. The significant elevation in serum aminotransferase (ALT&AST) activities, and total bilirubin with the rise of oxidative stress (MDA) and lower antioxidant capacity were all indicators of hepatotoxicity that was produced by AK as agreed with **Martines et al. (1988) Chaudhary et al. (2008) and Chan et al. (2020)** results. Free radicals generated by amikacincan destruct biological protein, lipid membranes, and DNA, producing a variety of pathogenic concerns as hepato-renal damage (**Parlakpinar et al. 2003; Valko et al. 2007; et al. 2009**). AK induced hepatic and renal damage may be ascribed to depletion of intracellular glutathione, mitochondrial damage, and oxidative stress produced by free radical generation (**Chaudhary et al. 2011; Asci et al. 2015**).

In existing study, NSO treatment lowered kidney and liver damage signs by reduction of tissue lipid peroxidation .Additionally, NSO therapy led to increase in active glutathione level as well as hepatorenal antioxidant enzymes, according to a prior study by **Bayoumi**

et al. (2020). NSO contains thymoquinone, an active antioxidant, as well as other related numerous volatile oils and thymol. Previous studies proved the the preventive and antioxidant properties of NSO against drugs, xenobioticsand chemical hazards (**Kanter et al. 2005; Barakat et al. 2010; Bayoumi et al . 2020**).

Because ALA has capability for scavenge free radicals, it is possible that ALA treatment decreased the toxicity of AK due to the decrease in MDA and the rise in antioxidant kidney and liver tissues levels. ALA is frequently used as a drug to prevent diabetic complications like polyneuropathy, nephropathy and cataracts (**El-Beshbishy et al. 2011; Wongmekia et al. 2013; Asci et al., 2015; Zhang and McCullough 2016**). ALA also includes amphipathic components that help antioxidant capability. It also neutralizes free radicals and regenerates other antioxidants, making it one of the essential antioxidants (**Golbidi et al. 2011; Feng et al. 2013**).

According to our research, AK-induced oxidative stress may be reduced by administering both NSO and ALA either directly by reducing lipid peroxidation and scavenging free radicals or indirectly by raising the antioxidant SOD and CAT enzymatic activities.

CONCLUSION

The antioxidant effect of NSO or ALA considerably reduced the hepato-renal damaging effect of AK. The combined effects of the two antioxidant drugs potentiate synergistic antioxidant and hepato-renal toxicity ameliorative capabilities.

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