Print ISSN: 2319-2003 | Online ISSN: 2279-0780

IJBCP International Journal of Basic & Clinical Pharmacology

DOI: http://dx.doi.org/10.18203/2319-2003.ijbcp20160104

Research Article

Evaluation of nephroprotective and nephrocurative activity of *Solanum nigrum* on gentamicin induced nephrotoxicity in experimental rats

Vimlesh Kushwaha*, Monica Sharma, Pinki Vishwakarma, Manish Saini, Shaily Bhatt, Kuldeep K. Saxena

Department of Pharmacology, L. L. R. M. Medical College, Meerut, UP, India-250004

Received: 7 December 2015
Revised: 8 December 2015
Accepted: 7 January 2016

***Correspondence to:**
Dr. Vimlesh Kushwaha,
Email: drvijv2008@gmail.com

ABSTRACT

Background: Aminoglycoside antibiotics are most commonly used drugs for the prevention and treatment of gram negative infections. Nephrotoxicity is the main side effect that restricts its long duration use. Modern medicines to treat nephrotoxicity are costly and also not vary effective. *Solanum nigrum* fruits, having potent antioxidant property can be used for nephroprotection as well as nephrocure.

Methods: The study was carried out in two phases. Nephroprotective phase, 54 rats were randomized in 3 groups named G10, G20 & G30 according to 10, 20 & 30 days of treatment. Each group was randomized in three subgroups i.e. control C group [received normal saline (2 ml/100 gm/day) daily for test duration], GT group [received normal saline (2 ml/100 gm/day) daily for test duration & intra-peritoneal gentamicin (40mg/kg) for last five days] & SNT group [received orally *S.nigrum* (200 mg/kg/day) daily for the test duration and intra-peritoneal gentamicin (40 mg/kg) for last five days]. Rats were sacrificed 24 hours after the last dose of gentamicin (on 11th, 21st and 31st day). In nephrocurative phase, 72 rats were randomised in two groups of 36 rats each. Group-1 received intra-peritoneal gentamicin (40 mg/dl) for five days. Group-2 received intra-peritoneal gentamicin (40 mg/dl) for five days and then *S.nigrum* (200 mg/kg/day) orally till the rats are sacrificed. Six rats from each group were sacrificed on 3rd, 5th, 7th, 10th, 12th and 14th day after administration of last dose of gentamicin. Blood sample were taken for evaluation of BUN and serum creatinine.

Results: There was significant decrease in BUN and serum creatinine values as compared to GT group in all test duration in phase-1. In phase two there was no significant difference of these markers in two groups.

Conclusions: *S.nigrum* fruits extract provide nephroprotection against gentamicin induced nephrotoxicity.

Keywords: Nephroprotective, Nephrocurative, *Solanum nigrum*, Gentamicin

INTRODUCTION

Aminoglycoside antibiotics are commonly used drugs to prevent and treat infections, particularly against gram negative bacterial infections since the beginning of twentieth century.[1,2] Besides their beneficial effects, aminoglycoside possess considerable side effects. Nephrotoxicity and ototoxicity are the common side effects that limits long duration therapy.[3] Gentamicin is highly nephrotoxic agent among all aminoglycosides.[4] Histopathological findings showed that administration of aminoglycosides caused apoptosis, intracellular oedema, mesangial cell contraction, basal membrane interruption, glomerulus narrowing of the Bowman's capsule and acute tubule necrosis.[5,6]

Nephrotoxic potential of various aminoglycosides is correlated with number of free amino group on their surface.[7] Kidneys are the primary route of elimination of gentamicin from the body. Because of small molecular weight (500 Dalton), high water solubility and less protein bound state of gentamicin facilitates its glomerular filtration.[8] A small part of filtered gentamicin gets actively reabsorbed in the proximal tubule.[9] Gentamicin enhances the production of superoxide anion and hydroxyl radical in renal cortical mitochondria. There is a role of hydroxyl radicals in pathogenesis of gentamicin toxicity by inducing suppression of Na^+K^+-ATPase activity and DNA synthesis in proximal tubules.[10]

