e-ISSN: 0975-5160, p-ISSN: 2820-2651

Available online on www.ijtpr.com

International Journal of Toxicological and Pharmacological Research 2024; 14(8); 15-25

Original Research Article

A Comparative Experimental Study to Evaluate Nephroprotective Effects of Nigella Sativa Oil in Gentamicin and Kanamycin Induced Nephrotoxicity in Rats.

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Received: 03-06-2024 / Revised: 18-06-2024 / Accepted: 03-07-2024

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Conflict of interest: Nil

Abstract

Background: Very frequently Drug-induced toxicity occurs in the kidney. Renal uptake, renal accumulation, and renal biotransformation of drugs could contribute to this susceptibility of kidneys to toxic damage. Aminoglycosides are often indicated in treatment of infections caused by aerobic gram-negative bacteria and resistant mycobacteria causing drug resistant TB. Nephrotoxicity is one of the most important side effects and therapeutical limitations of these antibiotics, especially gentamicin and kanamycin. Traditional medicinal plants and their products like nigella sativa oil may offer nephroprotection.

Materials and Methods: Plant material: nigella sativa oil experimental animals: healthy adult albino rats weighing 100-200g of either sex. Experimental models I) Aminoglycoside induced nephrotoxicity models a) Gentamicin induced nephrotoxicity. Nephrotoxicity was induced by Gentamicin in the dose of 80mg/kg/day through i.p route daily for 8 days. b) Kanamycin induced nephrotoxicity. Nephrotoxicity was induced by Kanamycin in the dose of 300mg/kg/day through i.p route daily for 14 days. II) Nigella sativa: Nephroprotective 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.

Results: In the animals treated with gentamicin and kanamycin, values of biochemical parameters were altered as compared with normal control values. Administration of kanamycin or gentamicin along with N. sativa oil in doses of 1ml/kg (low dose) and 2ml/kg(high dose) led to decrease in the level of alteration in values of biochemical parameters and markers of histopathological changes.

Conclusion: Nephrotoxicity induced by Gentamicin is more as compared to Kanamycin . Nigella sativa oil used for studying its nephroprotective effect is found to be nephroprotective against gentamicin and kanamycin induced nephrotoxicity.

Keywords: Aminoglycoside; Nephrotoxicity ; Experimental study; Gentamicin; Kanamycin; Nephroprotection; Nigella sativa oil.

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Introduction

NEPHROTOXICITY may be defined as renal disease or dysfunction that arises as a direct or indirect exposure to environmental and occupational agents as well as from diagnostic and therapeutic drugs. Very frequently Drug-induced toxicity occurs in the kidney. Renal uptake, renal accumulation, and renal biotransformation drugs could contribute to this susceptibility of kidneys to toxic damage. Many nephrotoxic substances produce mitochondrial dysfunction, leading to cellular apoptosis or necrosis (Kim HS et al 2020). [1] Many recent epidemiologic studies have shown that nephrotoxic drugs were contributing factors in 19% to 25% of cases of severe acute renal failure in critically ill patients (Uchino S et al.,2005 [2]; Mehta RL et al.,2004). [3]

Acute kidney injury (AKI), is currently defined as an absolute increase in serum creatinine level by 0.3 mg/dL or a relative increase of 50% over 48 h. AKI is a global health challenge of vast proportions, as approx. 13.3 million people worldwide are affected annually (Kwiatkowska E et al 2021).[4] Drug-induced AKI accounts for 19–26% of all hospitalized cases. Drugs can cause damage to different fragments of nephron like the glomerulus or the tubules. (Perazella MA et al 2018) [5]

Multitude of physiologic factors which are being implicated in the production of the nephrotoxic response caused by these drugs. Large renal blood flow (25% of the cardiac output) is one of the important features. This response can be attributed to function of concentration. Moreover high renal blood flow, exposes kidney to large quantities of whatever is in that blood, including nephrotoxicants. The ability of the kidney to concentrate the tubular fluid contents is a hallmark of renal function. Any nephrotoxic or potentially nephrotoxic compound present in the tubular fluid would be concentrated in a similar manner, which could contribute to direct damage to tubular epithelial cells or at least the creation of a concentration gradient that would facilitate the movement of the compound or compounds from the tubular fluid to the blood. (William O Brandt 1998).[6] There are epidemiological evidence which indicate that nephrotoxicity leading to acute and/or chronic renal failure presents a substantial financial burden to society (Weinberg J.M et al., 1991). [7]

