

Research Article

Biochemical assessment of nephroprotective and nephrocurative activity of *Withania somnifera* on gentamicin induced nephrotoxicity in experimental rats

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ABSTRACT

Background: Renal diseases are among the commonest cause of hospitalization in most of the countries. Acute renal failure (ARF) is a common and serious renal problem having high morbidity and mortality rate. So, prevention of occurrence and progression of acute renal failure (ARF) has become a very important issue. However modern system of medicine lacks reliable nephroprotective drugs. Ashwagandha (*Withania somnifera*), a traditional Indian plant having antioxidant property may be used for nephroprotection.

Methods: The study was carried out in two phases. In phase 1, evaluation of nephroprotection was done. 54 rats were randomised in 3 groups named G10, G20 and G30 according to 10, 20 and 30 days of treatment. In each of the main groups, rats were randomly assigned to any of the three subgroups i.e. control C group (received normal saline (2ml/100gm/day) orally once a day consecutively for test duration), gentamicin treated GT (received normal saline (2ml/100gm/day) orally once a day consecutively for test duration, Injection gentamicin (40mg/kg) was given intraperitoneally once daily for last five days) and *W. Somnifera* treated WST group (received *W. somnifera* orally (500mg/kg/day) as a single dose in morning for the test duration and injection gentamicin (40mg/kg) was given intraperitoneally once daily for last five days). Rats were sacrificed 24 hours after the last dose of gentamicin injection (on 11th, 21st and 31st day). In Phase-2 nephrocurative activity of *W. somnifera* was compared with the spontaneous reversal of gentamicin induced nephrotoxicity. 72 rats were randomized into two groups. In Group-1: 36 rats received intra-peritoneal gentamicin for five days in a dose of 40 mg/kg. In Group-2: 36 rats received intra-peritoneal gentamicin for five days in a dose of 40 mg/kg. From the 5th day onward these rats received *W. Somnifera* orally in a dose of 500mg/kg/day 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: BUN and serum creatinine values were significantly low 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: *Withania Somnifera* root extract have nephroprotective activity against gentamicin induced nephrotoxicity.

Keywords: Nephroprotective, Nephrocurative, *Withania somnifera*, Gentamicin

INTRODUCTION

The kidney is an essential excretory organ of our body. It is the prime target of several drugs, toxic xenobiotics or chemicals due to more vascularity (20%- 25% of cardiac output) and presence of cellular transport systems that cause accumulation of these compounds within the epithelial cells of nephron. Metabolites of the drugs that are excreted from kidney may also cause cellular damage leading to kidney dysfunction.¹

Drug induced nephrotoxicity, one of the most common renal problems, is an important concern especially in patients with impaired renal functions. It causes approximately 20% of community and hospital acquired episodes of acute renal failure.²

Several xenobiotics like aminoglycosides, cephalosporins, anticancer drugs (cisplatin), amphotericin B, analgesics etc. exert their toxic effects by one or more common pathogenic mechanism that can produce nephrotoxicity.³

Among several aminoglycoside antibiotics, the grade of nephrotoxicity caused has been reported to be in the following order as neomycin > gentamicin > tobramycin.⁴ Gentamicin nephrotoxicity occurs in about 15-30% of treated subjects. It manifests clinically as non-oliguric renal failure, with a slow rise in serum creatinine and hypoosmolar urinary output developing *after* several days of treatment.⁵

So, prevention of occurrence and progression of acute renal failure (ARF) has become a very important issue. Global estimates show that 80% of world population cannot afford synthetic pharmaceutical products, hence depend upon traditional medicines that are commonly derived from plants.⁶

Ashwagandha (*Withania somnifera*), also known as Indian Winter Cherry is an important ancient plant, the roots of which have been employed in Indian traditional systems of medicine, Ayurveda and Unani. The constituents of *Withania* roots are the steroidal alkaloids and steroidal lactones. Much of pharmacological activity in this plant has been attributed to two main withanolides i.e. withaferin A and withanolide D.⁷

Hence the present study was designed to investigate the nephroprotective as well as nephrocurative activity of *W. somnifera* because this herb is easily available throughout the country and being very common in nature hence not costly.⁸

METHODS

This was an experimental study conducted in Department of Pharmacology, L.L.R.M. Medical College, Meerut, UP, India from January 2014 to October 2014. The animals utilized in this study were wistar rats. Total

duration of study was ten months. The study was commenced after obtaining approval from Institutional Animal Ethical Committee of Lala Lajpat Rai Medical college, Meerut, India, registered under CPCSEA India.

Albino rats of either gender, weighing 150-200 gm were obtained from the rat rearing unit of the central animal house of the institute. The selected rats were housed in cages under controlled condition of temperature (25⁰ C) and alternating periods of light and darkness of 12 hours each. The rats had free access to standard rat pellet diet (Vet care India Ltd.) and tap water ad libitum. After one week of acclimatization, the animals were considered suitable for study.