Now days, there is increasing incidence of renal failure with the consequence of high morbidity and mortality in the world. So prevention of occurrence and progression of acute renal failure (ARF) has become a very important issue. But modern medicine lacks reliable nephroprotective drugs.[11] Medicinal plants have curative properties due to the presence of various complexes Chemical substances. Ancient literature has prescribed various herbs for the cure of kidney disease.[12]

Solanum nigrum (black nightshade) is a medicinal plant member of the *Solanaceae* family. *S.nigrum* commonly known as Makoi or black nightshade. In India, *S.nigrum* mixed with other herbal medicine has hepatoprotective effect in cirrhotic patients. Protective effect can be attributed to the diuretic, anti-inflammatory, anti-oxidative and immuno modulating properties of the component herbs.[13] Glycoprotiens are the main effective compound of this plant. Antioxidant potential of isolated glycoprotein has been evaluated by several methods like DPPH, superoxide radical & hydroxyl radical assay. From these results it has been suggested that glycoprotein of *S.nigrum* plant, has potent anti-oxidative potential.[14] Several studies showed the nephroprotective role of *S.nigrum* but nephrocurative property of this plant is still not evaluated. So this study was done to evaluate the nephroprotective as well as nephrocurative property of *S.nigrum*.

METHODS

This study was conducted in Department of Pharmacology, L.L.R.M. Medical College, Meerut, UP, India from Jan 2015 to Oct 2015. Total duration of study was ten months. The study was conducted after obtaining approval from Institutional Animal Ethical Committee of Lala Lajpat Rai Medical College, Meerut, India, registered under CPCSEA India.

Albino rats of either sex, weighing 150-200 gm were obtained from the central animal house of the institute. The selected rats were housed in cages under controlled condition of temperature (25 ^{0}C) and alternating periods of light and darkness of 12 hours each. The rats had free access to standard rat pellet diet and tap water ad libitum. The rats were considered suitable for study after 7 days of acclimatization. Gentamicin manufactured by Abott Healthcare Pvt. Ltd (Gentycin) was used to induce Gentamicin nephrotoxicity. Aqueous extract of dried fruits of Solanum *nigrum* was used as a test drug.

Solanum nigrum: The whole dry fruits were separated and powdered mechanically with an herb grinder. The dried powder was weighted and transferred to a thin conical flask containing distilled water and kept in a shaker for 24 hours under air tight condition. After 24 hours it was filtered using Whatmann no.1 filter paper and centrifuged. The supernatant was collected & evaporated till solid yield of the extract was obtained and this extract was used for study. When needed the extract was suspended in water and used in the study.[15]

The study was carried out in two phases.

Phase-1

Evaluation of nephroprotective activity:

Nephroprotection was evaluated for three periods of 10, 20,30 days and based on duration, the groups were named G10, G20, G30. Rats were randomized in three groups of 18 animals each. In each of the main groups, rats were randomized to any of the three subgroups i.e. control (C), gentamicin treated (GT), *S.nigrum* treated (SNT) groups of six rats each.

Group C- This was the control group and was given normal saline (2 ml/100 gm/day) orally once a day, every day for test duration.

Group GT- This group was given normal saline (2 ml/100 gm/day) orally once a day consecutively for test duration, Injection gentamicin (40 mg/kg) was given intraperitonealy once daily for last five days.

Group SNT- This group received *S.nigrum* orally (200 mg/kg/day) as a single dose in morning, before giving feed for the test duration and, injection gentamicin (40 mg/kg) was given intra-peritonealy once daily for last five days.

In phase 1, rats were sacrificed 24 hours after the last dose of gentamicin injection (on 11th 21st and 31st day).

Phase-2

Evaluation of Nephrocurative Activity:

In this phase, nephrocurative activity of *S.nigrum* was compared with the spontaneous reversal of gentamicin induced nephrotoxicity. For this phase 72 rats were randomized into two groups.

Group-1: 36 rats received intra-peritoneal gentamicin for five days in a dose of 40 mg/kg.

Group-2: 36 rats received intra-peritoneal gentamicin for five days in a dose of 40 mg/kg. From fifth day onwards these rats received *S.nigrum* orally in a dose of 200 mg/kg/day till the rats are sacrificed (as mentioned below)

In phase 2 six rats from each group were sacrificed on 3rd 5th, 7th 10th, 12th and 14th day after administration of last dose of gentamicin.