AMINOGLYCOSIDES group includes gentamicin, tobramycin, amikacin, netilmicin, kanamycin, streptomycin, paromomycin, and neomycin. Aminoglycosides are indicated in treatment of infections caused by aerobic gram-negative bacteria. Nephrotoxicity is one of the most important side effects and therapeutical limitations of aminoglycoside antibiotics, especially gentamicin. Despite rigorous patient monitoring, nephrotoxicity appears in 10–25% of therapeutic courses.(Lopez-Novoa JM et al 2011). [8] Aminoglycosides are bactericidal agents exhibiting concentration-dependent killing of pathogenic microorganisms. These agents undergo active transport into the bacterial cell where they inhibit protein synthesis on the 30S subunit of the ribosome. The development of multi-drug resistance among bacteria has now lead clinicians to re-examine the role of the aminoglycosides in the treatment of serious infections. (Wargo KA et al 2014). [9] The nephrotoxic potential of kanamycin is well-documented, but very few studies have reported data regarding their specific use in the treatment of Drug Resistant -TB, which requires a quite a long duration of treatment duration as compared to other indications (Perumal R et al 2018). [10] Due to their polar nature, the aminoglycosides do not penetrate into most cells, the CNS, or the eye. Concentrations of aminoglycosides in secretions and tissues are low. High concentrations are found only in the renal cortex and the endolymph and perilymph of the inner ear; the high concentration in these sites likely contribute to the nephrotoxicity and ototoxicity caused by these drugs. Chronic kidney disease (CKD) has emerged as a global public health burden for its increasing number of patients, high risk of progression to end-stage renal disease (ESRD), and poor prognosis of morbidity and mortality. (El Nahas AM et al 2005; Levey AS et al 2007) [11]. 10% of the population worldwide is affected by chronic kidney disease (CKD), and many die each year because they do not have access to affordable treatment. In developed countries, ESRD is a major cost driver for health-care systems, with annual growth of dialysis programs ranging between 6% and 12% over the past two decades and continuing to grow, particularly in developing countries. Over 2 million people now require renal replacement therapy to sustain life worldwide, but this likely represents less than 10% of those who need it (Eggers PW 2011). [12]

Nephroprotection is an emerging concept which aims at early detection and subsequent prevention of progression of kidney disease, mainly through lifestyle adjustment and the use of new pharmacological agents (Lameire N et al.,2005).[13]

The facts that 1) AKI in patients is increasing 2) Very few drugs solely offer renoprotection without causing serious adverse effects 3) Aminoglycosides are still widely used 4) Traditional medicinal plants may offer a suitable alternative for nephroprotection prompted us to carry out this study. The present animal study was undertaken with an aim to study nephroprotective activity of Nigella sativa oil against gentamicin and kanamycin induced nephrotoxicity in rats. Aminoglycosides namely A)Gentamicin and B) Kanamycin are used as nephrotoxicity inducing agents and the effect of Nigella sativa oil (low and high dose) in offering nephroprotection is being evaluated.

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

I) Aminoglycoside induced nephrotoxicity models

A. Gentamicin induced nephrotoxicity. Nephrotoxicity was induced by Gentamicin in the dose of 80mg/kg/day (Naidu et al 2000[14], Singh et al 2009[15]) through i.p route daily for 8 days.
B. Kanamycin induced nephrotoxicity. Nephrotoxicity was induced by Kanamycin in the dose of 300mg/kg/day (Luft et al 1978[16], Niizato et al 1976[17]) through i.p route daily for 14 days.

II) Nigella sativa Nephroprotective 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[18], Ali BH 2004[19], Danladi 2013[20]).

