Original article

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

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**A B S T R A C T**

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. Over all, *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, enzmyuria, 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...
(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 (withaferin C18 and VII), and withanolides (withaferin X and XI); 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 nephro-protective 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, Jawaharial 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 (acetoniitrile) 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
(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 × g for 5 min at 4 °C to separate the nuclear debris. The supernatant so obtained was centrifuged at 5000 × 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 ± 0.5 °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 × 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 H₂SO₄ and mixed carefully. After 10 min of standing at room temperature, 0.5 ml of tungstate solution was added to clear the brown haemolsysate. 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 × 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 sulfoxide 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 ± 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 *Withania somnifera* The chromatogram (Fig. 1) depicts presence of withanolides; withanolide A, withanolide B, withanolide IV, withanoside V, 12-deoxy withastramonolide and withaferin in the root extract of *Withania somnifera* and their relative percentage are mentioned in Table 1.

**Table 1**

<table>
<thead>
<tr>
<th>Peak</th>
<th>Number retention time</th>
<th>Active ingredient</th>
<th>Area</th>
<th>Area%</th>
</tr>
</thead>
</table>
| 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                 |                   | 5010 927 | 100.000 |}
Effect of Withania somnifera on body weight and relative kidney weight

Animals of gentamicin (GM) treatment shows significant \(^{(p < 0.05)}\) 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 \(^{(p < 0.05)}\) increase in body weight (Fig. 2A).

The relative kidney weight (g) of GM treatment alone significantly \(^{(p < 0.05)}\) increased as compare to control, NP and NC treatment. Interestingly, ameliorative effect of W. somnifera (NP and NC) significantly \(^{(p < 0.05)}\) retained relative kidney weight (g) against GM treatment (Fig. 2B).

Effect of Withania somnifera on forced motor activity

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

Effect of Withania somnifera on renal biochemical parameters

GM treatment significantly \(^{(p < 0.05)}\) increased serum concentrations of biochemical parameters: creatinine, blood urea nitrogen, total protein, albumin, \(\gamma\)-glutamyltransferase, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase as compared to control. However, interestingly per oral administration of W. somnifera (NP and NC) significantly \(^{(p < 0.05)}\) 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 \(^{(p < 0.05)}\) reduced in GM treatment as compared to control. Intriguingly, administration of alcoholic extract of W. somnifera significantly \(^{(p < 0.05)}\) moderated effects of GM by protecting serum concentrations of K\(^+\) and Ca\(^{2+}\).

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Cr (mg dl(^{-1}))</th>
<th>BUN (mg dl(^{-1}))</th>
<th>Total Prot. (g dl(^{-1}))</th>
<th>ALB (g dl(^{-1}))</th>
<th>GGT (U/l(^{-1}))</th>
<th>ALP (U/l(^{-1}))</th>
<th>ALT (U/l(^{-1}))</th>
<th>AST (U/l(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.32 ± 0.01</td>
<td>34.09 ± 1.09</td>
<td>7.12 ± 0.08</td>
<td>3.05 ± 0.06</td>
<td>2.52 ± 0.10</td>
<td>140.2 ± 2.1</td>
<td>46.29 ± 1.30</td>
<td>56.59 ± 1.60</td>
</tr>
<tr>
<td>GM</td>
<td>0.90 ± 0.04(^a)</td>
<td>78.17 ± 2.45(^a)</td>
<td>9.91 ± 0.59(^a)</td>
<td>3.74 ± 0.09(^a)</td>
<td>5.40 ± 0.23(^a)</td>
<td>256.51 ± 5.21(^a)</td>
<td>75.84 ± 3.70(^a)</td>
<td>83.01 ± 1.47(^a)</td>
</tr>
<tr>
<td>NP</td>
<td>0.37 ± 0.01(^b)</td>
<td>37.42 ± 0.35(^b)</td>
<td>7.46 ± 0.05(^b)</td>
<td>3.14 ± 0.05(^b)</td>
<td>3.09 ± 0.03(^b)</td>
<td>141.32 ± 1.45(^b)</td>
<td>47.61 ± 1.32(^b)</td>
<td>58.77 ± 1.32(^b)</td>
</tr>
<tr>
<td>NC</td>
<td>0.40 ± 0.02(^b)</td>
<td>39.92 ± 0.81(^b)</td>
<td>7.85 ± 0.11(^b)</td>
<td>3.16 ± 0.03(^b)</td>
<td>3.23 ± 0.10(^b)</td>
<td>143.54 ± 1.30(^b)</td>
<td>50.87 ± 1.00(^b)</td>
<td>59.76 ± 0.94(^b)</td>
</tr>
</tbody>
</table>

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 \(^{(p < 0.05, n = 6)}\) improvement in W. somnifera treatment rats as compared to gentamicin.

Table 3

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

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 \(^{(p < 0.05)}\) increased in GM treatment, whereas SOD and GSH levels significantly \(^{(p < 0.05)}\) reduced as compared to control. Administration of alcoholic extract of W. somnifera significantly \(^{(p < 0.05)}\) 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 \((p < 0.05)\) altered in renal tissues of gentamicin treatment. Withania somnifera treatment groups, nephroprotective and nephrocurative significantly \((p < 0.05)\) attenuated altered renal enzymes \((n = 6)\).

<table>
<thead>
<tr>
<th>Group</th>
<th>LPO (nM MDA g(^{-1}) of tissue)</th>
<th>GSH (µM GSH g(^{-1}) of tissue)</th>
<th>SOD (U g(^{-1}) of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>71.85 ± 1.08</td>
<td>71.74 ± 3.73</td>
<td>876.83 ± 0.32</td>
</tr>
<tr>
<td>GM</td>
<td>126.71 ± 1.45(^{a})</td>
<td>37.38 ± 4.06(^{a})</td>
<td>574.87 ± 0.36(^{a})</td>
</tr>
<tr>
<td>NP</td>
<td>73.83 ± 1.56(^{b})</td>
<td>60.77 ± 3.66(^{a})</td>
<td>843.34 ± 0.68(^{b})</td>
</tr>
<tr>
<td>NC</td>
<td>80.59 ± 0.51(^{b})</td>
<td>58.79 ± 4.67(^{a})</td>
<td>806.98 ± 0.71(^{a})</td>
</tr>
</tbody>
</table>

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 \((×200)\). These lesions were effectively ameliorated in W. somnifera treatment, nephroprotective group \((×100)\) (B), as compared to nephroprotective \((×200)\) (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 \((Karahan et al., 2005; Yaman and Balikci, 2010; Tavafi et al., 2012)\). 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 hydroethanolic 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 \((Davis and Kuttan, 2000; Jayaprakasham et al., 2003)\). 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 withaframolide 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 \((80 \text{ mg} \text{ kg}^{-1} \text{ IM})\) 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 alternations 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 \((Lopez-Giacoman and Madero, 2015; Abuezz et al., 2016; Teo and Endre, 2017)\). In our study, GM \((80 \text{ mg} \text{ kg}^{-1} \text{ b.wt.}, \text{i.p.})\) 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 withaframolide 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.
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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References


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 nephroprotec-
tive 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.