The test compounds were administered by the oral gavage method with animals fasted three to four hours prior to and one hour after administration. On the last day of study, rats of all groups were kept on fasting for 24 hours (during which tap water remained freely available) after which they were sacrificed under Ketamine (75

mg/kg) and Xylazine (10 mg/kg) anaesthesia given intra-peritoneally.[16] Blood samples were collected from abdominal aorta for performing bio-chemical tests.

Estimation of biochemical parameters

The collected blood sample was centrifuged & separated serum was used for estimation of renal biochemical marker.

1. Blood urea nitrogen (BUN)
2. Serum creatinine

Blood urea nitrogen (BUN): Serum urea was estimated by Liquimax urea reagent kit, spectrophotometrically (marketed by Avecon PVT. LTD.) from the serum. The kit utilises Marshall method.[17]

Serum creatinine: Serum creatinine was estimated spectrophotometrically by Auto Zyme Creatinine reagent kit (Marketed by Accurex Biomedical PVT. LTD.) from the serum. The kit utilises initial rate method using alkaline picrate.[18]

Statistical analysis

Mean ± SE was calculated for each group. The statistical analysis was done by using one way analysis variation (ANOVA) followed by post Hoc Test. p values < 0.05 were considered as significant. P Values were estimated by referring to appropriate tables.[19]

RESULTS

Nephroprotective study (Phase-1)

A. Effect of Solanum nigrum on serum BUN

In *S.nigrum* pre-treated group (SNT), values of BUN ranged between 24.46 ± 0.73 to 40.29 ± 1.97 mg/dl and were significantly low as compared to group GT (p value <0.001) for all test durations of 10, 20,and 30 days. When compared to group C, the BUN level was significantly higher at 10 days (p value <0.001) but not significant (p value > 0.05) at 20 and 30 days pre-treatment (Table-1).

B. Effect of Solanum nigrum on serum creatinine

In *S.nigrum* pre-treated groups (SNT), values of serum creatinine ranged between 0.55 ± 0.02 to 0.97 ± 0.02 mg/dl and were significantly low (p value <0.001) as compared to GT group for all test durations of 10, 20,and 30 days. When compared to group C, the serum creatinine level was significantly higher at 10 days (p value <0.001) and not significant (p value > 0.05) at 20 and 30 days pre-treatment (Table-2).

There were significantly lower levels of serum creatinine and BUN at 20 and 30 days treatment than after 10 days with *S.nigrum*. This showed that *S.nigrum* takes more than 10 days to become fully effective.

Nephrocurative study (phase-II)

A. BUN levels

BUN in group II took nearly 10-14 days to became normal and this was not significantly different (p value >0.05) as compared to spontaneous reversal in group I. The BUN levels between group I & II after 14 days were not significantly different (p value > 0.05) (Table-3).

B. Serum creatinine level

Serum creatinine in group II took nearly 10-14 days to became normal and was not significantly different (p value >0.05) as compared to spontaneous reversal in group I . The serum creatinine levels of group I & II after 14 days were not significantly different (p value > 0.05) (Table-4).

Table 1: BUN levels in C, GT & SNT groups at different test durations (n=6).

Duration of pre-treatment (days)	BUN(mg/dl) Mean ± SE		
	C	GT	SNT
10	22.45 ± 0.40	63.91 ± 0.96$^{\alpha}$	40.29 ± 1.97$^{\theta\zeta}$
20	24.13 ± 0.41	64.08 ± 0.52$^{\beta}$	24.67 ± 0.95$^{\iota\rho}$
30	23.61 ± 0.41	65.77 ± 1.16$^{\gamma}$	24.46 ± 0.73$^{\kappa\pi}$

$^{\alpha}$p<0.001as compared to 10 days of control.
$^{\beta}$p<0.001as compared to 20 days of control.
$^{\gamma}$p<0.001as compared to 30 days of control.
$^{\theta}$p<0.001as compared to 10 days of control.
$^{\iota}$p>0.05 as compared to 20 days of control.
$^{\kappa}$p>0.05 as compared to 30 days of control.
$^{\xi}$p<0.001 as compared to 10 days of GT.
$^{\rho}$p<0.001as compared to 20 days of GT.
$^{\pi}$p<0.001as compared to 30 days of GT.