EXPERIMENTAL DESIGN. Healthy adult albino Wistar rats of either sex weighing 100-200gm were randomly divided into 9 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): (n=5)	Distilled water 1ml/kg (From day 1-14.)	Intra-peritoneally
Group II (normal control): (n=5)	Nigella sativa oil – 1ml/kg (From day 1-16)	Oral
Group III (normal control): (n=5)	Nigella sativa oil – 2ml/kg (From day 1-16)	Oral
Group IV: (n=5) (Gentamicin negative control)	Gentamicin (80mg/kg). (From day 1-8)	Intra-peritoneal
Group V: (n=5) (Gentamicin and low dose- -nigella sativa oil treatment group)	Low dose Nigella sativa oil. (1ml/kg). (From day 1-10) + Gentamicin(80mg/kg) (From day 3-10)	Oral Intra-peritoneally
Group VI: (n=5) (Gentamicin and high dose- nigella sativa oil treatment group)	High dose Nigella sativa oil. (2ml/kg) (From day 1-10) + Gentamicin(80mg/kg) (from Day 3-10)	Oral Intra-peritoneal
Group VII: (n=5) (Kanamycin negative control group)	Kanamicin. (300mg/kg)	Intra-peritoneally
Group VIII: (n=5) (Kanamycin and low dose- -nigella sativa oil treatment group)	Low dose Nigella sativa oil. (1ml/kg). (from Day 1-16) + Kanamycin(300mg/kg) (from Day 3-16)	Oral Intra-peritoneal
Group IX: (n=5) (Kanamycin and high dose- -nigella sativa oil treatment group)	High dose Nigella sativa oil. (2ml/kg). (from Day 1-10) + Kanamycin (300mg/kg) (from Day 3-16)	Oral Intra-peritoneal

Administration of Nigella sativa oil was started two days prior wherever aminoglycoside antibiotic was co-administered (Ali BH 2004).

Twenty four hours after the last dose in respective treatment group was administered following procedures were carried out:

I) Blood samples were collected for measurement of following biochemical parameters: 1) Blood urea nitrogen (BUN). 2) Blood urea 3) Serum creatinine

II) Animals were dissected and sacrificed (under pentobarbitone sodium 50mg/kg i.p. anaethesia). The kidneys were dissected out for histopathological examination

III) Oxidative stress studies were carried out on renal tissue samples from rats of all groups for following parameters 1) Malondialdehyde (MDA) 2) Catalase 3) Reduced glutathione (GSH).

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 '-').

Statistical Analysis

The results are presented as Mean ± Standard Deviation (SD). The groups were compared by one-way Analysis of Variance (ANOVA) followed by post hoc "Dunnett's Multiple comparison test" to analyze statistical significance. A "p" value of less than 0.05 ($p<0.05$) was considered to be significant.

Results

Biochemical Parameters

Effect of Distilled water Nigella Sativa oil low dose (1ml/kg) and Nigella sativa oil high dose (2ml/kg): Administration of Distilled water alone for 14 days, Nigella sativa oil alone in low dose (1ml/kg p.o) and high dose (2ml/kg p.o) for 16 days produced no significant change in BUN, Blood urea and Serum creatinine levels as compared to normal control.

S. No.	Groups	BUN (mg/dl)	Blood Urea (mg/dl)	Serum creatinine (mg/dl)
I.	Normal Control	19.14 ± 1.02	40.96 ± 2.19	0.61 ± 0.03
II.	LNSO	18.64 ± 2.54	39.88 ± 7.68	0.57 ± 0.08
III.	HNSO	19.04 ± 1.76	40.74 ± 5.32	0.59 ± 0.05

The values are expressed as Mean ± Standard Deviation (SD). LNSO = Low dose (1ml/kg) Nigella sativa oil. HNSO = High dose (2ml/kg) Nigella sativa oil.

A. Gentamicin Induced Nephrotoxicity Model

In the normal control group, which was given only distilled water; BUN, Blood urea and Serum creatinine were within normal range. In the animals treated with gentamicin, the level of BUN, Blood urea and serum creatinine were significantly elevated ($p<.001$). There was a decrease in the levels BUN and Blood Urea in the groups treated with low dose Nigella sativa oil along with gentamicin and this decrease was significant ($p<0.05$) when compared with negative (gentamicin) control group whereas as the decrease in the levels of serum creatinine was not significant. In the treatment group which received high dose of Nigella sativa oil along with gentamicin it was seen that decrease in the levels of BUN, Blood Urea and serum creatinine were highly significant ($p<0.001$) when compared with levels of BUN, Blood Urea and serum creatinine in the negative(gentamicin) control group.

S. No.	Groups	BUN (mg/dl)	Blood Urea (mg/dl)	Serum creatinine (mg/dl)
I.	Normal Control	19.14 ± 1.02	40.96 ± 2.19	0.61 ± 0.03
II.	GNCG	65.79 ± 4.25$^{\#}$	140.80 ± 9.10$^{\#}$	2.37 ± 0.34$^{\#}$
III.	GLNSO	56.91 ± 5.57^{a}	121.80 ± 11.9^{a}	2.08 ± 0.16
IV.	GHNSO	40.75 ± 2.90^{c}	87.20 ± 6.22^{c}	1.53 ± 0.08^{c}

