

Original article

Effect of *Withania somnifera* on gentamicin induced renal lesions in rats



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ABSTRACT

Gentamicin induced renal complications are well known in humans and animals. Medicinal properties of *Withania somnifera* (L.) Dunal, Solanaceae, are recognized to improve renal functions. However, the pharmacological function of *W. somnifera* is not completely understood. We sought to unravel medicinal therapeutic function of *W. somnifera* on gentamicin-induced nephrotoxicity in wistar rats. Twenty-four adult male wistar rats evenly divided into four groups to evaluate *in vivo* nephroprotective and nephrocurative function of *W. somnifera* in gentamicin induced nephrotoxic rats. Experimental design as follows: Group I, saline control for 21 days; Group II, gentamicin nephrotoxic control for eight days; Group III, alcoholic extract of *W. somnifera* for 13 days + simultaneous administration of gentamicin and *W. somnifera*, from day 14 to 21 (nephroprotective) and Group IV, gentamicin for 8 days + alcoholic extract of *W. somnifera* from day 9 to 21 (nephrocurative). End of experiment, respective serum and kidney tissue samples used to analyze renal function. *Withania somnifera* as a nephroprotective and nephrocurative molecule significantly restore the renal function on gentamicin-induced nephrotoxicity. This phenomenon is accompanied with significantly reduced blood urea nitrogen, creatine, alkaline phosphatase, gamma-glutamyl transferase, albumin, total protein, calcium, potassium and kidney malondialdehyde concentrations. Additionally, *W. somnifera* significantly increased antioxidant activities of glutathione and superoxide dismutase to protect renal tissue damage from gentamicin in wistar rats. Overall, *W. somnifera* treated nephroprotective animal shows improved recovery compared to nephrocurative. The nephroprotective or nephrocurative effect of *W. somnifera* could be due to inherent antioxidant and free-radical-scavenging principle(s). In the near future, biologically active compounds of *W. somnifera* (withanolides) could appear as a novel therapeutic molecule for renal disorders.

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Introduction

Gentamicin (GM) is an important aminoglycoside antibiotic used reliably to treat serious and life threatening infections (*Mycobacterium* infection, septicemia, complicated urinary tract infection, endocarditis, peritonitis, etc.) mainly caused by gram-negative bacteria in human and animals. However, its clinical use is limited due to incidence of nephrotoxicity and ototoxicity (Krause et al., 2016; Jiang et al., 2017). Nephrotoxicity induced by gentamicin manifests clinically as nonoliguric renal failure, with a slow rise in creatinine, blood urea nitrogen, also accompanied with

proteinuria, enzymuria, glycosuria and electrolyte impairments (Mingeot-Leclercq and Tulkens, 1999).

Although the exact pathophysiology of gentamicin induced nephrotoxicity is still unclear and it seems to be linked with generation of destructive reactive oxygen species (ROS) in renal cells. Eventually, overproduction of ROS potentiates oxidative damage of biomolecules (lipids, proteins, DNA) by altering malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST) and glutathione peroxidase (GPx) in prime renal failure (Khan et al., 2011).

Herbal products have a special place in the world of pharmaceuticals with their safety, efficacy and cost effectiveness. The World Health Organization reported that 80% of the world's population depends on medicinal plants for their healthcare needs and more than 30% of the pharmaceutical preparations based on plants

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(Jeyanthi and Subramanian, 2009). *Withania somnifera* (L.) Dunal belongs to Solanaceae family, commonly known as Ashwagandha, Indian ginseng and Winter cherry used in ayurvedic and indigenous medicine for over 3000 years.

The biologically active chemical constituents of *W. somnifera* are alkaloids (isopelletierine, anferine), steroidal lactones (withanolides, withaferins), saponins (sitoindoside VII and VIII) and withanolides (sitoindoside XI and X); used widely for therapeutic purpose of immunomodulatory, antitumor, anti-inflammatory, antioxidant, anti-ageing, antidepressant, anxiolytic, hematopoietic and aphrodisiac.

To our knowledge, as of today there is no profound nephroprotective or nephrocurative evidence on traditional medicine. Accordingly, the present study is design to understand pharmacological renal function of alcoholic extract *W. somnifera* on gentamicin-induced nephrotoxicity in Wistar rats.

