

## Evaluation and comparison of nephroprotective effect of *Hemidesmus indicus* Linn. and *Withania somnifera* Linn. on gentamicin induced nephrotoxicity in Albino rats

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**Received:** 08 January 2018

**Revised:** 09 February 2018

**Accepted:** 27 February 2018

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### ABSTRACT

**Background:** Nephrotoxicity is a known deleterious effect of Aminoglycoside antibiotics. This class of antibiotics is used very frequently. It is well known that Gentamicin causes renal tubular necrosis in the proximal tubular cells. Phytochemicals are being studied extensively and have proved to be of benefit in various diseases. The use of phytochemicals would minimize the adverse effects encountered with these antibiotics. Comparison between various phytochemicals would help in optimizing dosage and duration of treatment. In the present study we studied and compared the nephroprotective effect of *Hemidesmus Indicus* Linn. and *Withania Somnifera* Linn. on Gentamicin induced nephrotoxicity in Albino rats.

**Methods:** Wistar albino rats weighing 180-250Gms were utilized for the present study. The root extract of these *Hemidesmus Indicus* Linn. and *Withania Somnifera* Linn. was administered orally to rats for 10 days concurrently with Gentamicin. Nephrotoxicity was assessed following a single dose injection of Gentamicin. Nephroprotective effect was assessed after 10 days of administering *Hemidesmus Indicus* Linn. and *Withania Somnifera* Linn. Renal function was assessed using Blood Urea, Serum Creatinine, SOD (Superoxide Dismutase) and MDA (Malondialdehyde) as parameters.

**Results:** Statistically significant improvement in renal function ( $p < 0.05$ ) was seen after administration of *Hemidesmus Indicus* and *Withania Somnifera* in Gentamicin treated rats. There was significant reduction in blood urea, serum creatinine and MDA levels with an increase in SOD levels.

**Conclusions:** *Withania Somnifera* and *Hemidesmus Indicus* had nephroprotective effect. However, *Withania Somnifera* was found to be more nephroprotective than *Hemidesmus Indicus*.

**Keywords:** Gentamicin, Nephrotoxicity, Phytochemicals

### INTRODUCTION

Aminoglycoside is a medicinal and bacteriologic category of gram-negative antibacterial therapeutic agents that inhibit protein synthesis and contain as a portion of the molecule an amino-modified glycoside (sugar).<sup>1</sup>

They are useful primarily in infections involving aerobic, Gram-negative bacteria, such as *Pseudomonas*, *Acinetobacter* and *Enterobacter*. In addition, some

*Mycobacteria*, including the bacteria that cause tuberculosis, are susceptible to aminoglycosides. Streptomycin was the first effective drug in the treatment of tuberculosis, though the role of aminoglycosides such as streptomycin and amikacin has been eclipsed (because of their toxicity and inconvenient route of administration) except for multiple-drug-resistant strains. The most frequent use of aminoglycosides is empiric therapy for serious infections such as septicemia, complicated intraabdominal infections, complicated urinary tract

infections, and nosocomial respiratory tract infections. Usually, once cultures of the causal organism are grown and their susceptibilities tested, aminoglycosides are discontinued in favor of less toxic antibiotics.<sup>2</sup>

Gentamicin is a broad-spectrum antibiotic belonging to aminoglycoside group. It is very effective against gram-negative and some gram positive bacterial infections.

Gentamicin is well recognized, to produce renal tubular necrosis mainly in the proximal tubule. This drug causes generation of reactive oxygen species (ROS), which induces cell injury and necrosis via lipid peroxidation.

