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Original Research Article

Evaluation of Nephroprotective Potential of *Nigella sativa* Oil against Kanamycin Induced Nephrotoxicity in Rats.

Murtuza Bohra[*1], Akash Vishwe[2], Pushpraj Gour[3], Richa Malani[4], Preeti Rajak[5], Rabia Riaz[6]

[1]Associate Professor, Department of Pharmacology, JIET Medical College and Hospital, Jodhpur, Rajasthan

[2]Associate Professor, Department of Pharmacology, Index Medical College Hospital and Research Centre, Indore, MP

[3]Associate Professor, Department of Pharmacology, RKDF Medical College, Hospital and Research Center, Bhopal, MP

[4]Assistant Professor, Department of Pharmacology, Index Medical College Hospital and Research Center, Indore, MP

[5]Assistant Professor, Index Medical College Hospital and Research Center, Indore, MP

[6]Assistant Professor, Department of Jarahat, Ajmal Khan Tibbiya College AMU, Aligarh, UP

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Corresponding Author: Dr. Murtuza Bohra

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Abstract

The objective of this study is to evaluate the nephroprotective role of *Nigella sativa oil* against Kanamycin-induced nephrotoxicity in rats. The nephroprotective potential was evaluated on basis histopathological changes in renal tissue. Healthy adult albino rats of either sex (100-200 g) were randomly and equally into six groups of five animals each. Group I animals (normal control: DWNC) were administered distilled water intra peritoneally for 14 days. Group II animals (LNSO) were administered low dose nigella sativa oil 1ml/kg orally for 16 days. Group III animals (HNSO) were administered High dose nigella sativa oil 2ml/kg orally for 16 days. Group IV animals (KNCG)Kanamycin negative control group. were administered Kanamycin (300mg/kg) From day 1-14 Intra-peritoneally. Group V animals(KLNSO) were administred Low dose of Nigella sativa oil (1ml/kg) orally (From day 1-16) + Kanamycin (300mg/kg) (From day 3-16) Intra-peritoneally. Group VI animals (KHNSO) were administered high dose Nigella sativa oil. (2ml/kg) orally (From day 1-16) + Kanamycin (300mg/kg) (From day 3-16) intra-peritoneally. On the 16 th day(14th day for normal control group) the rats were sacrificed. The kidney was removed for histolopathological evaluation . Kanamycin treated group i.e Group IV showed increased damage to renal tissue as compared to normal control group Group I. Also renal tissue histological examination reveals decreased nephrotoxicity in Group V and Group VI compared with Group IV. It is seen that Nigella sativa oil has a nephroprotective role against Kanamycin-induced nephrotoxicity in rats as is evident from histopatholgical examinations of tissue samples.

Introduction

The aminoglycoside group includes kanamycin , capreomycin, amikacin, gentamicin, tobramycin, amikacin, netilmicin, streptomycin, paromomycin, and neomycin. Aminoglycosides use in combination is the continually increasing in the lieu of rising multidrug-resistant bacteria. Aminoglycosides are used in treatment of zoonotic infections such as plague and tularemia. Aminoglycoside mechanism of action involves three stages. In the first stage polycationic aminoglycosides bind to the negatively charged components of the bacterial membrane, such as the phospholipids and teichoic acids of Gram-positive organisms and the phospholipids and lipopolysaccharide (LPS) of Gram-negative organisms, followed by displacement of cations responsible for cross bridging and stabilization of the lipid components of bacterial membrane and their removal leads to disruption of the outer membrane, enhanced permeability, and initiation of aminoglycoside uptake. Inhibition of protein synthesis and mistranslation of proteins occurs once aminoglycoside molecules enters the cytoplasm. Inhibition of protein synthesis and mistranslation contributes to accelerated cell death (Ramirez et al 2016). [1] Kanamycin was first isolated from *Streptomyces kanamyceticus in 1957*, Krause et al 2016 [2] Kanamycin is indicated in serious gram-negative infections in which Pseudomonas

