

## Evaluation of Role of Atorvastatin in Cisplatin Induced Nephrotoxicity in Wistar Rats

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**Abstract:** This study was carried out to investigate the ameliorative effect of Atorvastatin on Cisplatin-induced Nephrotoxicity in Wistar rats. Twenty Four (24) male rats were used for the experiment. Group I was administered per oral daily with Phosphate Buffered Saline (PBS) for 10 days. Group II was administered single intraperitoneal dose of Cisplatin 7.5mg kg<sup>-1</sup> body weight on fifth day, Group III was administered daily per oral dose of Atorvastatin 3 mg kg<sup>-1</sup> body weight for 10 days and on fifth day single intraperitoneal dose of Cisplatin 7.5 mg kg<sup>-1</sup> body weight. Group IV was administered daily per oral dose of Atorvastatin 10 mg kg<sup>-1</sup> body weight for 10 days and on fifth day a single intraperitoneal dose of Cisplatin 7.5 mg kg<sup>-1</sup> body weight. After the last day of treatment animals were sacrificed and blood samples collected by cardiac puncture and used for analysis of serum creatinine, blood urea nitrogen and total protein. The antioxidant parameters of Catalase, GSH and TBARS were measured in kidneys. The result showed a significantly reduced serum concentration of creatinine, urea and total protein in groups treated with Cisplatin only when compared to PBS. It is concluded that the decrease in creatinine, blood urea and total protein were ameliorated by the administration of atorvastatin which may be due to its antioxidant properties.

**Key words:** Nephrotoxicity • Cisplatin • Atorvastatin • Oxidative Stress

### INTRODUCTION

Cisplatin [cis- DDP, diaminedichloroplatinum (II)] is a potent cytotoxic drug that Nephro-, neuro- and ototoxicity with clinical resistance, are frequent reasons for treatment discontinuation of cisplatin therapy [1]. Increased generation of reactive oxygen species along with decreased antioxidant defense and tissue nitric oxide levels promote the development and progression of cisplatin mediated nephrotoxicity [2, 3]. Though intensive hydration and/or forced diuresis are some of remedies to protect kidney from cisplatin, several studies were performed with different pharmacological agents to investigate their possible protective effects against cisplatin induced renal cell injury and nephrotoxicity [4]. Thus there is an increasing interest toward the use of new therapeutic agents with cytoprotective and antioxidant properties against Cisplatin induced nephrotoxicity.

Atorvastatin is a member of the statin class of 3-hydroxy-3-methylglutaryl-coenzymeA (HMG-CoA) reductase inhibitors, statins have revolutionized the treatment of hypercholesterolemia. It was reported that Atorvastatin improves tubular status in non-diabetic patients with chronic kidney disease [5]. Its role in inhibition of osteoclastogenesis and bone destruction in RA patients has reported by Jeong Yeon Kim *et al.* (2012). Neuroprotective effect of atorvastatin by inhibition of caspase dependent proapoptotic pathway was proposed by Gao *et al.* (2009). Cellular antioxidant effects of atorvastatin *in vitro* and *in vivo* has proposed by Wassmann *et al.* (2002). But, there is no study up to our knowledge investigated the nephroprotective capacity of Atorvastatin on this model of nephrotoxicity. Thus, the present work was aimed to study the possible protective role of Atorvastatin against cisplatin induced nephrotoxicity in rats.

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## MATERIALS AND METHODS

**Chemicals:** Cisplatin, 5,5-Dithio-bis (2-nitrobenzoic acid) (DTNB), 2-Thiobarbituric Acid (TBA), reduced Glutathione (GSH), catalase etc. were purchased from Sigma-Aldrich Co. St.Louis, MO, USA. Atorvastatin was kind gift from Hetero drugs Pvt. Ltd. Hyderabad, India. All other chemicals were of analytical grade and were purchased commercially.

**Animals:** Male albino Wistar rats weighing between 170-200 g were obtained from Sanzyme scientific, Hyderabad, India. The animals were housed in poly acrylic cages (38×23×10 cm) with not more than six animals per cage, at an ambient temperature of 18±2°C with 12hrs. The rats were accessed with standard chow diet and water *ad libitum*. The maintenance and the handling of animals were performed according to Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA) guidelines and the Institutional Animal Ethical Committee (IAEC) approved all the experimental procedures (IAEC NO: 1047/ac/07/CPCSEA), Vaagdevi college of Pharmacy, Hanamkonda, Warangal andhra Pradesh, India.

