Histomorphological Effects of Withania Somnifera Root Extract against Cisplatin Induced Renal Lesions in Rats

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ABSTRACT

Aim: To analyze the nephroprotective effects of W. Somnifera root extract against cisplatin induced nephrotoxicity in albino Wistar rats through gross and histopathological parameters.

Methodology: This experimental study was carried out at the Baqai Medical University, Karachi from November 2018 till February 2019. Eighty adult male Albino Wistar rats were divided into equal four groups. Group A served as control, group B received inj. Cisplatin 1mg/kg intraperitoneally for 7 days, while group C was given W. Somnifera root extract 500mg/kg orally for 15 days before cisplatin treatment and thereafter concurrently with cisplatin for last 7 days. Group D received only W. Somnifera root extract 500mg/kg orally for 22 days. Their initial and final body weights were recorded. On 8th day of experiment group B and on 23rd day group A, C & D were sacrificed.

Results: Albino rats of cisplatin treated group B showed significant (p<0.05) reduction in final body weight as compared to group A and D whereas insignificant (p>0.05) change was recorded in WS+CP treated group C. Similarly there was a significant (p<0.05) increase of absolute & relative kidney wt. of group B. However, no significant (p>0.05) change in kidney weight of others groups were recorded. Stained section of group B showed significant (p<0.05) histopathological alterations in renal parenchyma. Degenerative changes were more marked in renal corpuscle and proximal convoluted tubule (PCT). However there was preservation of renal architecture of group C (p>0.05).

Conclusion: It is concluded from our study that pretreatment with W. Somnifera has prevented the cisplatin mediated tubular damage in kidney.

Keywords: Cisplatin, W. Somnifera, renal corpuscles, proximal convoluted tubules, nephrotoxicity.

INTRODUCTION

Nephrotoxicity is a renal dysfunction & one of the most common kidney associated problems as a result of direct or indirect exposure to medicine, industrial or environmental factors i.e. heavy metals, fungal toxins and chemicals¹. These agents can adversely affect the kidney resulting in acute or chronic renal failure. Incidence of drug induced nephrotoxicity has been increasing with the increasing number of therapeutic agents. There is list of medications like carbon tetrachloride, heavy metals (mercury & lead), ethylene glycol, insecticides, NSAID’s, some antibiotics & chemotherapeutic agents (cisplatin) which are nephrotoxic. Each of them has specific toxins which can cause death of renal tubular epithelial cells².

Cisplatin is one of chemotherapeutic agent widely used for treatment of almost 50% of solid tumors³. However limitations in therapeutic use of Cisplatin are its severe side effects i.e. gastrotoxicity & nephrotoxicity. As Cisplatin is cleared through kidney and concentration in proximal tubular cells is 5 times higher than the serum concentration, thus its accumulation in kidney contributes to its nephrotoxicity which is manifested as Acute Kidney Injury (AKI) in 20-30% of patients⁴. Main mechanism for Cisplatin induced acute kidney injury involves Oxidative stress, inflammation, proximal tubular injury and vascular injury⁵.

In order to prevent acute kidney injury, many plants have been used as potential nephroprotective agents throughout the world. Withania Somnifera (WS) commonly known as Ashwagandha, belongs to the family of Solanaceae, and provides protection against nephrotoxicity. It is widely used in traditional system of medicine like Unani medicine and Ayurvedic more than 3000 years. WS is known for its contributing factor to strengthen immune system but beside this, it has anti-inflammatory, antibacterial, antiuretic, antiarthritic, anticancer, neuroprotective and nephroprotective effects⁶. However all parts of plant (leaves, fruits, seeds, shoots & roots) have been used traditionally for medicinal purposes but roots of plant are well known for its nephroprotective activity⁷. Withaferin A & Withanolides are most active ingredients found in the roots which contribute to the biological activity of this plant⁸. Nephroprotective and nephrocurative effect of W. Somnifera root extract against gentamicin induced renal lesion validates its use to cure renal ailments⁹.
Keeping in mind the above mentioned facts, current study was planned to observe the protective effect of *W. Somnifera* root extract on renal tissue against Cisplatin induced nephrotoxicity through gross and histological analysis.