aeruginosa is not a likely causative agent (Brewer N. S. 1977). [3] Active electron transport is required for it's uptake into cells, thus it's inactivate against anaerobic bacteria. MDR-TB treatment guidelines recommend inclusion of one of the following agents: kanamycin, amikacin, or capreomycin,(a cyclic peptide antibiotic that is often considered as an aminoglycoside owing to its mechanism of action) during the intensive phase of therapy. Although choice of agent depends on previous injectable use (if any) and the likelihood of resistance. Certain strains of mycobacteria show discordant resistance between Kanamycin and amikacin, such that strains resistant to kanamycin are still susceptible to amikacin Krüüner et al. 2003. [4] With the advent of extensively drug resistant TB these antibiotics are an increased area of focus and importance. Shah et al 2007 [5]. Kanamycin is one such agent which possesses potent bactericidal activity against *M. tuberculosis* (Ho et al. 1997)[6]. With rapidly increasing deployment of aminoglycosides like kanamycin for resistant and severe bacterial infections, concern rises over its long term use related adverse effects and toxicity. Nephrotoxicity induced by this drug if frequently responsible for its discontinuation despite obtaining positive treatment results. Up to 10% of administered parenteral dose of aminoglycosides gets concentrated in cells of Proximal renal tubules (Galløe et al 1995). [7] They then mediate nephrotoxic effects through inhibition of mitochondrial ribosomes, analogous to their bactericidal effect on the ribosomal units of bacteria (Hobbie et al 2008). [8] This injury may progress to renal failure, particularly in the presence of hypovolemia. Plasma t 1/2 of aminoglycosides is a few hours as compared to several days in the proximal tubule cells; this exposure leads to an exponential risk for renal toxicity. Estimated aminoglycoside-induced nephrotoxicity stands at 20.6% during standard therapy for gram-negative infections, and rises to almost 50% when drug exposureis more than 14 days (Leehey et al 1999). [9] Among large number of phytoconstituents that have been evaluated for nephroprotection in kidney diseases, only few phytoconstituents possess *in vitro* and *in vivo* activities and fewer are under human trials. Nigella sativa oil obtained from seeds of Nigella sativa is one such source which has nephroprotective potential activity and is studied here (Hannan et al 2021). [10] Increasing number of studies experimental, clinical and relevant data published subsequently could greatly increase chances of discovering a potent nephroprotective agent with fewer or no side effects.

Materials and Methods

Plant Material Nigella sativa oil – Nigella sativa oil (Kalonji oil, Mohammedia products, Aamirnagar, Shah Sahab Mohalla, Karimnagar – 505001, A.P., India) was procured from local market at Aligarh. As per manufacturer's information, it was prepared by steam distillation.

Experimental Animals: Healthy adult albino rats weighing 100-200g of either sex were procured from Central Animal House, J.N. Medical College, AMU, Aligarh. The animals were placed in polypropylene cages bedded with paper strips. They were maintained at a temperature of 27°C ± 2°C, also, the animals were kept under 12 hours dark-light cycle and were fed with standard pellet diet and water ad libitum. The paper strips were changed every day to maintain proper hygiene and comfort for animals. The animals were acclimatized for one week under laboratory conditions before experimentation. Ethical clearance for experimental study of the animals was obtained from the Institutional animal ethical committee, JNMC, AMU, Aligarh before commencement of the experiments.

IAEC Approval The study protocol was approved by the Institutional Animal Ethics Registration no.: 401/RO/C/2001/CPCSEA. All animal experiments were carried out as per the rules and regulations of IAEC & CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) under the "Guidelines for Care and Use of Animals in Scientific Research" (INSA 1992 & 2000).

Experimental Models

Kanamycin induced nephrotoxicity. Nephrotoxicity was induced by Kanamycin in the dose of 300mg/kg/day (Luft et al 1978 [11], Niizato et al 1976 [12]) through i.p. route daily for 14 days.
II) Nigella sativa oil Nephroprotectivity model

Nigella sativa oil was administered in two different doses in the study i) low dose 1ml/kg body weight/day and ii) high dose 2ml/kg body weight/day for evaluating the nephroprotective role of the Nigella sativa oil (Hafez 2013 [13], Ali BH 2004 [14], Danladi 2013 [15].

Experimental Design: Healthy adult albino wistar rats of either sex weighing 100-200gm were randomly divided into 6 groups of 5 rats each as follows: (n = number of rats in each group)

Groups	Treatment and Duration	Route of Drug Administration.
Group I (normal control: DWNCG) : (n=5)	Distilled water 1ml/kg (From day 1-14.)	Intra-peritoneally
Group II (LNSOG) : (n=5)	Nigella sativa oil – 1ml/kg (From day 1-16)	Oral
Group III (HNSOG) : (n=5)	Nigella sativa oil – 2ml/kg (From day 1-16)	Oral
Group IV : (n=5) (Kanamycin negative control group: KNCG)	Kanamicin.(300mg/kg)	Intra-peritoneally
Group V : (n=5) (Kanamycin and low dose- -nigella sativa oil treatment group: KLNSOG)	Low dose Nigella sativa oil.(1ml/kg).(from Day 1-16) + Kanamycin(300mg/kg) (from Day 3-16)	Oral Intra-peritoneal
Group VI : (n=5) (Kanamycin and high dose- -nigella sativa oil treatment group: KHNSOG)	High dose Nigella sativa oil.(2ml/kg).(from Day 1-10) +Kanamycin (300mg/kg) (from Day 3-16)	Oral Intra-peritoneal

Wherever kanamycin was co-administered with Nigella sativa oil either in high dose and or low dose. Nigella sativa oil administration was started two days prior (Ali BH 2004). [14]

Twenty four hours after the last dose in respective treatment group was administered, study animals were dissected and sacrificed (under pentobarbitone sodium 50mg/kg i.p. anaethesia). The kidneys were dissected out for histopathological examination