**Experimental Design and Treatment Protocol:** Animals were divided into four groups, with six rats in each and were treated as follows (Group I) Normal control: Phosphate buffered saline (5mL kg<sup>-1</sup>) was administered orally for 10 days and a single intraperitoneal injection of Phosphate buffered saline on 5<sup>th</sup> day; (Group II) Cisplatin control: Phosphate buffered saline (5 mL kg<sup>-1</sup>) was administered orally for 10 days and a single intraperitoneal (i.p.) injection of Cisplatin (7.5 mg kg<sup>-1</sup>) on 5<sup>th</sup> day; (Group III) Atorvastatin (3 mg kg<sup>-1</sup>) + Cisplatin (7.5 mg kg<sup>-1</sup>): Rats were fed with Atorvastatin (3 mg kg<sup>-1</sup>) dissolved in Phosphate buffered saline orally for 10 days and a single dose of Cisplatin (7.5 mg kg<sup>-1</sup>, i.p.) on 5<sup>th</sup> day, 1 hr. prior to low dose of Atorvastatin. (IV) Atorvastatin (10 mg kg<sup>-1</sup>) + Cisplatin (7.5 mg kg<sup>-1</sup>): Rats were fed with Atorvastatin (3 mg kg<sup>-1</sup>) dissolved in Phosphate buffered saline per oral for 10 days and a single dose of Cisplatin (7.5 mg kg<sup>-1</sup>, i.p.) on 5th day, 1 hr. prior to high dose of Atorvastatin.

**Sampling and Biochemical Assays:** Animals were sacrificed 5 days after the Cisplatin dose (on 6<sup>th</sup> day). Blood samples were collected and the kidney tissues were immediately removed from all the groups. A 10% homogenate of kidney tissue was prepared in ice cold

Phosphate Buffer Saline (PBS, 0.05M, pH 7). Serum was separated by centrifugation at 4000 rpm (4 °C) for 15 min and stored at -80°C until analysis. A part of homogenate was mixed with equal volume of 10% Trichloroacetic Acid (TCA) and was centrifuged at 5000 rpm for 10 min and supernatant was used for the estimation of Malondialdehyde (MDA). The remaining part of homogenate was centrifuged at 17,000 rpm for 60 min at 4°C and supernatant was used for the estimation of total protein, Catalase (CAT) and Reactive Oxygen Species (ROS).

**Assessment of Renal Function:** BUN (blood urea nitrogen), Creatinine and total protein levels were measured in serum by commercial diagnostic kits using auto analyzer.

**Lipid Peroxidation:** Malondialdehyde (MDA), an index for lipid peroxidation in kidney homogenate, was determined based on the reaction with thiobarbituric acid [9] at wave length 532 nm, quantified using an extinction coefficient of 1.56×10<sup>5</sup>M<sup>-1</sup> cm<sup>-1</sup> and was expressed as nanomoles of MDA per g of tissue.

**Catalase and Reduced Glutathione Activity:** The activity of catalase in kidney tissue was determined by measuring the rate of decomposition of hydrogen peroxide at 240 nm [10] and the activity was expressed as U/mgprotein. Reduced glutathione contents were measured following its reaction with 5, 5-dithiobis-(2-nitrobenzoic acid) in phosphatebuffer, pH 8 [11].

**Histopathological Studies of the Kidney:** All kidney samples were processed and embedded in paraffin. Sections were cut at 5 μm thicknesses on a rotary microtome, mounted and stained with hematoxylin and eosin. Special staining techniques viz. Periodic Acid Schiff (PAS) stain to study the glomerular changes and Congo red stain for amyloid deposits were also performed. These sections were evaluated for histological changes under light microscopy (Nikon E800 research microscope).

**Statistical Analysis:** Results were expressed as Mean ± S.D. The statistical significance of differences among various experimental groups were calculated by ANOVA followed by Dunnett's multiple comparison tests. Analysis was performed using the statistical software Graph Pad version 5.0 (San Diego, CA, USA). Results were considered significant when *p*<0.05.

## RESULTS

**Effect of Atorvastatin on Renal Function:** In the present study, Blood urea nitrogen, serum creatinine and total protein levels were significantly ( $p < 0.001$ ) elevated in Cisplatin control group compared to normal, conforming Cisplatin induced renal damage. Atorvastatin treatment at doses (3 and 10 mg kg<sup>-1</sup>) shows a dose dependent protection against Cisplatin induced renal damage. Blood urea nitrogen, serum creatinine and total protein results were presented in Table 1.

**Effect of Atorvastatin on Lipid Peroxidation:** Cisplatin treatment significantly ( $p < 0.05$ ) increased the Malondialdehyde (MDA) (Fig. 2A) levels in kidney tissues of Cisplatin control group. Atorvastatin significantly ( $p < 0.001$ ) attenuated the increase in MDA levels at both doses compared to Cisplatin control group.

### Effect of Atorvastatin on Catalase, Reduced Glutathione:

Table 2 shows the changes in the activities of enzymatic antioxidants Catalase (CAT), reduced Glutathione (GSH) in kidney tissues of control and experimental rats (Table 2). Cisplatin per se treated rats showed significant ( $p < 0.05$ ) decrease in activities of enzymatic antioxidants compared to normal control rats. Treatment of Atorvastatin (3 mg kg<sup>-1</sup> and 10 mg kg<sup>-1</sup>) along with Cisplatin significantly increased the activities of, Catalase and reduced glutathione in dose dependent manner.