Material and methods

Plant materials and alcoholic extraction

Withania somnifera (L.) Dunal, Solanaceae, roots obtained commercially from Department of Aromatic and Medicinal Plants, Agriculture College, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.), India. The roots were identified and authenticated by Prof. Kappali S.A., Botanist, Department of Botany, Basaveshwar Science College, Bagalkot, Karnataka, India. The voucher specimen was prepared and deposited (Bsc/Pharmacy/81/2010) in the Department of Pharmacology, Hangal Shri Kumareswar College of Pharmacy, Bagalkot, Karnataka, India. *Withania somnifera* roots, subject to shade dry for fine powder preparations. These powders utilized to extract pharmacologically active phytochemicals by alcoholic extract method using Soxhlet apparatus. After extraction, excess alcoholic solvent evaporated at 60 °C until the extracts concentrated to a paste and dried by vacuum drying method at 35 °C.

Quantification of bioactive compound using high performance liquid chromatography (HPLC)

Withania somnifera extract (500 mg) taken in a clean and dried flask, 50 ml of HPLC grade methanol was added and refluxed for 10 min, cooled and sonicated for 6 min. The final volume of 100 ml made up with methanol. HPLC analysis performed on a Waters Alliance 2690 HPLC system, equipped with a 2998 Photodiode Array Detector (Waters, Milford, MA, USA). For all separations, a C18 column (250 × 4.60 mm, 5 μm particle size) from Phenomenex (Torrance, CA, USA) was used. The mobile phase consisted of solvent A; dissolved 0.14 g of anhydrous potassium dihydrogen orthophosphate (KH₂PO₄) in 900 ml of HPLC grade water, added 0.5 ml of orthophosphoric acid and volume was made to 1000 ml with water. The mixture was filtered through 0.45 μm membrane and sonicated for 3 min. The sonicated content was quantitatively transferred to HPLC column with solvent B (acetonitrile) which were applied in the following gradient elution: 95A/5B, 55A/45B, 20A/80B, 20A/80B, 55A/45B, 95A/5B, 95A/5B from respective time of 0.01, 18, 25, 28, 35, 40 and 45 min. The separation flow rate and sample volume were set to 1.5 μl and 20 μl, respectively and all separations were monitored at 227 nm. Then injected three times the standard preparation and calculated the mean area and the RSD. Then after injected 20 μl of sample preparation and recorded the chromatogram at 227 nm.

Animal procurement and care

Healthy male Wistar rats (8–10 weeks, 180–200 g) procured from laboratory animal breeding centre, college of Veterinary Science and Animal Husbandry, Jabalpur (M.P.), India. All animals were maintained as per the protocol outlined in the publication of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Institutional Animal Ethics Committee (IAEC) bearing approval No. 268/IAEC/MPPCVV/2010 approved the experimental procedures.

Acute oral toxicity study of *Withania somnifera*

The acute oral toxicity study was conducted according to the OECD guideline 423 (Acute toxic class method). The limit test dose of *W. somnifera* (2000 mg kg⁻¹), administered by gavage using a stomach tube. Animals observed individually at least once during the first 30 min, with special attention for first 4 h and daily thereafter for a total of 14 days. The extract was found to be devoid of mortality at 2000 mg kg⁻¹. Hence, 2500 mg kg⁻¹ was considered as LD₅₀ cutoff value and 1/5th (500 mg kg⁻¹, *p.o.*) of the dose selected for the screening of nephroprotective and nephrocurative activities.

Experimental protocol

Gentamicin sulphate (40 mg ml⁻¹ injection, Wockhardt Ltd., Mumbai, India) and all other chemicals used in this experiment meets analytical grade. Twenty-four experimental rats randomly divided into four different groups (*n* = 6): Group I, normal saline (1 ml kg⁻¹, once a day for 21 days, *i.p.*); Group II, GM (80 mg kg⁻¹, once a day for 8 days, *i.p.*); Group III, *W. somnifera* (500 mg kg⁻¹, once a day for 13 days, *p.o.*) pre-treatment + simultaneous administration of gentamicin and *W. somnifera* (*i.p.*, 80 mg kg⁻¹ and *p.o.*, 500 mg kg⁻¹, once a day from day 14 to day 21) is called as nephroprotective/NP group; Group IV, GM (80 mg kg⁻¹, once a day for 8 days, *i.p.*) pre-exposure + *W. somnifera* (500 mg kg⁻¹, once a day from day 9 to day 21, *p.o.*) is called as nephrocurative/NC group.