Table 2: Serum creatinine levels in C, GT & SNT groups at different test durations (n=6).

Duration of pre-treatment (days)	Serum Creatinine (mg/dl) (Mean± SE)		
	C	GT	SNT
10	0.56 ± 0.01	1.89 ± 0.04$^{\alpha}$	0.97 ± 0.02$^{\theta\xi}$
20	0.58 ± 0.02	2.00 ± 0.03$^{\beta}$	0.62 ± 0.02$^{\iota\rho}$
30	0.60 ± 0.02	2.20 ± 0.02$^{\gamma}$	0.55 ± 0.02$^{\kappa\mu}$

$^{\alpha}$p<0.001as compared to 10 days of control.
$^{\beta}$p<0.001as compared to 20 days of control.
$^{\gamma}$p<0.001as compared to 30 days of control.
$^{\theta}$p<0.001as compared to 10 days of control.
$^{\iota}$p>0.05 as compared to 20 days of control.
$^{\kappa}$p>0.05 as compared to 30 days of control.
$^{\xi}$p<0.001as compared to 10 days of GT.
$^{\rho}$p<0.001as compared to 20 days of GT.
$^{\pi}$p<0.001as compared to 30 days of GT.

Table 3: BUN levels in group-1 & group-2 at different test durations (n=6).

Days of Sacrifice	BUN(mg/dl) Mean ± SE	
	Group-1 (C)	Group-2 (SNT)
3	70.76 ± 0.66	69.87 ± 0.88$^{\eta}$
5	64.56 ± 0.77	63.46 ± 0.68$^{\theta}$
7	54.77 ± 0.83	53.38 ± 0.65$^{\iota}$
10	35.44 ± 0.53	34.64 ± 0.49$^{\kappa}$
12	25.83 ± 0.75	24.07 ± 0.34$^{\lambda}$
14	24.09 ± 0.72	23.83 ± 0.74$^{\mu}$

$^{\eta}$p>0.05 as compared to 3 days of group-1.
$^{\theta}$p>0.05 as compared to 5 days of group-1.
$^{\iota}$p>0.05 as compared to 7 days of group-1.
$^{\kappa}$p>0.05 as compared to 10 days of group-1.
$^{\lambda}$p>0.05 as compared to 12 days of group-1.
$^{\mu}$P>0.05 as compared to 14 days of group-1.

Table 4: Serum creatinine levels in group-1 & group 2 at different test durations (n=6).

Days of Sacrifice	S. Creatinine(mg/dl) (Mean± SE)	
	Group-1 (C)	Group-2 (WST)
3	1.24 ± 0.01	1.22 ± 0.02$^{\nu}$
5	1.06 ± 0.02	1.06 ± 0.02$^{\xi}$
7	0.86 ± 0.01	0.79 ± 0.01$^{\pi}$
10	0.90 ± 0.01	0.69 ± 0.01$^{\rho}$
12	0.58 ± 0.02	0.59 ± 0.01$^{\varsigma}$
14	0.54 ± 0.01	0.56 ± 0.02$^{\sigma}$

$^{\nu}$p>0.05 as compared to 3 days of group-1.
$^{\xi}$p>0.05 as compared to 5 days of group-1.
$^{\pi}$p>0.05 as compared to 7 days of group-1.
$^{\rho}$p>0.05 as compared to 10 days of group-1.
$^{\varsigma}$p>0.05 as compared to 12 days of group-1.
$^{\sigma}$p>0.05 as compared to 14 days of group-1.