Commercially available injectable preparation (Genticyn manufactured by Abbott Healthcare Pvt. Ltd.) was used to induce Gentamicin nephrotoxicity. Aqueous extract of *Withania somnifera* as a test drug was used.

Withania somnifera-The plant's root was dried for two days at 40⁰C, crushed and powder was separated. 50 gm powder was extracted in 250 ml of distilled water for 18 hours in a soxhlet apparatus. Extract of the root of *W. Somnifera* in distilled water was collected. The extract was dried and stored at 0⁰-4⁰C. When needed the extract was suspended in water and used in the study.⁹

The study was carried out in two phases and in each phase new sets of rats were utilized.

Phase 1

Evaluation of nephroprotective activity

Evaluation of nephroprotection was done for three treatment periods of 10, 20, 30 days; and based on duration the groups were named G10, G20, G30. Rats were randomised in three groups of 18 animals each. In each of the main groups, rats were randomly assigned to any of the three subgroups i.e. control(C), gentamicin treated (GT), *W. Somnifera* treated (WST), groups of six rats each.

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

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

Group WST: This group received *W. somnifera* orally (500mg/kg/day) as a single dose in morning, before giving feed for the test duration and, injection gentamicin (40mg/kg) was given intra-peritoneally 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 of study the nephrocurative activity of *W. somnifera* was compared with the spontaneous reversal of gentamicin induced nephrotoxicity. For this phase 72 rats were randomised 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 *W. Somnifera* orally in a dose of 500 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.

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 (75mg/kg) and Xylazine (10mg/kg) anaesthesia given intra-peritoneally.¹⁰ Blood samples were collected from abdominal aorta for performing bio-chemical tests.

Estimation of biochemical parameters

The collected blood sample was centrifuged and separated serum was used for estimation of renal function.

- Blood urea nitrogen (BUN)
- 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 utilizes Marshall method.¹¹

Serum creatinine

Serum creatinine was estimated spectrophotometrically by Auto Zyme Creatinine reagent kit (Marketed by Accurex Biomedical Pvt. Ltd) from the serum. The kit utilizes initial rate method using alkaline picrate.¹²

Statistical analysis

Mean \pm SE was calculated for each group to observe the general trend of the group. The statistical analysis was carried out 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.¹³

RESULTS

A. Effect of *Withania somnifera* on serum BUN

In *Withania somnifera* pre-treated groups (WST) BUN ranged between 24.77 \pm 0.70 to 40.67 \pm 2.02 mg/dl and was lower as compared to group GT, and reduction was significant (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) and was comparable to group C level (p value >0.05) at 20 and 30 days pre-treatment (Table-1).

Table 1: Bun levels in control, Gentamicin and *Withania somnifera* pre-treated groups at different test durations (n=6).

Duration of pre-treatment (days)	BUN (mg/dl) Mean \pm SE		
	C	GT	WST
10	22.45 \pm 0.40	63.91 \pm 0.96 ^a	40.67 \pm 2.02 ^{ελ}
20	24.13 \pm 0.41	64.08 \pm 0.52 ^β	24.77 \pm 0.70 ^{ζμ}
30	23.61 \pm 0.41	65.77 \pm 1.16 ^γ	24.86 \pm 0.75 ^{ην}

^ap<0.001as compared to 10 days of control; ^εp<0.001as compared to 10 days of control; ^λp<0.001as compared to 10 days of GT; ^βp<0.001 as compared to 20 days of control; ^ζp>0.05 as compared to 20 days of control; ^μp<0.001as compared to 20 days of GT; ^γp<0.001as compared to 30 days of control; ^ηp>0.05 as compared to 30 days of control; ^νp<0.001as compared to 30 days of GT.

B. Effect of *Withania somnifera* on serum creatinine

In *Withania somnifera* pre-treated groups (WST) serum creatinine ranged between 0.60 \pm 0.02 to 0.99 \pm 0.02 mg/dl and was lower as compared to GT group, and the decrease was significant (p value <0.001) 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 comparable to group C (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 *Withania somnifera*, exhibiting a time dependent limitation of serum creatinine and BUN rise.

Reversal of gentamicin toxicity (phase-II)

1. BUN levels

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

Table 2: Serum Creatinine levels in control, Gentamicin and *Withania somnifera* pre-treated groups at different test durations (n=6).

Duration of pre-treatment (days)	Serum creatinine (mg/dl) (Mean± SE)		
	C	GT	WST
10	0.56±0.01	1.89±0.04 ^a	0.99±0.02 ^{ελ}
20	0.58±0.02	2.00±0.03 ^β	0.65±0.01 ^{ζμ}
30	0.60±0.02	2.20±0.02 ^γ	0.60±0.02 ^{νν}

^ap<0.001as compared to 10 days of control; ^εp<0.001as compared to 10 days of control; ^λp<0.001as compared to 10 days of GT; ^βp<0.001as compared to 20 days of control; ^ζp>0.05 as compared to 20 days of control; ^μp<0.001as compared to 20 days of GT; ^γp<0.001as compared to 30 days of control; ^νp>0.05 as compared to 30 days of control; ^{νν}p<0.001as compared to 30 days of GT.