Forced motor activity

Experimental rats were habituated and completed six training sessions (three sessions daily at 15 min resting interval) on rotarod at 5–20 rpm for 5 min (Rotarod EM-35, Microteknik, India). On the test day of experiment, trained rats were placed on the rotating rod at 10 rpm for 5 min and the latency to fall (the time it took for the rat to fall from the rod) was measured. The variations in animals were used to identify and compare alterations in motor coordination (Hernández-López et al., 2017).

Relative kidney weight

Rats were fasted overnight on day 21 of experiment and the live body weight (g) was recorded on day 22 before euthanizing. Kidney was isolated and weighed (g) (absolute organ weight) to calculate relative organ weight of each animal.

$$\text{Relative kidney weight (g)} = \frac{\text{Absolute kidney weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100$$

Serum and tissue sampling

Experimental rats were fasted overnight and anesthetized using isoflurane to collect blood samples (via retro-orbital plexus) for serum isolation. The harvested serum stored at –80 °C until analysis of biochemical parameters using Erba Chem 5 Plus V2 Biochemistry Analyzer. Before harvesting kidneys, all animals were perfused

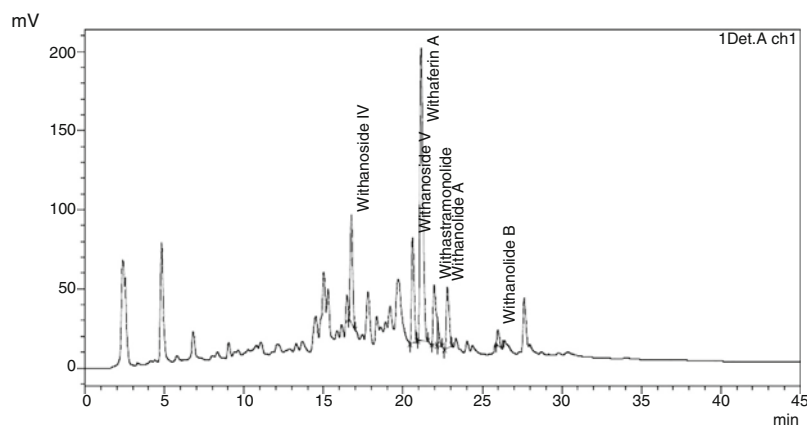


Fig. 1. Chromatogram of *Withania somnifera* root extract by using high performance liquid chromatography.

(0.9% sodium chloride) and then stored at -80°C (for estimation of oxidative stress markers) and 10% neutral buffered formaldehyde solution (for histopathological studies).

Assessment of electrolytes

Serum samples subject to analyze electrolytes, Na^{+} and K^{+} by using Flame Photometer (Flame Photometer 128, Systronic). Sodium and potassium standards were prepared for the analysis of samples by plotting a straight-line graph as guided by Hawk's Physiological Chemistry.

Assessment of oxidative stress

Kidneys homogenized in ice cold 0.9% sodium chloride using a homogenizer. The homogenates were centrifuged at $800 \times g$ for 5 min at 4°C to separate the nuclear debris. The supernatant so obtained was centrifuged at $5000 \times g$ for 15 min at 4°C to get the postmitochondrial supernatant (Karadeniz et al., 2008). These samples were used to analyze lipid peroxidation (MDA), glutathione (GSH) and superoxide dismutase (SOD) activity by following in-house laboratory protocol using Helios double beam spectrophotometer. The brief protocol of MDA, GSH and SOD quantification is as follows.

Determination of MDA: One ml of 33% (dilution in PBS) of kidney tissue supernatant was incubated at $37 \pm 0.5^{\circ}\text{C}$ for 2 h. To each sample 1 ml of 10% (w/v) trichloroacetic acid was added. After thorough mixing, the reaction mixture was centrifuged at $100 \times g$ for 10 min. To 1 ml of supernatant 1 ml of 0.67% (w/v) of thiobarbituric acid was added and kept in boiling water bath for 10 min, cooled and diluted with 1 ml of distilled water. Blank was made by adding all the reagents except the kidney tissue supernatant. The absorbance was read at 535 nm (Shafiq-Ur-Rehman, 1984).