The values are expressed as Mean ± Standard Deviation (SD) where "a" is $p<0.05$ and "c" is $p<0.001$ when compared with the Gentamicin negative control group. Also "#" is $p<0.001$ when comparison is made with Normal control group. GNCG = Gentamicin negative control. GLNSO= Gentamicin + Low dose (1ml/kg) N. sativa oil. GHNSO= Gentamicin + High dose (2ml/kg) N. sativa oil

B. Kanamycin Induced Nephrotoxicity Model

The normal control group received only distilled water and upon measurement BUN, blood urea and serum creatinine were within normal range. In the animals treated with kanamycin alone BUN, Blood urea and Serum creatinine were significantly elevated ($p<.001$). Administration of kanamycin along with

N. sativa oil in doses of 1ml/kg (low dose) and 2ml/kg(high dose) led to decrease in the levels of BUN, Blood Urea and serum creatinine as compared with the negative (Kanamycin) control group (group X) which were significant . In the treatment group which was given low dose of Nigella sativa oil the decrease in levels of BUN and Blood urea was highly significant ($p<0.001$) and the decrease in levels of Serum creatinine was significant upto $p<0.01$ when the values were compared with the levels of BUN, Blood Urea and Serum creatinine in the negative(kanamycin) control group.

S. No.	Groups	BUN (mg/dl)	Blood Urea(mg/dl)	Serum creatinine (mg/dl)
I.	Normal Control	19.14 ± 1.02	40.96 ± 2.19	0.61 ± 0.03
II.	KNCG	58.29 ± 2.13$^{\#}$	124.74 ± 4.57$^{\#}$	1.69 ± 0.08$^{\#}$
III.	KLNSO	50.58 ± 1.71^{c}	108.25 ± 3.68^{c}	1.47 ± 0.06^{b}
IV.	KHNSO	37.54 ± 1.97^{c}	80.33 ± 4.22^{c}	1.14 ± 0.08^{c}

The values are expressed as mean. "b" is $p<0.01$, and "c" is $p<0.001$ when compared with the Kanamycin negative control group. Also"#" is $p<0.001$ when comparison is made with Normal control group. KNCG = Kanamycin negative control group. KLNSO= Kanamycin+ Low dose (1ml/kg) N. sativa oil. KHNSO= Kanamycin + High dose (2ml/kg) N. sativa oil

Parameters of Oxidative Stress.

Effect of Nigella Sativa oil low dose (1ml/kg) and Nigella sativa oil high dose (2ml/kg): Administration of Nigella sativa oil alone in low dose 1ml/kg and high dose 2ml/kg did not show much deviation in parameters MDA, Catalase and GSH from normal control. The values were statistically similar to normal control ($p>0.05$).

S. No.	Groups	MDA (nmoles/g wet tissue wt.)	Catalase (nmoles H_2O_2 consumed/min /mg protein)	GSH (nmoles/mg protein)
I.	Normal Control	48.5 ± 1.57	131.19 ± 2.36	18.35 ± 0.45
II.	LNSO	46.15 ± 2.10	135.57 ± 2.65	18.90 ± 1.07
III.	HNSO	46.80 ± 1.40	133.21 ± 3.80	18.75 ± 0.84

The values are expressed as Mean ± Standard Deviation (SD). LNSO = Low dose (1ml/kg) Nigella sativa oil. HNSO = High dose (2ml/kg) Nigella sativa oil.

Gentamicin Induced Nephrotoxicity Model

In the normal control group, which was given only distilled water, the levels of MDA, Catalase and GSH were measured. In the animals treated with gentamicin the levels of MDA were significantly elevated, whereas Catalase and GSH levels were significantly decreased as compared with normal control group. Administration of N. sativa oil in doses of 1ml/kg (low dose) and 2ml/kg(high dose) led to decrease in the levels of MDA which was highly significant ($p<0.001$). The increase levels of catalase was highly significant ($p<0.001$) as compared with the negative (gentamicin) control group (group IV). Increase levels of GSH was highly significant ($p<0.001$) in group which received Nigella sativa oil in high dose along with gentamicin whereas the increase in levels of GSH in group receiving Nigella sativa oil in low dose was significant upto $p<0.05$, when compared with levels of GSH in the negative (gentamicin) control group (group IV).