**MATERIAL & METHODS**

This experimental study was done in the Department of Anatomy and Animal house of Baqai Medical University, Karachi after research approval from Ethical committee and Board of Advanced Studies and Research (BASR), Baqai Medical University, duration of study was 3 months (November 2018-February 2019). A total number of 80 healthy adult, 14-16 weeks old male Albino Wistar rats, weighing 200-250gm were taken. Before conducting the study, rats were acclimatized for 1 week at constant room temperature of 21-24°C, humidity (60-70%). To produce nephrotoxicity, Cisplatin injections (Inj. Cisplasul 50mg/50ml) were purchased from local pharmacy.

The dry roots of *W. Somnifera* were purchased from local market in Karachi. Botanical identification was accomplished by Department of Pharmacognosy, University of Karachi and a voucher number (WSR -01-18/18) was generated.

Ethanol extract of *W. Somnifera* roots was prepared and used for our experimental study. **Experimental Protocol**: Eighty male albino rats were divided into four groups, (20 per group) labeled as A, B, C & D. Group A served as control group with no intervention, Group B received inj. Cisplatin intraperitoneally (1mg/kg) for seven days11. Group C were given *W. Somnifera* root extract (500mg/kg orally via gastric gavage) for 22 consecutive days and cisplatin (1mg/kg intraperitoneally) for last 7 days (16th to 22nd day)12. Whereas group D was given only *W. Somnifera* root extract (500mg/kg orally) for 22 days, to evaluate any harmful effect of *W. Somnifera* on rat kidneys. Dosing was done after overnight fasting, around 9am in morning.

Animals’ weight was recorded at the beginning and at the end of experiment. Animals of Group A, C & D were anesthetized and sacrificed on 23rd day and group B was sacrificed on 8th day of study. Kidneys were dissected out and weighed (absolute organ weight in g) to calculate relative organ weight of each rat13.

Relative organ weight (kidney) = \(
\frac{\text{Absolute organ (kidney) weight (gm)}}{\text{Final body weight of animal (gm)}} \times 100
\)

One half of kidney was placed in 10% formalin and the other one in alcoholic formalin. After paraffin embedding of fixed kidney tissue, 4um thick sections were prepared. Representative sections were stained with Hematoxylin and Eosin (H&E) and Periodic Acid Schiff (PAS) stains and covered with cover slip. Stained sections were examined under light microscope at 10x and 40x magnification to observe morphological and morphometric changes. Micrometry was done manually with stage micrometer and ocular micrometer scale under light microscope. Data was analyzed statistically on SPSS version 22. Mean of body weight, organ weight (Absolute & relative) and diameter of renal structure (Renal corpuscle & Proximal convoluted tubule) was calculated and expressed as Mean±SD. Comparison between groups were analyzed by using one way analysis of variance (ANOVA) followed by post hoc Tukey’ test. P value of less than 0.05 was considered significant (p<0.05).

**RESULTS**

The mean (±SD) initial and final body weight, kidney weight (absolute & relative) and diameter (Renal corpuscle and Proximal convoluted tubule) of Group A, B, C & D is shown in Table 1. Mean comparison between groups showed a significant (p<0.05) reduction in final body weight of Cisplatin treated group B as compared to group A and D. Meanwhile insignificant (p>0.05) change of final body weight was recorded in WS+CP treated group C. Similarly there was a significant (p<0.05) increase of absolute & relative kidney wt. of group B. However, no significant (p>0.05) change in kidney weight of others groups were detected, as shown in Table 2.

The histological study of H&E and PAS stained section of renal tissue is shown in figure 1 & 2 respectively. Group A and D showed normal architecture of kidney while renal parenchyma of cisplatin treated group B exhibited severe significant (p<0.05) histopathological alterations. Renal corpuscles appeared hypertrophied with congested and shrunken glomeruli and disrupted basement membrane (BM) & brush borders (BB). There were severe tubular degenerative changes which were more pronounced in PCT. However there was preservation of renal architecture of WS+CP treated group C. (p>0.05)