The kidney is an essential excretory organ of our body. It plays a vital role in homeostasis by excreting the metabolic waste products and excess necessary substances. It is the prime target of several drugs, toxic xenobiotics or chemicals due to high rate of blood flow (20%-25% of cardiac output) and presence of cellular transport systems that causes accumulation of these compounds within the nephron epithelial cells. Metabolites of the drugs that are excreted from kidney may also cause cellular damage leading to kidney dysfunction. Several xenobiotic substances 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.<sup>3,4</sup>

Nephrotoxicity is defined as renal dysfunction occurring as a direct result of exposure to external agents such as drugs and environmental chemicals.<sup>5</sup>

Nephrotoxicity and ototoxicity are the major limitations of Aminoglycoside use in clinical practice. Complications caused by aminoglycoside toxicity are one of the most common reasons for prolonging hospital stays.<sup>6</sup> Data shows that up to 30% of patients treated with GM for more than 7 days show signs of nephrotoxicity.<sup>7</sup>

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated by xenobiotics, such as toxicants and drugs, play crucial role in the initiation, and progression of nephrotoxicity and renal injuries.<sup>8</sup>

Antioxidant agents can ameliorate these effects in the kidney through modification of the oxidant-antioxidant balances.<sup>9</sup>

An antioxidant is defined as any substance which when present at low concentrations delays or prevents oxidations of cell contents like lipids, proteins, carbohydrates and DNA. Superoxide dismutase (SOD), Catalase (CAT) and Glutathione reductase are the first line endogenous antioxidants in the human body.<sup>10</sup>

Antioxidant minerals include Selenium (Se), Magnesium (Mg), Copper (Cu) and Zinc (Zn).<sup>11</sup> Oxidative stress results when the balance between ROS and antioxidant defense is lost.<sup>12</sup> Plants have efficient complex enzymatic

and non-enzymatic antioxidant defense systems to avoid the toxic effects of free radicals. Enzymatic systems include SOD, catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR), while non-enzymatic systems consist of low molecular weight antioxidants (ascorbic acid, glutathione, proline, carotenoids, phenolic acids, flavonoids, etc.) and high molecular weight secondary metabolites such as tannins. Commonly used plants with antioxidant properties are *Curcuma Domestica* (Turmeric), *Ocimum Sanctum* (Tulsi), *Withania Somnifera* (Ashwagandha), *Embellica Officinalis* (Amla) etc.<sup>13</sup>

Malondialdehyde is a highly reactive compound that is not typically observed in pure form. It results from lipid peroxidation of polyunsaturated fatty acids. The degree of lipid peroxidation can be estimated by the amount of malondialdehyde in tissues.<sup>14</sup>

Free oxygen radicals can induce lipid peroxidation in cells, and malondialdehyde (MDA) is formed during oxidative degeneration and accepted an indicator of lipid peroxidation.

Superoxide dismutase (SOD) is an antioxidant enzyme. It protects cells against oxygen free radical damage by providing reducing equivalents for several enzymes. Aminoglycoside antibiotics are known to facilitate the generation of oxygen free species both in vivo and in vitro which plays an important role in aminoglycoside induced nephrotoxicity.<sup>15</sup>

The World Health Organization found that 80% of the world's population depends on medicinal plants for their healthcare needs, and more than 30% of the pharmaceutical preparations are based on plants.

*Hemidesmus indicus* Linn. R. Br is a twining shrub, belonging to the family Asclepiadaceae. Roots of this taxon are being used in folk medicine as well as in ayurvedic and unani preparations. They are prescribed against the diseases of blood, inflammation, diarrhea, respiratory disorders, skin diseases, syphilis, fever, bronchitis, asthma, eye diseases; epileptic fits in children, kidney and urinary disorders, loss of appetite, burning sensation and rheumatism.<sup>16-18</sup>

*Withania somnifera*, also known as ashwagandha /Winter cherry/ Indian ginseng is widely used in Ayurvedic medicine. It belongs to the family of Solanaceae. The leaves, fruits, seeds, shoots and roots of this plant have all been used traditionally. The roots of *Withania somnifera* contain 35 chemical constituents. Most of the biological actions have been attributed to the active ingredients i.e Withaferin A and withanolides. The roots of this plant are known to promote health and longevity by augmenting defense against some diseases, arresting the aging process, revitalizing the body in debilitated condition, increasing the capability of an individual to resist adverse

environment factors. No side effects have been reported so far with this plant.<sup>19,20</sup>

Due to the serious adverse effects of Aminoglycosides on kidney, the present study was designed to compare the effect of *Withania somnifera* and *Hemidesmus Indicus* on gentamicin induced nephrotoxicity in experimental animals.