S. No.	Groups	MDA (nmoles/g wet tissue wt.)	Catalase (nmoles H_2O_2 consumed/min /mg protein)	GSH (nmoles/mg protein)
I.	Normal Control	48.5 ± 1.57	131.19 ± 2.36	18.35 ± 0.45
II.	GNCG	126.5 ± 3.64$^{\#}$	91.40 ± 1.33$^{\#}$	8.40 ± 0.49$^{\#}$
III.	GLNSO	110.65 ± 1.40^{c}	97.31 ± 1.75^{c}	9.52 ± 0.54^{a}
IV.	GHNSO	90.83 ± 3.01^{c}	116.65 ± 2.25^{c}	11.28 ± 0.81^{c}

The values are expressed as Mean ± Standard deviation (SD) where "a" is $p<0.05$, and "c" is $p<0.001$ when compared with the Gentamicin negative control group. Also "#" is $p<0.001$ when

comparison is made with Normal control group. GNCG = Gentamicin negative control. GLNSO= Gentamicin + Low dose (1ml/kg) N. sativa oil. GHNSO= Gentamicin + High dose (2ml/kg) N. sativa oil.

Kanamycin Induced Nephrotoxicity Model

In the normal control group, which was given only distilled water and the parameters MDA, Catalase and GSH were measured. In the animals treated with kanamycin the level of MDA were significantly elevated, whereas Catalase and GSH levels were significantly decreased as compared with normal control group. Administration of N. sativa oil in doses of 1ml/kg and 2ml/kg led to decrease in the level of MDA, whereas levels of Catalase and GSH showed an increase as compared with the negative (kanamycin) control group (group X) which were significant. When levels of MDA in treatment groups receiving Nigella sativa oil in low dose and high dose along with kanamycin were compared with levels of MDA in negative (kanamycin) control group it was seen that decrease in levels of MDA in treatment groups was highly significant ($p<0.001$). The levels of catalase in negative(kanamycin) control group were lower as compared to treatment groups and the difference was significant upto $p<0.01$ in low dose group whereas the difference in levels when compared with high dose treatment group was highly significant ($p<0.001$). In the treatment groups which received Nigella sativa oil in low dose there was an increase in level of GSH which was significant($p<0.05$) and the increase in level of GSH was highly significant ($p<0.001$) in treatment group which received high dose of Nigella sativa oil along with kanamycin.

S. No.	Groups	MDA (nmoles/ g wet tissue wt.)	Catalase (nmoles H_2O_2 consumed/ min/mg protein)	GSH (nmoles/mgprotein)
I.	Normal Control	48.5 ± 1.57	131.19 ± 2.36	18.35 ± 0.45
II.	KNCG	109.3 ± 1.95$^{\#}$	100.80 ± 1.22$^{\#}$	9.92 ± 0.47$^{\#}$
III.	KLNSO	101.25 ±1.07^{c}	106.19 ± 1.23^{b}	11.17 ± 0.67^{a}
IV.	KHNSO	87.84 ± 2.18^{c}	114.91 ± 3.33^{c}	12.61 ± 0.84^{c}

The values are expressed as Mean ± Standard Deviation (SD) where "a" is $p<0.05$, "b" is $p<0.01$ and "c" is $p<0.001$ when compared with the Kanamycin negative control group. Also "#" is $p<0.001$ when comparison is made with Normal control group. . KNCG = Kanamycin negative control group. KLNSO= Kanamycin+ Low dose (1ml/kg) N. sativa oil. KHNSO= Kanamycin + High dose (2ml/kg) N. sativa oil.

Histopathology

Kidney sections showing inflammatory cells infiltration, atrophied glomerulus, degeneration, desquamation of tubular epithelium and hyaline casts are suggestive of tubular necrosis and interstitial edema.

Effect of N. sativa oil on Gentamicin induced nephrotoxicity in rats Histopathological grading.

Histopathological features	Normal control group	GNCG	GLNSO	GHNSO
Glomerular and tubular congestion,	-	++++	+++	++
Interstitial oedema	-	+++	++	+
Inflammatory cells infiltration	-	+++	++	++
Tubular necrosis	-	++++	+++	++
Tubular casts(hyaline)	-	+++++	++	+

GNCG = Gentamicin negative control.

GLNSO= Gentamicin + Low dose(1ml/kg) N. sativa oil.

GHNSO= Gentamicin + High dose(2ml/kg) N. sativa oil

Effect of N. sativa oil on Kanamycin induced nephrotoxicity in rats

Histopathological grading

Histopathological features	Normal control group	KNCG	KLNSO	KHNSO
Glomerular and tubular congestion	-	++++	+++	++
Interstitial oedema	-	+++	++	+
Inflammatory cells infiltration	-	+++	++	++
Tubular necrosis	-	++++	++	+
Tubular casts(hyaline)	-	+++	+	-

KNCG = Kanamycin negative control group.