Determination of GSH: Kidney tissue supernatant (0.2 ml) was added to 4 ml of 0.08 N H_2SO_4 and mixed carefully. After 10 min of standing at room temperature, 0.5 ml of tungstate solution was added to clear the brown haemolysate. The tube was stoppered and the mixture was shaken vigorously for 5 min in order to avoid bubble formation on the top of supernatant. The suspension was then centrifuged for 15 min at $100 \times g$ at room temperature. After centrifugation, 2 ml of supernatant, 2.5 ml of TRIS buffer and 0.2 ml of DTNB [5,5-dithiobis(2-nitrobenzoic acid)] reagent was added and mixed well. Absorbance was measured at 412 nm (Beutler et al., 1963).

Determination of SOD: In a set of test tubes 650 μl of PBS was taken. To this, 30 μl MTT [3-(4,5-dimethyl thiazol-2-4) 2,5-diphenyl tetrazolium bromide] was added. Then, 75 μl of pyrogallol was

added to both the tubes. This mixture was incubated for 5 min at room temperature. To stop this reaction, 750 μl of dimethyl sulphoxide was added to both the tubes and finally 10 μl of sample was added to the second tube to find out the percentage of reduction of MTT formation. The colour development was read at 570 nm (Madesh and Balasubramanian, 1998).

Histopathology

Kidneys fixed in 10% neutral buffered formaldehyde solution (pH 7), dehydrated in graded anhydrous ethanol and embedded in paraffin. Fine sections of 5 μm of thickness were obtained, mounted on glass slides and counter-stained with haematoxylin and eosin (H and E) for light microscopic analyses (Cardiff et al., 2014). Veterinary pathologist who was unaware of the treatments carried out interpretation of histopathological changes.

Statistical analysis

The data were analyzed using Student's *t*-test or one-way analysis of variance (ANOVA). All the values are presented as mean \pm SE. The probability (*p*) values of ≤ 0.05 were considered statistically significant.

Results

Quantification of withanolides by HPLC

A HPLC analysis was performed to estimate withanolides in the root extract of *W. somnifera*. The chromatogram (Fig. 1) depicts presence of withanolides; withanolide A, withanolide B, withanoside IV, withanoside V, 12-deoxy withastramonolide and withaferin in the root extract of *W. somnifera* and their relative percentage are mentioned in Table 1.

Table 1
Percentage of withanolides in the root extract of *Withania somnifera* by HPLC.

Peak	Number retention time	Active ingredient	Area	Area%
1	16.753	Withanoside IV	703 633	14.042
2	20.606	Withanoside V	715 829	14.285
3	21.140	Withaferin A	2414 057	48.176
4	21.960	Withastramonolide	472 909	9.438
5	22.150	N/A	131 542	2.625
6	22.786	Withanolide A	472 719	9.434
7	25.965	Withanolide B	100 238	2.000
		Total	5 010 927	100.000

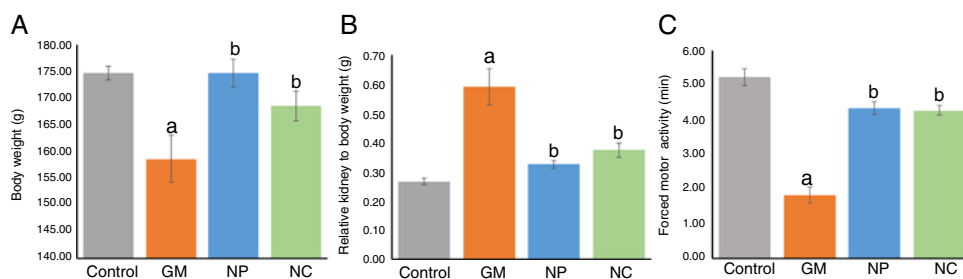


Fig. 2. Effect of *Withania somnifera* on gentamicin induced nephrotoxic changes. (A) Body weight (g); (B) relative kidney weight (g); (C) forced motor activity (min) shows significant ($^b p \leq 0.05$, $n = 6$) improvement in *W. somnifera* treatment rats as compared to gentamicin.