DISCUSSION

Drug induced renal injuries constitute approximately 20 percent of community and hospital acquired episodes of acute renal failure.[20,21] In elderly, the incidence of drug-induced nephrotoxicity may be as high as 66 percent.[22] The nephrotoxicity of aminoglycoside antibiotics, and especially that of the most commonly used compound, gentamicin, is well documented.[23] The number of patients developing nephrotoxicity increases with duration of therapy reaching 50% if the therapy is continued for 14 days or more.[24]

In this study nephrotoxicity was induced by gentamicin because nephrotoxicity is rapidly induced by this and presents with well-established morphological and biochemical changes.[25] Gentamicin induced nephrotoxicity has spontaneous reversal potential, so nephrocurative study can be done.[26] The elevation of BUN and serum creatinine have been used in gentamicin induced nephrotoxicity models as indicators of renal injury because both have been found to correlate well with the extent of injury.[27]

In present study pre-treatment with *S.nigrum* the BUN and serum creatinine levels were significantly decreased in groups treated for 10 or 20 or 30 days as compared to GT group (p value <0.001). When compared with saline treated group, the BUN and serum creatinine values are significantly raised on day 10. But with 20 and 30 days of pre-treatment, the levels of biomarkers markedly improved and were almost equal to that in saline treated group C (p value > 0.05) (Table 1 & 2). It could be inferred that the plasma concentration of *S.nigrum* require more than 10 days to reach a protective level. Several studies have reported that oxygen-free radicals are considered to be important mediators of gentamicin induced acute renal failure. Treatment with *S.nigrum* fruit extract significantly modulated the nonenzymatic antioxidants to near normal, which may be due to their quenching and free radical scavenging action. This antioxidant property of *S.nigrum* is mainly because of the glycoprotein present in it.[28]

In phase II study, the nephrocurative activity of *S.nigrum* was compared with the spontaneous reversal of gentamicin induced nephrotoxicity. The BUN & serum creatinine levels in *S.nigrum* treated groups were not significantly different from the gentamicin alone treated group at all test durations i.e. 3rd ,5th ,7th, 10th, 12th and 14th day (Table 3 & 4). It means *S.nigrum* do not show any nephrocurative activity in this study. There may be many reasons behind the failure of *S.nigrum* to show nephrocurative activity. First, it may not have so potent antioxidant activity to provide nephrocure. Secondly, the duration for nephrocurative activity was very less (only 14 days), as the best nephroprotection was seen only at 20 days.

CONCLUSION

This study clearly indicate that *S.nigrum* fruit extract had nephroprotective potential. To see the nephrocurative potential of *S.nigrum*, some more study will be needed in future.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Karahan I, Atessahin A, Yilmaz S, Ceribas AOC, Sakin F. Protective effect of lycopene on gentamicin-induced oxidative stress and nephrotoxicity in rats, Toxicology. 2005;3(215):198-204.
2. Mwengee W, Butler T, Mgema S, Mhina G, Almasi Y, Bradley C, Formanik JB, Rochester CG.