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

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

Discussion

Aminoglycosides drugs are known to cause nephrotoxicity. Major portion of the injected drug is excreted via urine. Results have indicated that aminoglycosides are taken up by receptor-mediated endocytosis following the binding of aminoglycosides to the brush-border membrane. (Baylis C et al 1977). [21] It is proposed that long term treatment leads selective accumulation of these drugs in renal tissues which is responsible for their nephrotoxic effects. This causes disturbances in renal functions as evident by derangements in biochemical parameters like BUN(Blood Urea Nitrogen),Serum urea and Serum creatinine levels.

Natural substances obtain from medicinal plants or other natural sources may provide for a useful nephroprotective agent with fewer side effects. Several such agents: *Rheum officinalis* (Yokozawa et al 1991), [22] *Zingiber officinale* (Narora et al.,1992), [23]; Honey(Abd Ali A.R.2012), [24] Allium sativum (Abdelaziz I et al 2011) [25] and more have been tested for their role in improving renal function or in offering nephroprotection in various studies. In this study Nigella sativa oil was selected for evaluating it's nephroprotective role although studies have been conducted to test for nephroprotection offered by this amazing plant source but very few studies have been conducted on the oil obtained from seeds of Nigella sativa in regards to nephroprotective activity against nephrotoxicity induced by various aminoglycosides. Treatment with Nigella Sativa oil led to improvement in renal functions as evident by measurement of biochemical parameters.

In the present study it was found that administration of gentamicin led to derangements in biochemical parameters. These findings were in accordance with studies conducted by Luft FC et al 1978 and Saleem U et al 2012. [26]The Kanamycin treated group show similar effects on biochemical parameters and it was seen that derangement in the values of biochemical is less marked when compared with gentamicin treated animals.

The present study also evaluated the effect on biochemical parameters related to renal function upon administration of Nigella sativa oil in low dose and a high dose alone. It was seen that the administration of Nigella sativa oil alone in low dose 1ml/kg body weight and a high dose 2ml/kg body weight did not show much deviation in values of biochemical parameters and the values were more or less same as values obtained in normal control group.

Effects on levels of biochemical parameters: bun, blood urea and s. creatinine due to co-administration of nigella sativa oil and aminoglycosides

Gentamicin treatment Groups:

Nigella sativa oil when administered along with gentamicin led to dose dependant decrease in BUN, S.urea and S.creatinine as compared to group treated with gentamicin alone. When Nigella sativa oil was administered in low dose (1ml/kg) along with gentamicin there was decrease in level of Blood Urea was significant ($P<0.05$),whereas the decrease in level of BUN and S.creatinine was not significant as compared to group which was treated with gentamicin alone. In the group treated with high dose (2ml/kg) of Nigella sativa oil there was decrease in levels of BUN,Blood urea and S.creatinine and this difference was significant ($p<0.01$) when compared with group treated with Gentamicin alone.

Kanamycin Treatment Groups:

Similar results were obtained when of Nigella sativa oil was given alongwith kanamycin which led to dose dependant decrease in BUN, S.urea and S.creatinine as compared to group treated with kanamycin alone. Low dose (1ml/kg) of N. sativa oil along with Kanamycin showed decrease in level of BUN and Blood Urea and this decrease in values was significant ($p<0.001$),whereas the decrease in level of S. creatinine was significant upto $p<0.01$ as compared to group receiving Kanamycin only.

Aminoglycoside are taken up by renal proximal tubular cells and remain there for an extended period this leads to renal damage in the form of structural and functional impairment of the plasma membrane, mitochondria and lysosome (Mingeot-Leclerq et al 1999). [27] Histopathological studies strongly support the concept that tubular necrosis (and related phenomena) is the primary cause of functional toxicity. It is possible that no single

change or alteration is important per se but that tubular cells eventually die because of the simultaneous occurrence of multiple changes (Kaloyanides 1984). [28]

Histopathological Examination

Histopathological examination revealed that there was damage to the nephrons of cortical region, mainly in PCT due to administration of gentamicin and kanamycin. This is in accordance with various other studies conducted to evaluate nephrotoxic potential of these aminoglycosides. On the basis of histopathological examination and grading gentamicin was found to be the more nephrotoxic than kanamycin.

Histopathological examination revealed that, in the renal tissue of group which was given kanamycin alone, severe damage to renal tissue was evident. The degree of damage seen was lesser when compared with gentamicin (only) treated group.