Effect of *Withania somnifera* on body weight and relative kidney weight

Animals of gentamicin (GM) treatment shows significant ($^a p \leq 0.05$) reduction in body weight as compare to normal control on day 22. However, as compare to GM treatment, animals of NP and NC shows significant ($^b p \leq 0.05$) increase in body weight (Fig. 2A). The relative kidney weight (g) of GM treatment alone significantly ($^a p \leq 0.05$) increased as compare to control, NP and NC treatment. Interestingly, ameliorative effect of *W. somnifera* (NP and NC) significantly ($^b p \leq 0.05$) retained relative kidney weight (g) against GM treatment (Fig. 2B).

Effect of *Withania somnifera* on forced motor activity

Gentamicin treatment significantly ($^a p \leq 0.05$) reduced forced motor activity (min) as compare to control, NP and NC treatment. Intriguingly, the forced motor activity fortified significantly ($^b p \leq 0.05$) in NP and NC treatment as compare to GM treatment alone (Fig. 2C).

Effect of *Withania somnifera* on renal biochemical parameters

GM treatment significantly ($^a p \leq 0.05$) increased serum concentrations of biochemical parameters: creatinine, blood urea nitrogen, total protein, albumin, γ -glutamyltransferase, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase as compared to control. However, interestingly per oral administration of *W. somnifera* (NP and NC) significantly ($^b p \leq 0.05$) reduced elevated serum concentrations of biochemical parameters by GM (Table 2).

Effect of *Withania somnifera* on renal electrolytes

Restorative effect of *W. somnifera* on GM induced serum electrolytes (Na^+ , K^+ and Ca^{2+}) changes depicted in Table 3. *W. somnifera* (NP and NC) and GM treated group did not show any appreciable changes in Na^+ concentrations as compared to control. However, K^+ and Ca^{2+} levels were significantly ($^a p \leq 0.05$) reduced in GM treatment as compared to control. Intriguingly, administration of alcoholic extract of *W. somnifera* significantly ($^b p \leq 0.05$) moderated effects of GM by protecting serum concentrations of K^+ and Ca^{2+} .

Table 2

Effect of *Withania somnifera* on GM induced serum biochemical changes. Results of renal biochemical parameters significantly ($^a p \leq 0.05$) increased in gentamicin treatment rats. *Withania somnifera* treatment groups, nephroprotective and nephrocurative significantly ($^b p \leq 0.05$) reduced the biochemical changes in gentamicin induction ($n = 6$).

Group	Cr (mg dl ⁻¹)	BUN (mg dl ⁻¹)	Total Prot. (g dl ⁻¹)	ALB (g dl ⁻¹)	GGT (IU l ⁻¹)	ALP (IU l ⁻¹)	ALT (IU l ⁻¹)	AST (IU l ⁻¹)
Control	0.32 ± 0.01	34.09 ± 1.09	7.12 ± 0.08	3.05 ± 0.06	2.52 ± 0.10	140.2 ± 2.11	46.29 ± 1.30	56.59 ± 1.60
GM	0.90 ± 0.04 ^a	78.17 ± 2.45 ^a	9.91 ± 0.59 ^a	3.74 ± 0.09 ^a	5.40 ± 0.23 ^a	256.51 ± 5.21 ^a	75.84 ± 3.70 ^a	83.01 ± 1.47 ^a
NP	0.37 ± 0.01 ^b	37.42 ± 0.35 ^b	7.46 ± 0.05 ^b	3.14 ± 0.05 ^b	3.09 ± 0.03 ^b	141.32 ± 1.45 ^b	47.61 ± 1.32 ^b	58.77 ± 1.32 ^b
NC	0.40 ± 0.02 ^b	39.92 ± 0.81 ^b	7.85 ± 0.11 ^b	3.16 ± 0.03 ^b	3.23 ± 0.10 ^b	143.54 ± 1.30 ^b	50.87 ± 1.00 ^b	59.76 ± 0.94 ^b

Table 3

Effect of *Withania somnifera* on gentamicin induced serum electrolyte changes. Results of serum electrolyte parameters (potassium and calcium) significantly ($^a p \leq 0.05$) decreased in gentamicin treatment rats. *Withania somnifera* treatment groups, nephroprotective and nephrocurative significantly ($^b p \leq 0.05$) increased the altered serum electrolytes in gentamicin induction ($n = 6$).