Treatment of plague with gentamicin or doxycycline in a randomized clinical trial in Tanzania. Clini Infect Dis. 2006;5(42):614-21.
3. Pavle R, Slavimir V, Nenad S, Ljubinka JV, Dusan S, Milan S, Ivan I. Salicylic acid attenuates gentamicin-induced nephrotoxicity in rats. Sci World J. 2012;2012:1-6.
4. Suzuki SS, Takamura J , Yoshida Y, Shinzawa N, Tamatani SR. Comparison of gentamicin nephrotoxicity between rats and mice, Comp Biochem Physiol. 1995;1:15-28.
5. Martínez-Salgado C, Henández-López, FJ, Novoa-López JM. Glomerular nephrotoxicity of aminoglycosides. Toxicol Appl Pharmacol. 2007;223(1):86- 98.
6. Souza VB, Oliveira RFL, Ferreira AAA, Araújo Júnior RF. Alterações renais por aminoglicosídeos. Arq Med. 2008;22(4-5):131-5.
7. Williams PD, Bennett DB, Gleason CR, Hottendorf GH. Correlation between renal membrane binding and nephrotoxicity of aminoglycosides. Antimicrob Agents Chemother. 1987;31:570-4.
8. Faber J, Rudhardt M, Blanchard P, Regamey C, Chauvin P. Persistence of sisomicin and gentamicin in renal cortex and medulla compared with other organs and serum of rats. Kidney Int. 1976; 10:444-449.
9. Gyselynck AM, Forrey A, Cutler R. Pharmacokinetics of gentamicin: Distribution and plasma renal clearance. J Infect Dis. 1971;124:70-6.
10. Du XH, Yang CL. Mechanism of gentamicin nephrotoxicity in rats and protective effect of zinc induced metallothionein synthesis. Nephrol Dial Transplant. 1994;9:135-40.
11. Zhang HA, Wang M, Zhou J, Yao QY, Ma JM, Jiang, CL. Protective effect of Ginsenoside against acute renal failure and expression of tyrosine hydroxylase in the locus coeruleus. Physio Res. 2010;59:61-70.
12. Gaikwad K, Dagle P, Choughule P, Joshi YM, Kadam V. A review on some nephroprotective medicinal plants. Int J Pharm Sci Res. 2012;3(8):2451-4.
13. Fallah H, Alavian Heshmat R, Heydari MR. The efficacy of Liv-52 on liver cirrhotic patients: a randomized, double-blind, placebocontrolled first approach. Phytomedicine. 2005;12:619-24.
14. Lim K, Heo H, Lee S. Cytotoxic effect of glycoprotein isolated from *Solanum nigrum L.*, through the inhibition of hydroxyl radical induced DNA-binding activities of NF-kappa B in HT-29 cells. Envi Toxico Pharm. 2004;17:45-54.
15. Hui-Mei L, Hsien-Chun T, Chau-Jong W, Jin-Jin L, Chia- Wen L, Fen-Pi C. Hepatoprotective effects of *Solanum nigrum Linn* extract against CCl_4-iduced oxidative damage in rats. Chem Biol Interact. 2008;171:283-93.
16. Zbigniew K, Przemystaw K, Morys J. General anaesthesia in rats undergoing experiments on the central nervous system. Folia Morphol. 2001;60(4):235-42.
17. Marshall EK. A new method for the determination of urea in blood. J Biol Chem. 1993;15:487-94.
18. Pardue HL. Kinetic aspects of analytical chemistry. Anal Chem Acta. 1989;216:69-107.
19. Ghosh MN, editor, Fundamentals of experimental pharmacology 4th ed. Kolkata: Hilton & Company;2008:235-7.
20. Kaufman J, Dhakal M, Patel B, Hamburger R. Community-acquired acute renal failure. Am J Kidney Dis. 1991;17(2):191-8.
21. Nash K, Hafeez A, Hou S. Hospital-acquired renal insufficiency. Am J Kidney Dis. 2002;39(5):930-6.
22. Kohli HS, Bhaskaran MC, Muthukumar T. Treatment-related acute renal failure in the elderly: a hospital-based prospective study. Nephrol Dial Transplant. 2000;15(2):212-7.
23. Cuzzocrea S, Mazzon E, Dugo L, Serraino I, Di PR, Britti D. A role for superoxide in gentamicin mediated nephropathy in rats. Eur J Pharmacol. 2002;450:67-76.
24. Luft FC. Clinical significance of renal changes engendered by aminoglycosides in man. J Antimicrob Chemother. 1984;13:23-30.
25. Seale TW, Rennert OM, Mechanism of antibiotic-induced nephrotoxicity. Aim Clin Lab Sci. 1982;12:1-10.
26. Gyselynck AM, Forrey A, Cutler R. Pharmacokinetics of gentamicin: distribution and plasma renal clearance. J Infect Dis. 1971;124:70-6.
27. Bennet WM. Aminoglycoside nephrotoxicity. Nephron. 1983;35:73-7.
28. Lee SJ, Ko JH, Lim K, Lim KT. 150 kDa glycoprotein isolated from *Solanum nigrum Linn* enhances activities of detoxicant enzymes and lowers plasma cholesterol in mouse. Pharm Res. 2005;51:399-408.

Cite this article as: Kushwaha V, Sharma M, Vishwakarma P, Saini M, Bhatt S, Saxena KK. Evaluation of nephroprotective and nephrocurative activity of *Solanum nigrum* on gentamicin induced nephrotoxicity in experimental rats. Int J Basic Clin Pharmacol 2016:5:74-8.