It was also seen that administration of low dose of Nigella sativa oil in gentamicin and kanamycin treated animals led to decrease in the damage as compared to damage caused by gentamicin alone or kanamycin alone, also when the Nigella sativa oil was given in a higher dose along with gentamicin or with kanamycin it led to a further reduction in observed nephrotoxicity and the evident damage was graded as lesser when compared with that observed in the group treated with gentamicin or kanamycin administered along with low dose of Nigella sativa oil.

Histopathological examination revealed that, in the renal tissue of group which was given kanamycin alone, severe damage to renal tissue was evident. The degree of damage seen was lesser when compared with gentamicin (only) treated group which was very severe.

In the present study the histopathology of renal tissues obtained from the groups receiving only Nigella sativa oil in low dose and high dose was also observed for histopathological changes. And it was seen that the histopathological appearance of these samples were similar to those of the normal control group. These results were in accordance with results of the study conducted by Zaoui et al (2002). [29]

Owing to the similarity in chemistry amongst various aminoglycosides the mechanism of causing nephrotoxicity by aminoglycosides is similar. 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). [30] It is proposed that nephrotoxicity 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), [31] and Karahan et al (2005), [32] 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) [33] 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 gentamicin and kanamycin as documented in our study is in conjuction with study performed by Salgueiro SR et al 2014 [34] 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

Effects On Levels of Oxidative Stress Markers: MDA, Catalase And GSH Due To Administration of Nigella Sativa Oil Concurrently With Aminoglycosides.

Gentamicin treatment Groups:

In the normal control group, which was given only distilled water; the levels of MDA, Catalase and GSH were measured. In the animals treated with gentamicin alone, the level of MDA were significantly elevated, whereas Catalase and GSH levels were significantly decreased as compared with normal control group. Administration of N. sativa oil in doses of 1ml/kg and 2ml/kg led to dose dependent decrease in the level of MDA, whereas levels of Catalase and GSH showed a dose dependent increase as compared with the negative (gentamicin) control group (group IV). The decrease in the level of MDA was significant ($p<0.001$) in Nigella sativa oiltreated groups and this decrease was more in groups treated with higher dose of Nigella sativa oil. The increase in levels of catalase was significant ($p<0.001$) in groups treated with Nigella sativa oil and the increase was more with higher dose of Nigella sativa oil. The increase in GSH levels was significant ($p<0.05$) in group receiving low dose of Nigella sativa oil and was significant upto $p<0.001$ in group receiving high dose of Nigella sativa oil.

Kanamycin Treatment Groups:

In the normal control group, which was given only distilled water the parameters MDA, Catalase and GSH were measured. In the animals treated with kanamycin alone, the level of MDA were significantly elevated, whereas Catalase and GSH levels were significantly decreased ($p<.001$) as compared with normal control group. Administration of N.

sativa oil in doses of 1ml/kg and 2ml/kg led to dose dependent decrease in the level of MDA, whereas levels of Catalase and GSH showed a dose dependent increase as compared with the negative (kanamycin) control group (group X) which were significant. The decrease in the level of MDA was significant ($p<0.001$) in Nigella sativa oil treated groups and this decrease was more in groups treated with higher dose of Nigella sativa oil. The increase in levels of catalase was significant ($p<0.01$) in group receiving low dose of Nigella sativa oil and was significant upto $p<0.001$ in group receiving high dose of Nigella sativa oil. The increase in GSH levels was significant ($p<0.05$) in group receiving low dose of Nigella sativa oil and was significant upto $p<0.001$ in group receiving high dose of Nigella sativa oil.

The effect on oxidative stress parameters upon administration of Nigella sativa oil in low dose and a high dose alone was also evaluated in the present study.It was seen that the administration of Nigella sativa oil alone in low dose 1ml/kg and a high dose 2ml/kg body weight did not show much deviation in these parameters and the values were statistically similar to normal control($p>0.05$). This signifies thatsignificant deviation in parameters of oxidative stress from the normal amongst the treatment groups which were administered Nigella sativa oil along with aminoglycosides were due to the effect of aminoglycosides themselves.

Electrophiles may bind covalently to nucleophilic sites abundantly present in biological macromolecules, such as proteins, lipids, RNA, and DNA, this may lead tofunctional inactivation of these macromolecules. Nonprotein thiols, mainly glutathione (GSH, 5-10 mM) in the cells can protect against covalent binding.