Table 1: Descriptive Statistics of Group A, B, C&D

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group A (Mean± SD)</th>
<th>Group B (Mean± SD)</th>
<th>Group C (Mean±SD)</th>
<th>Group D (Mean± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Body Wt.(gm)</td>
<td>217.95±21.090</td>
<td>218.95±22.988</td>
<td>215.50±24.04</td>
<td>207.10±15.134</td>
</tr>
<tr>
<td>Final Body Wt. (gm)</td>
<td>234.80±21.683</td>
<td>188.55±25.276</td>
<td>204.50±24.85</td>
<td>221.80±13.942</td>
</tr>
<tr>
<td>Absolute Kidney Wt. (gm)</td>
<td>0.59±0.100</td>
<td>0.73±0.10414</td>
<td>0.60±0.082</td>
<td>0.55±0.04607</td>
</tr>
<tr>
<td>Relative Kidney Wt. (gm)</td>
<td>0.25±0.02</td>
<td>0.39±0.04110</td>
<td>0.28±0.017</td>
<td>0.24±0.01251</td>
</tr>
<tr>
<td>Renal Corpuscle Diameter (µm)</td>
<td>91.87±2.13</td>
<td>109.72±3.3969</td>
<td>98.93±1.67</td>
<td>87.53±15.235</td>
</tr>
<tr>
<td>PCT Diameter(µm)</td>
<td>22.14±1.08</td>
<td>31.98±1.653</td>
<td>24.70±0.797</td>
<td>22.07±0.9108</td>
</tr>
</tbody>
</table>
Table 2: Statistical Analysis of Mean Final Body Wt., kidney Wt. (Absolute & Relative) & Diameter (Renal Corpuscle & PCT) of Rats between different groups.

<table>
<thead>
<tr>
<th>Groups comparison</th>
<th>Mean Final Body Weight (gm)</th>
<th>Mean Absolute Kidney Weight (gm)</th>
<th>Mean Relative Kidney Weight (gm)</th>
<th>Mean Renal Corpuscle Diameter (µm)</th>
<th>Mean PCT Diameter (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B &amp; A</td>
<td>-46.25</td>
<td>0.000*</td>
<td>0.146</td>
<td>0.000*</td>
<td>0.139</td>
</tr>
<tr>
<td>B &amp; C</td>
<td>-15.95</td>
<td>0.000*</td>
<td>0.136</td>
<td>0.002*</td>
<td>0.102</td>
</tr>
<tr>
<td>B &amp; D</td>
<td>-33.25</td>
<td>0.000*</td>
<td>0.180</td>
<td>0.001*</td>
<td>0.143</td>
</tr>
<tr>
<td>C &amp; A</td>
<td>-30.3</td>
<td>0.061</td>
<td>0.009</td>
<td>0.738</td>
<td>0.037</td>
</tr>
<tr>
<td>C &amp; D</td>
<td>-17.3</td>
<td>0.067</td>
<td>0.043</td>
<td>0.044</td>
<td>0.040</td>
</tr>
<tr>
<td>A &amp; D</td>
<td>13</td>
<td>0.080</td>
<td>0.034</td>
<td>0.177</td>
<td>0.003</td>
</tr>
</tbody>
</table>

(P value ≤ 0.05 is considered statistically significant *)

Figure 1: Photomicrograph from kidney tissue sections stained with H&E x 100
Group A (Ct) showing normal renal structure, with normal Renal corpuscle (RC) & proximal convoluted tubule (PCT) diameter. Group B (CP) exhibit congested glomeruli with increased RC & PCT diameter. Group C (CP+Ws) showing preservation of renal parenchymal architecture and normal RC & PCT diameter. Group D (Ws) showing normal healthy renal tissue.

Figure 2: Photomicrograph from kidney tissue sections stained with PAS-H x 100
Group A (Ct) showing RC and renal tubules with well-defined basement membrane (BM) and intact brush borders (BB) of PCT. Group B (Cp) showing dilated proximal convoluted tubule (PCT) and degenerated tubular epithelial lining cells along with disruption of basement membrane (BM) and brush borders (BB). Group C (CP+Ws) showing preservation of renal parenchyma with mild degeneration of renal tubules with intact basement membrane (BM) and brush borders (BB). Group D (Ws) showing normal healthy renal tissue.
DISCUSSION

This study was designed to analyze the morphometric changes of cisplatin induced nephrotoxicity and to evaluate the protective effect of *W. Somnifera* root extract against nephrotoxicity through histopathology slides.

Cisplatin toxicity occurs as a result of increased production of reactive oxygen species (ROS), accumulation of lipid peroxidation products & suppression of antioxidant system, in kidney. It reacts with glutathione (cellular antioxidant) and results in its depletion leading to further accumulation of endogenous ROS and oxidative stress within cell. *W. Somnifera* has been traditionally used in the treatment of certain diseases i.e. rheumatoid arthritis, behavioural disorders and infertility, for a long time. Bioactive components of WS roots, Sitoindosides VII-X and Withaferin A, enhances free radical scavenging enzymes such as superoxide dismutase(SOD), catalase(CAT), glutathione peroxidase (GPx), which inhibits lipid peroxidation & generation of free radicals and hence improve histological architecture of kidney.