Histological Examination

All the kidney tissue specimens were preserved in 10% Formalin and processed in the Neuroanatomy & Histology Research Laboratory, Department of Anatomy, J.N. Medical College, A.M.U., Aligarh. Histopathological features which were observed in various normal control, negative control and treatment groups are as follows 1) Glomerular and tubular congestion 2) Interstitial oedema 3)Inflammatory cells infiltration 4) Tubular necrosis 5) Tubular casts(hyaline) and graded present (as '+' to '+++++') or absent (as '-')

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Histopathological grading

Histopathological features	DWNCG	LNSO/HNSO	KNCG	KLNSO	KHNSO
1)Glomerular and tubular congestion,	-	-	++++	+++	++
2)Interstitial edema	-	-	+++	++	+
3)Inflammatory cells infiltration	-	-	+++	++	++
4)Tubular necrosis	-	-	++++	++	+
5)Tubular casts(hyaline)	-	-	+++	+	-

DWNCG = Distilled water normal control group.

LNSO = Low dose (1ml/kg) Nigella sativa oil group

HNSO = High dose (2ml/kg) Nigella Sativa oil group

KNCG = Kanamycin negative control group.

KLNSO= Kanamycin+ Low dose(1ml/kg) N. sativa oil.

KHNSO= Kanamycin + High dose(2ml/kg) N. sativa oil

Discussion

Histopathological Examination: Histopathological examination revealed that there was damage to the nephrons more so in renal cortical areas, mainly in PCT region of nephrons due to administration kanamycin. This is in accordance with various other studies conducted to evaluate nephrotoxic potential of this aminoglycoside. Simultaneous administration of low dose of Nigella sativa oil in animals treated with kanamycin led to decrease in the damage to renal tissue as compared to that caused by kanamycin alone, also when the Nigella sativa oil was given in a higher dose along with kanamycin increased reduction in nephrotoxicity was observed. In the present study the histological examination for renal tissues

obtained from the groups receiving Nigella sativa oil (only) in low dose and high dose was also performed. And it was seen that the histological appearance of these samples were similar to those of the normal control group and were devoid of any pathology. The results obtained were in accordance with those reported in the study conducted by Zaoui et al (2002). [16] Aminoglycosides group comprises of chemically similar compounds, thus mechanism of nephrotoxicity by various aminoglycosides is more or less similar and varies only with respect to severity. Around 10% of parenteral dose of aminoglycosides reportedly gets accumalated in cells of Proximal renal tubules (Galløe et al 1995). [7] There they cause nephrotoxic effects by inhibition of mitochondrial ribosomes, analogous to their bactericidal effect on the ribosomal units of bacteria (Hobbie et al 2008). [8] This injury may progress to renal failure, particularly in the presence of hypovolemia. Plasma t 1/2 of aminoglycosides is a few hours as compared to several days in the proximal tubule cells; this exposure leads to an exponential risk for renal toxicity Aminoglycosides are polycationic molecules capable of binding to phospholipids of the renal brush border and particularly the basolateral membranes, thus inhibiting the activity of lysosomal phospholipases (Commandeur et al 1990). [17] It is proposed that nephrotoxicty resulting from aminoglycosides could be attributed to increase in oxidative stress and subsequent oxidative damage to cell membrane and other organelles of the cell. This was evident in studies conducted by Walker et al (1999) [18], and Karahan et al (2005) [19], who reported that gentamicin induced increase in oxidative free radicals were responsible for injury to renal tissues. Likewise it was reported from Rengaraju et al (2016) [20] that gentamicin induces oxidative stress in rat kidney, as evidenced by the significant increase in lipid peroxidation and significant decline of endogenous antioxidants such as reduced Glutathione, Superoxide dismutase and Catalase. The histopathological effects of kanamycin as documented in our study is in conjunction with study performed by Salgueiro SR et al 2014 [21] who described the glomerular effects of kanamycin through the use of chronic models of kanamycin nephrotoxicity in rats, in terms of increased mesangial matrix at the expense of increasing the number of mesangial cells and also the presence of glomerular synechiae.

Nephroprotective Role of Nigella Sativa: Several studies have shown nephroprotective activity of Nigella sativa. In the present study Nigella sativa oil was seen to have a nephroprotective role on the basis of observations obtained from histopathological examination of renal tissues obtained from study animals. From the results of present study it was evident that Nigella sativa oil given concomitantly with aminoglycosides led to decrease in damage to renal tissue which was evident from histopathological findings. Similar results were reported by Rehman et al (2012)[22] with Nigella sativa oil was concomitant administration which led to protectivities effects on morphological and histological aspects in tubular parts of kidney. Yaman et al (2010)[23] attributed the nephroprotective effect of Nigella sativa oil to it's ability to attenuate oxidative stress through attenuation of lipid peroxidation. Salama et al (2011)[24] who reported that the beneficial effects of the use of the N. sativa seeds and thymoquinone (one of its constituent) might be related to their cytoprotective and antioxidant actions. Further studies for quantification of concentration of aminoglycosides in renal tubular cells are warranted, when aminoglycosides are administered alone and when they are administered concurrently with nigella sativa oil.

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