Group	Sodium (mEq l ⁻¹)	Potassium (mEq l ⁻¹)	Calcium (mg dl ⁻¹)
Control	138.53 ± 1.74	6.20 ± 0.74	7.91 ± 0.10
GM	142.52 ± 2.68	4.55 ± 1.01 ^a	5.14 ± 0.07 ^a
NP	140.50 ± 1.48	6.33 ± 0.78 ^b	7.21 ± 0.10 ^b
NC	140.75 ± 1.84	5.68 ± 0.87 ^b	7.16 ± 0.20 ^b

Effect of *Withania somnifera* on renal tissue enzymes

The effect of *W. somnifera* on GM-induced changes in lipid peroxides (LPO), superoxide dismutase (SOD) and glutathione (GSH) kidney tissue is depicted in Table 4. Lipid peroxide concentration significantly ($^a p \leq 0.05$) increased in GM treatment, whereas SOD and GSH levels significantly ($^a p \leq 0.05$) reduced as compared to control. Administration of alcoholic extract of *W. somnifera* significantly ($^b p \leq 0.05$) subverted nephrotoxic effects of GM by reducing LPO and increasing SOD and GSH concentrations.

Effect of *Withania somnifera* on renal histopathological changes

Gross examinations of kidneys in GM treated animals were evidently pale and swollen in character (Fig. 3A). Inquisitively, *W. somnifera* with its antioxidant and rejuvenate function effectively reduce the renal lesions of GM in nephroprotective and nephrocurative treatment (Fig. 3B and C). Haematoxylin and eosin (H and E) staining of kidney in GM treatment showed an extensive and marked congestion, interstitial haemorrhages and tubular necrosis (Fig. 3A). On the other hand, tubular injuries were also markedly reduced in nephroprotective treatment (Fig. 3B). However, histological lesions in nephrocurative treatment showed minimal to mild interstitial nephritis with slightly detached (flattened) but otherwise normal tubular cells (Fig. 3C).

Discussion

Gentamicin is a bactericidal broad-spectrum antibiotic, commonly used in veterinary practice to treat acute serious infections. Despite of therapeutic application, its usage is limited due to its

Table 4

Effect of *Withania somnifera* on gentamicin induced renal tissues enzyme changes. Results of LPO and GSH and SOD significantly (^a $p \leq 0.05$) altered in renal tissues of gentamicin treatment. *Withania somnifera* treatment groups, nephroprotective and nephrocurative significantly (^b $p \leq 0.05$) attenuated altered renal enzymes ($n = 6$).

Group	LPO (nM MDA g ⁻¹ of tissue)	GSH (μM GSH g ⁻¹ of tissue)	SOD (U g ⁻¹ of tissue)
Control	71.85 ± 1.08	71.74 ± 3.73	876.83 ± 0.32
GM	126.71 ± 1.45 ^a	37.38 ± 4.06 ^a	574.87 ± 0.36 ^a
NP	73.83 ± 1.56 ^b	60.77 ± 3.66 ^b	843.34 ± 0.68 ^b
NC	80.59 ± 0.51 ^b	58.79 ± 4.67 ^b	806.98 ± 0.71 ^b