Aim of the study was to compare the effect of Hemidismus Indicus and Withania Somnifera on Gentamicin induced nephrotoxicity in albino rats.

**Objectives**

- To induce nephrotoxicity with Gentamicin.
- To evaluate the effect *Hemidesmus indicus* on gentamicin induced nephrotoxicity.
- To evaluate the effect of *Withania somnifera* on gentamicin induced nephrotoxicity.
- To compare the effect of *Hemidesmus indicus* and *Withania somnifera* on gentamicin induced nephrotoxicity.

Duration of the study was 1 month. There were 32 albino rats used in the study. It was an experimental study. The study was carried out in Animal House, Department of Pharmacology, JNMC, Wardha.

**METHODS**

Wistar albino rats of either sex weighing 180-250gm were utilized for the study. Experiment was performed after due permission from the Institutional Animal Ethics Committee (IAEC).

**Table 1: Experimental animals were grouped.**

Group	Treatment given	Duration
I	Normal saline 10ml/kg intraperitoneally (IP) daily	10 days
II	Gentamicin 80mg/kg body weight intraperitoneally ((IP) daily	10 days
III	<i>Hemidesmus Indicus</i> 7g/kg/day PO along with Inj. Gentamicin 80mg/kg body weight intraperitoneally (IP) daily	10 days
IV	<i>Withania Somnifera</i> 500mg/kg/day PO along with Gentamicin 80mg/kg body weight daily intraperitoneally (IP) daily	10 days

All rats were kept under observation for 1 week prior to the experiment to permit the animals to adjust to the environment. Blood samples were collected through retrorbital puncture a day before starting the study and on the 10<sup>th</sup> day. These samples were centrifuged for 10

minutes at 3000rpm and sera were obtained. Samples were analyzed for blood urea, serum creatinine, MDA and SOD levels. All specimens of sera were stored at -20<sup>0</sup> Celsius until use. Results were measured using a spectrophotometer.

**RESULTS**

Statistical analysis was done by using descriptive and inferential statistics using one way ANOVA and Multiple comparison Tukey test and software used in the analysis was SPSS17.0 version and p<0.05 is considered as level of significance.

**Table 2: Comparison of blood urea in four groups.**

Group	N	Mean	Std. deviation	Std. error	Minimum	Maximum
Group I	8	18.80	1.62	0.57	16.13	20.83
Group II	8	77.13	3.62	1.28	70.76	81.13
Group III	8	40.30	3.78	1.33	35.12	46.34
Group IV	8	33.69	2.43	0.85	30.45	36.45

Mean blood urea level in group I was 18.80±1.62, in group II it was 77.13±3.62, in group III it was 40.30±3.78 and in group IV it was 33.69±2.43. By using one way ANOVA statistically significant variation was found in mean urea level among four groups (F=546.47, p-value=0.0001). On comparing blood urea level in four groups using Multiple Comparisons: Tukey Test statistically significant variation was found in mean urea level among four groups (p<0.05).

**Table 3: Comparison of serum creatinine in four groups.**

Group	N	Mean	Std. deviation	Std. error	Minimum	Maximum
Group I	8	0.84	0.08	0.03	0.72	0.94
Group II	8	3.00	0.56	0.19	2.30	3.80
Group III	8	1.62	0.23	0.08	1.30	2.00
Group IV	8	1.57	0.23	0.08	1.20	1.90

**Table 4: Comparison of SOD in four groups.**

Group	N	Mean	Std. deviation	Std. error	Minimum	Maximum
Group I	8	1.74	0.12	0.04	1.57	1.95
Group II	8	0.05	0.01	0.006	0.03	0.08
Group III	8	0.75	0.11	0.040	0.56	0.91
Group IV	8	0.82	0.07	0.025	0.74	0.93

Mean serum Creatinine level in group I was  $0.84 \pm 0.08$ , in group II it was  $3.00 \pm 0.56$ , in group III it was  $1.62 \pm 0.23$  and in group IV it was  $1.57 \pm 0.23$ . By using one way ANOVA statistically significant variation was found in mean creatinine level among four groups ( $F=60.07$ ,  $p$ -value= $0.0001$ ). On comparing serum Creatinine level in four groups using Multiple Comparisons: Tukey Test statistically significant variation was found in mean creatinine level among four groups ( $p < 0.05$ ).