Results of our study demonstrated the cisplatin induced nephrotoxicity in group B. Animals in this group were ill looking and lethargic with loss of body hair, similar changes were reported by Nasr et al. There was reduction in food and water consumption and a significant decrease in body weight of rats which is in accordance with the observation of Dalia et al. The weight loss could be due to loss of appetite and gastrointestinal toxicity induced by cisplatin as reported by Dalia et al. Another reason of weight loss might be due to dehydration which occurs as a result of polyuria due to renal tubular injury as discussed by Darwish et al. and Ponzeshki et al.

Animals in group C demonstrated that *W. Somnifera* root extract when administered along with cisplatin reduced the toxic effects produced by cisplatin. Rats of this group appeared healthy, and active. There was moderate weight gain due to increased food consumption. This finding was in agreement with the result of Tiwari et al. and Robin et al.

Examination of the animals' organ with the naked eye revealed no gross change in kidneys of animals of all groups. There was significant increase in absolute kidney weight of cisplatin treated group B as compared to control group A and D. Similar changes were observed by Ku et al. & Abdelmeguid et al. who stated increase in kidney weight due to edema of renal tissue as a result of acute renal failure induced by cisplatin. There was also increased kidney/body relative weight of the same group in comparison to the control groups. Confirming our results Lee et al. stated there is significant increase in kidney weight to body weight ratio in cisplatin treated rats. The increase in kidney/body relative weight might be due to renal parenchymal edema produced as result of cisplatin induced renal inflammation.

However animals of *W. Somnifera* pretreated and Cisplatin treated group C showed decrease absolute and relative kidney weight. Our finding was in consistent with the results of Shimmi et al who reported decrease in kidney weight due to anti-inflammatory activity of withanolide, which is one of the major bioactive phytochemical of *W. Somnifera* roots. Free radical scavenging activity of Withanolides inhibits lipid peroxidation and subsequent cellular damage. This property may be helpful in decreasing absolute and relative kidney weight.

Microscopic study of control group A and D showed normal architecture of renal tissue. However section of cisplatin treated group B depicted distortion of renal parenchyma. Degenerative changes were more marked in renal corpuscle and proximal convoluted tubule. Hypertrophied renal corpuscle with wide urinary space and shrunken glomeruli were seen, which was in agreement with results of Akca et al. This might be due to cisplatin induced elevated oxygen free radicals which cause mitochondrial dysfunction, protein degradation, DNA injury which may lead to cell death and organ damage.

The kidney section of *W. Somnifera* pretreated and Cisplatin treated group C showed preservation of renal architecture. Renal corpuscles were slightly dilated and some glomeruli were still congested and hypercellular but these changes were less marked as compared to group B. These findings were in accordance with the study of Govindappa et al. who also stated that *W. Somnifera* root extract has antioxidant and free radical scavenging properties which reduces the renal lesion and provide nephroprotection.

Stained section of cisplatin treated group B revealed characteristic features of tubular necrosis which is more marked in PCT. Their tubular lumen appeared dilated as compared to the control group. Our results were supported by Motamedi et al. who also reported similar findings in PCT of cisplatin treated kidneys, due to increase oxidative stress. In another study Ibrahim et al. reported similar distortion in renal architecture and stated that as the cisplatin accumulates in renal cells particularly of PCT, chloride atoms of cisplatin is replaced by water molecule and resulting compound will react with GSH in cytoplasm.
and DNA in nucleus which leads to cellular damage. This could be one of the mechanisms which results in cisplatin induced renal damage. 

CONCLUSION

Our study concluded the nephroprotective medicinal value of *W. Somnifera* root extract against cisplatin induced acute renal failure by a significant improvement in renal histopathology.

Limitations of study: We concluded the protective effect of roots of *W. Somnifera* on drug induced nephrotoxicity. However analysis of *W. Somnifera* roots extract was proposed to be carried out using HPLC (High Performance Liquid Chromatography) but unable to do so due to inaccessibility to the equipment.

Conflict of interest – None

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REFERENCES