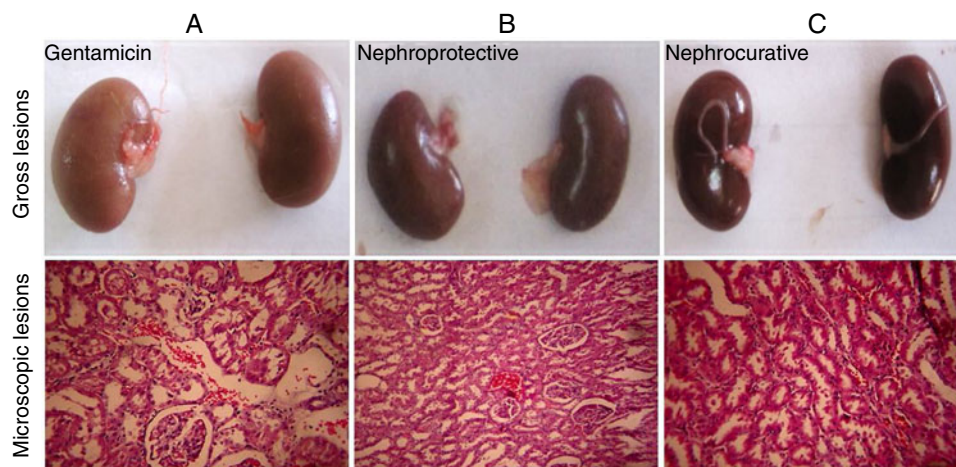


Fig. 3. Effect of *Withania somnifera* on renal gross and histopathological changes in gentamicin treatment. Gross lesions of kidney shows diffused pale and swollen condition in gentamicin treated animals (A), which are effectively ameliorated in nephroprotective (B) and nephrocurative (C) treatment of *W. somnifera*. (A) Haematoxylin and eosin (H and E) staining of kidney sections shows marked congestion, interstitial haemorrhages, desquamated epithelial cells and tubular necrosis in gentamicin treated group ($\times 200$). These lesions were effectively ameliorated in *W. somnifera* treatment, nephroprotective group ($\times 100$) (B), as compared to nephroprotective ($\times 200$) (C) group, where slightly flattened tubular cells observed ($n = 6$).

severe acute nephrotoxicity (Ahn et al., 2012). Several studies have reported that oxygen-free radicals are prime offenders of GM induced acute renal failure (Karahan et al., 2005). However, the exact mechanism by which GM induces renal damage is elusive.

Among therapeutic antioxidants, deferoxamine, methimazole, vitamin E, vitamin C, diethyl dithiocarbamate, L-histidinol and thymoquinone are extensively studied to protect renal physiological function (Karahan et al., 2005; Yaman and Balıkcı, 2010; Tavafi et al., 2012). Uncertainly, none of these compounds has proved to be clinically efficient in patients. Recently, with this concern attention is focused on natural antioxidant sources that are able to ameliorate gentamicin-induced nephrotoxicities. These natural antioxidants act as an alternative medicine to synthetic antioxidants, which may cause serious or deleterious adverse effects (Sodimbaku et al., 2016).

W. somnifera is such a plant containing rich antioxidant principles to treat renal disorders. In support to our findings, investigators have shown that hydromethanolic extract of *W. somnifera* exerts therapeutic efficiency in regulation of lead nitrate induced nephrotoxicity in Swiss albino mice and that could be attributed to (i) presence of natural antioxidants (ii) its free radical scavenging and antioxidant properties and (iii) excess removal of urea related compounds (Davis and Kuttan, 2000; Jayaprakasam et al., 2003). It has also been reported that the root extract of *W. somnifera* tends to reverse the changes in lipid peroxidation and damage to cells (Dhuley, 2000). With these scientific backgrounds, we have isolated and quantified biologically active compounds of *W. somnifera* as withanolides (withanolide A, withanolide B, withanoside IV-V and 12-deoxy withastramonolide and withaferin A) by HPLC. After that, we focused to understand renal protective and renal curative role of *W. somnifera* in Wistar rats.

In our findings GM treatment significantly decrease the live body weight of rats and similar findings were reported in rats

administered with GM (80 mg kg⁻¹ IM) for six consecutive days (Ali et al., 1992). This could be due to cumulative accumulation of GM in renal tissues, which leads to decrease in food intake and body weight due to consequence of renal injuries. This impairment causes subsequent loss of tubular cells to reabsorb water that leads to dehydration and loss of body weight. Unfortunately, urine volume of rats was not measured in this experiment. However, interestingly there were no significant alterations in kidney and body weight ratios of rats treated with *W. somnifera* in NP and NC group. The alleviation of GM-induced live body weight reduction denotes the palliative effect of *W. somnifera* on nephrotoxicity.

Serum creatinine and BUN are well-known biomarkers to detect early phase of renal damage and in chronic renal damage, the elevated levels of total protein, ALP, AST and ALT are remarkable (Lopez-Giacoman and Madero, 2015; Abuelezz et al., 2016; Teo and Andre, 2017). In our study, GM (80 mg kg⁻¹ b.wt., i.p.) significantly increased the levels of creatine, blood urea nitrogen, total protein, ALP, AST and ALT levels in serum. Additionally, GGT levels also increased in serum due to damage in renal brush border of epithelial cells in the proximal tubule and it is a sensitive indicator of GM toxicity (McMahon and Waikar, 2013). Intriguingly, the concentration of serum biochemical parameters significantly reduced in *W. somnifera* treatment. This could be due to antioxidant properties of withanolides viz., withanolide A-B, withanoside IV-V, 12-deoxy withastramonolide and withaferin. These alterations in biochemical parameters were well correlated with the renal histopathological lesions (Bhattacharya et al., 1997; Jeyanthi and Subramanian, 2009).