However, after excessive covalent binding, nonprotein thiols can become depleted depletion of the cofactor GSH leads to destruction of vital macromolecules. Free radicals also bind covalently to cellular macromolecules, abstract hydrogen atoms from macromolecules, and/ or donate their unpaired electron to molecular oxygen. Removal of hydrogen atoms of lipids and reaction of the lipid radicals with molecular oxygen initiates the process of lipid peroxidation, which may lead to detrimengtal effects on fluidity, permeability, and/or integrity of membranes (Girotti 1985). [35] Increase of malonaldehyde levels by gentamicin are indicative for lipid peroxidation (Ramsammy et al 1987).[36] The elevated level of MDA, a marker of lipid peroxidation, indicates increased free-radical generation in the GS-induced nephrotoxicity (Hayashi et al 1988 [37], Kuhad et al 2006). [38] Depletion of reducing equivalents (NADPH, GSH) may shift the redox state of cellular constituents to the oxidized state causing oxidative stress. The hydroxyl radical is believed to be responsible for most of the serious damage caused by redox cycling, e.g., peroxidation of membranal lipids and protein and DNA damage (Kappus 1986). [39] Gentamicin may enhance the formation of reactive oxygen species. Gentamicin inhibits the mitochondrial state 3 respiration of isolated mitochondria, while it stimulated state 4 respiration. Because this effect was inhibitable with catalase, production of hydrogen peroxide might be involved in gentamicin nephrotoxicity (Walker et al 1987). [40] Scavengers of hydroxyl radicals (dimethyl sulfoxide, sodium benzoate, dimethyl thiourea or iron chelator deferoxamine) inhibit Fenton reaction protected rats against acute renal failure of gentamicin in vivo, which suggests a pivotal role for hydroxyl radicals in causation of nephrotoxicity due to gentamicin.

Nephroprotective Role of Nigella Sativa:

Several studies have shown nephroprotective activity of Nigella sativa. In the present study Nigella sativa oil administration was seen to have a nephroprotective role on the basis of observations obtained from biochemical analysis and histopathological examination. In the present study it was evident from the resultsthat Nigella sativa oil given concomitantly with aminoglycosides led to decrease in deviation in biochemical parameters when compared with negative control group. And there was decrease in damage to renal tissue which was evident from histopatholical findings. These positive findings in favour of the Nigella sativa oil

Co-administration can be due to it's ability to decrease the damage to the cells of renal tissue by decreasing the MDA levels, increasing the Catalase and GSH levels which would inturn lead to decrease in oxidative stress to the cells and thus leading to lesser cellular damage. Moreover, concurrent administration of Nigella sativa oil might also be hindering the mechanism of uptake and concentration of aminoglycosides by renal tubular cells. Similar results were reported by Rehman et al (2012).[41] when Nigella sativa oil was concomitantly administered with gentamicin which led to morphological and histopathological changes to tubular parts of kidneys by potentiating the existing antioxidant defense mechanism at the levels of tubular parts of kidneys along with the reduction of serum creatinine and urea levels. Also Salama et al (2011)[42] who reported that the beneficial effects of the use of the N. sativaseeds and thymoquinone (one of its constituent) might be related to their cytoprotective and antioxidant actions. Yaman et al (2010)[43] attributed the nephroprotective effect of Nigella sativa oil to it's ability to attenuate oxidative stress through attenuation of lipid peroxidation. Further studies for quantification of concentration of aminoglycosides in renal tubular cells and endolymph are warranted, when aminoglycosides are administered concurrently with nigella sativa oil.

Conclusion

The present study entitled "Study of nephroprotective activity of Nigella sativa oil against aminoglycosides induced nephrotoxicity in rats" is an experimental study carried out on albino rats. Fourty five rats were equally and randomly divided in 9 groups. The work was carried out in Department of Pharmacology and Department of Anatomy J.N. Medical College, AMU, Aligarh. In the present study the aminoglycosides namely gentamicin and kanamycin were used to induce nephrotoxicity in rats. It was evident from biochemical parameters, parameters for oxidative stress and histopathological examination of renal tissues that above drugs are nephrotoxic. Nephrotoxicity induced by **Gentamicin is more as compared to Kanamycin**

Nigella sativa oil used for studying its nephroprotective effect is found to be **nephroprotective** against gentamicin and kanamycin induced nephrotoxicity. Nephroprotective effect could be attributed to it's ability to decrease oxidative stress, as increased oxidative stress thought to be the main mechanism involved in causing nephrotoxicity due aminoglycoside administration.

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