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

Days of sacrifice	BUN (mg/dl) Mean ± SE	
	Group-1 (C)	Group-2 (WST)
3	70.76 ± 0.66	70.11 ± 0.61 ^a
5	64.56 ± 0.77	65.25 ± 0.64 ^β
7	54.77 ± 0.83	55.92 ± 0.61 ^γ
10	35.44 ± 0.53	34.60 ± 0.32 ^δ
12	25.83 ± 0.75	25.01 ± 0.61 ^ε
14	24.09 ± 0.72	24.09 ± 0.66 ^ζ

^ap>0.05 as compared to 3 days of group-1; ^βp>0.05 as compared to 5 days of group-1; ^γp>0.05 as compared to 7 days of group-1; ^δp>0.05 as compared to 10 days of group-1; ^εp>0.05 as compared to 12 days of group-1; ^ζp>0.05 as compared to 14 days of group-1.

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

Days of sacrifice	Serum creatinine (mg/dl) (Mean±SE)	
	Group-1(C)	Group-2 (WST)
3	1.24±0.01	1.26±0.02 ^a
5	1.06±0.02	1.04±0.01 ^β
7	0.86±0.01	0.75±0.01 ^γ
10	0.90±0.01	0.84±0.01 ^δ
12	0.58±0.02	0.58±0.01 ^ε
14	0.54±0.01	0.52±0.01 ^ζ

^ap>0.05 as compared to 3 days of group-1; ^βp>0.05 as compared to 5 days of group-1; ^γp>0.05 as compared to 7 days of group-1; ^δp>0.05 as compared to 10 days of group-1; ^εp>0.05 as compared to 12 days of group-1; ^ζp>0.05 as compared to 14 days of group-1.

2. Serum creatinine level

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

creatinine levels of group I and II after 14 days were not significantly different (p value >0.05) (Table-4).

DISCUSSION

Nephrotoxicity induced by gentamicin is a commonly employed experimental model for evaluation of nephroprotective activity of the drugs. There are three reasons to select this model in this study. Firstly the gentamicin nephrotoxicity is a major concern in clinical practice and accounts for nearly 10% of all cases of drug induced nephrotoxicity and 30% of gentamicin treated patients develop nephrotoxicity.^{14,15} Secondly, nephrotoxicity is rapidly induced in rats and presents with established morphological changes and biochemical markers.¹⁶ Thirdly, gentamicin induced nephrotoxicity has spontaneous reversal potential, hence provides an opportunity to study the nephroprotective as well as nephrocurative properties of *Withania somnifera*.¹⁷

In present study pre-treatment with *Withania somnifera* the BUN and serum creatinine levels were significantly decreased in group treated for 10 or 20 or 30 days as compared to GT group. When compared with saline treated group, the BUN and serum creatinine values are significantly raised on day 10. In group with 20 and 30 days of pre-treatment, the levels of biomarkers markedly improved and were almost equal to the findings in saline treated group C (Tables 1 and 2). It could be inferred that the plasma concentration of *Withania somnifera* require some time to reach a protective level.

In phase II study, the nephrocurative activity of *W. somnifera* was compared with the spontaneous reversal of gentamicin induced nephrotoxicity. The BUN and serum creatinine levels in *Withania somnifera* 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 (Tables 3 and 4).

The failure of *Withania somnifera* to offered nephrocurative could have been due to two reasons. First, the gentamicin accumulation within the renal cells damages a significant number of cells beyond repair, and such affected cells might not take up the active ingredient of *Withania somnifera*.¹⁸ Secondly, the curative effect was studied after administration of drugs only for 14 days which may not be a sufficient period for *Withania somnifera* as nephroprotective activity of the drug was best seen after 20 days. However, the mean levels of BUN and serum creatinine were lesser than that in gentamicin treated group, though the difference was insignificant. This means the drug has no nephrocurative property at these test doses and durations.

Gentamicin is actively concentrated in the renal cortex and proximal tubular cells (achieves maximum concentration). After entering the cortical cells it binds to lysosomes with formation of myeloid bodies/secondary lysosomes thus impairing mitochondrial function,

interfering with the tubular transport, increasing oxidative stress and forming free radicals.¹⁹ Much of *Withania somnifera* pharmacological activity has been attributed to two main withanolides, withaferin A and withanolide D. These two compounds have potent antioxidant property.

CONCLUSION

Therefore the present study carries a scope for further evaluation of *Withania somnifera* with other dose levels and extended test duration in order to conclusively establish *Withania somnifera* not only as nephroprotective but also as potential nephrocurative drug also

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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