Gentamicin, independently of cell injury, inhibits variety of cell membrane transporters of both the brush border and the basolateral membranes leading to electrolyte abnormalities. Transport inhibition not only affects tubular reabsorption but also compromises cell viability, which ultimately results in necrosis or apoptosis

(Rai et al., 2003; Raghavan and Weisz, 2016). The significant reduction in calcium and potassium concentration and simultaneous non-significant slight increase in sodium ions observed in GM treatment. GM probably by antagonizing calcium ions reduces the release of acetylcholine from the motor nerve endings and produce curare-like effects (Lee et al., 2013; Rhee et al., 2015). This could mitigate forced motor activities in rats. Nephroprotective effects of *W. somnifera* shows improved ameliorative action on the kidneys as compared to their nephrocurative effects. The observations of Jeyanthi and Subramanian (2009); also supports our findings by reporting the ameliorative effect of *W. somnifera* on electrolyte abnormalities in GM intoxicated rats.

Several investigators have reported that, aminoglycoside as a classic antibiotic capable of causing nephrotoxicity by inducing reactive oxygen species (Randjelovic et al., 2017; Perazella, 2018). The elevated level of MDA (a marker of lipid peroxidation in tissues) results in the reduction of polyunsaturated fatty acid content, which serves as substrate of free radicals particularly, hydrogen peroxide and superoxide. These aggravated free radicals affect antioxidant functions of SOD, catalase, GSH and GPx (Niki et al., 2008). Thus, there is an imbalance between oxidant and antioxidant status leading to cellular damage. Other report also shows that, cationic interaction of aminoglycosides with anionic phospholipid (kidneys) induces nephrotoxicity (Wiland and Szechciński, 2003). By following ionic interaction process, GM as a catalyst might form chelation complex with iron (iron-GM complex), to induce oxidative free radicals in renal tissues (Yanagida et al., 2004). Accordingly, our result also shows an increase in lipid peroxidation products (MDA) and decrease in nonenzymic (GSH) and enzymic (SOD) antioxidants in renal tissues of GM treatment. However, interestingly *W. somnifera* retains MDA, GSH and SOD levels in NP and NC groups and is probably due to (i) detoxification of potential reactive oxygen species and (ii) restoration of non-enzymatic and enzymatic antioxidant functions.

These oxidative changes well correlated with the renal histological changes, by revealing interstitial haemorrhages, epithelial granular degeneration and marked tubular necrosis in GM treated kidney (Sayed-Ahmed and Nagi, 2007; Yaman and Balikci, 2010). However, co-administration of *W. somnifera* effectively reverse renal damage with marked reduction in tubular damage induced by GM. Overall, our result shows that renal injury induced by gentamicin is a manifest of elevated MDA, total protein, BUN and Cr levels with decreased electrolytes and antioxidant enzymes. *W. somnifera* with its antioxidant function significantly alleviates adverse effects of GM.

Study limitation

We showed the ameliorative effect of *W. somnifera* extracts on nephrotoxicity. However, more studies are required to understand mechanistic pathways of specific bioactive compounds of *W. somnifera* on gentamicin induced nephrotoxicity.

Conclusion

The present study specifies nephroprotective and nephrocurative medicinal values of *W. somnifera* root extract. Hence, validates its traditional use as an ethnomedicine to cure renal ailments. In the near future, bioactive compounds of *W. somnifera* could appear as a novel biomolecule to protect oxidative stress.

Author's contribution

All authors contributed significantly to the work presented in this manuscript. YPS and VG supervised the whole experiments.

PKG and VG conceptualized the project and performed the experiments. PKG, VG, SMT and RHL organized, analyzed and interpreted the results. PKG and SMT wrote the manuscript draft. RHL and SMT read the manuscript and provided critical assessment and conceptual insights.

Conflicts of interest

The authors declare no conflicts of interest.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

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