Mean SOD level in group I was  $1.74 \pm 0.05$ , in group II it was  $0.05 \pm 0.01$ , in group III it was  $0.75 \pm 0.11$  and in group IV it was  $0.82 \pm 0.07$ . By using one way ANOVA statistically significant variation was found in mean SOD level among four groups ( $F=442.71$ ,  $p$ -value= $0.0001$ ). On comparing SOD level in four groups using Multiple Comparisons: Tukey Test statistically significant variation was found in mean SOD level among four groups ( $p < 0.05$ ).

**Table 5: Comparison of MDA in four groups.**

Group	N	Mean	Std. deviation	Std. error	Minimum	Maximum
Group I	8	1.23	0.08	0.02	1.15	1.38
Group II	8	2.79	0.36	0.12	2.13	3.16
Group III	8	1.71	0.15	0.05	1.51	1.93
Group IV	8	1.61	0.12	0.04	1.45	1.81

Mean MDA level in group I was  $1.23 \pm 0.08$ , in group II it was  $2.79 \pm 0.36$ , in group III it was  $1.71 \pm 0.15$  and in group IV it was  $1.61 \pm 0.12$ . By using one way ANOVA statistically significant variation was found in mean MDA level among four groups ( $F=79.74$ ,  $p$ -value= $0.0001$ ). On comparing MDA level in four groups using Multiple Comparisons: Tukey Test statistically significant variation was found in mean MDA level among four groups ( $p < 0.05$ ).

## DISCUSSION

This study groups included albino rats with normal renal function. They were given Gentamicin by intraperitoneal route for 10 days. Significant nephrotoxicity was seen with Gentamicin in comparison with the control group. Study parameters of Blood urea, serum creatinine and MDA levels were significantly higher in gentamicin treated group. This group also showed a marked reduction in SOD levels. Study groups III and IV were concurrently administered *Hemidesmus Indicus* at a dose of 7g/kg/day PO and *Withania Somnifera* at a dose of 500mg/kg/day PO for 10 days. Renal function was monitored on Day 1 and then on Day 10.

Our results concluded that *Hemidesmus Indicus* and *Withania Somnifera* had a significant nephroprotective effect. This was evident by a reduction in Blood Urea,

Serum Creatinine. An increase in SOD level and decrease in MDA level in comparison with the control group showed significant reduction in oxidative stress.

These results confirmed that Gentamicin produced nephrotoxicity as previously reported by authors.<sup>21</sup> These changes reflected the severity of renal insufficiency which occurred in association with the sudden fall in glomerular filtration rate because of the majority of administered GM enters specifically the proximal tubular epithelial cells, binds to anionic phospholipids in the target cells inducing abnormalities in the function and metabolism of multiple intracellular membranes and organelles then developed injury in the proximal tubular epithelial cells of kidney that caused acute renal failure.<sup>22</sup>

Russo A et al reported that phytochemicals present in *Withania somnifera* are responsible in overcoming oxidative stress.<sup>23</sup> This result are also in accordance with these earlier reports.

## CONCLUSION

This study concludes that *Hemidesmus indicus* at a dose of 7g/kg/day and *Withania somnifera* at a dose of 500mg/kg showed nephroprotective effect in Gentamicin induced nephrotoxicity. We also concluded that *Withania Somnifera* is more nephroprotective than *Hemidesmus indicus*.

*Funding: No funding sources*

*Conflict of interest: None declared*

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

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**Cite this article as:** Padhye MR, Jogdand SD, Bhattacharjee J. Evaluation and comparison of nephroprotective effect of *Hemidesmus indicus* Linn. and *withania somnifera* Linn. on gentamicin induced nephrotoxicity in Albino rats. Int J Basic Clin Pharmacol 2018;7:691-5.