**Histopathology:** There was no histological alteration in the kidney morphology of normal (Fig. 1A). Cisplatin control rats showed prominent multiple tubular necrosis, degeneration, inflammatory cell infiltration, vacuolization and loss of architecture of tubules (Fig. 1B). Atorvastatin at 3 mg kg<sup>-1</sup> is showing normal glomerulus with degenerating tubules and Atorvastatin at 10 mg kg<sup>-1</sup> showing predominantly normal glomerulus with occasional degenerating tubules (Fig. 1C and D).

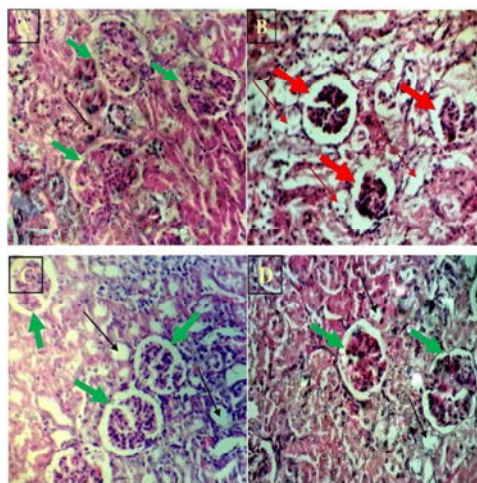


Fig. 1: Photomicrographs of rat kidney tissue 40 X: (A) Normal control group showing normal glomerulus (thick arrows) and tubules (thin arrows). (B) Cisplatin control group well defined degenerating tubular structures with vacuolization and loss of architecture. (C) Cisplatin+Atorvastatin (3 mg kg<sup>-1</sup>) showing normal glomerulus with degenerating tubules. (D) Cisplatin+Atorvastatin (10 mg kg<sup>-1</sup>) showing predominantly normal glomerulus with occasional degenerating tubules

Table 1: Effect of atorvastatin treatment (3 mg/kg/day p.o and 10 mg/kg/day p.o) on kidney function tests of normal control and cisplatin injected rats

Treatment groups	Creatinine (mg/dL)	Blood urea nitrogen (mg/dL)	Total Protein(g/dL)
Group I	0.486±0.040	16.961±0.3641	5.983±0.708
Group II	2.926±0.316***	32.0733±0.8143***	14.066±0.850***
Group III	2.495±0.115**	30.547±0.8246**	12.472±0.651**
Group IV	1.230±0.105***	24.305±0.749***	7.400±0.464***

All values are shown as Mean ± SD and n = 6

\*  $p < 0.05$ -Statistically significant

\*\*  $p < 0.01$ -Statistically very significant (\*\* $p < 0.01$ )

\*\*\*  $p < 0.001$ -Statistically very highly significant (\*\*\* $p < 0.001$ ) in response to Control animals

Table 2: Effect of atorvastatin treatment (3 mg/kg/day p.o and 10 mg/kg/day p.o) on markers of oxidative stress on normal control and Cisplatin injected groups

Treatment groups	Catalase (% H <sub>2</sub> O <sub>2</sub> scavenging activity)	MDA (n moles/mg of tissue)	GSH (μ moles/gm tissue)
Group I	64.161±5.690	16.961± 0.3641	69.307±6.450
Group II	23.624±4.322***	32.0733±0.8143***	17.578±6.666***
Group III	37.521±5.680**	30.547±0.8246**	30.180±4.488**
Group IV	48.722±6.020***	24.305±0.749***	56.801±5.242***

All values are shown as Mean ± SD and n = 6

\*p<0.05-Statistically significant

\*\*p<0.01-Statistically very significant (\*\*p<0.01)

\*\*\*p<0.001-Statistically very highly significant (\*\*\*p<0.001) in response to Control animals

## DISCUSSION

The results demonstrate that daily Atorvastatin treatment markedly ameliorate Cisplatin-induced renal damage as shown in microscopic examination and biochemical parameters. Cisplatin is one of the widely used cytotoxic agent in the treatment of several forms of cancer. In spite of clinical usefulness, drawbacks in using this drug as it causes nephrotoxicity and neurotoxicity. Other less frequent toxic effects like hepatotoxicity, which observed after administration of high doses of Cisplatin, can also modify the clinical situation in patients [12-14]. The mechanism of nephrotoxicity due to Cisplatin nephrotoxicity is the combined result of the carriage of cisplatin into renal epithelial cells, grievance to nuclear and mitochondrial DNA, stimulation of a multiple cell death and survival pathways and commencement of a robust inflammatory response [15]. In the present study, Cisplatin injection produced severe degeneration in glomeruli, proximal and distal tubules, Tubular swelling and necrosis are in line with earlier studies, which show similar findings [16]. The renal function tests such as blood urea nitrogen, serum creatinine and serum protein were elevated in Cisplatin injected animals as compared to normal control group. It is unlikely that increased Reactive Oxygen Species (ROS) production in the renal tissue may be responsible for this damage of the organ as reflected by the change in the levels of MDA and activities of reduced glutathione and Catalase in the study. Additional player in the induction of these changes is depletion of thiol groups in the kidney. Both these effects, in concert, led to the development of Cisplatin-induced nephrotoxicity. It is evident from prior clinical data that Cisplatin administration results in elevation in serum creatinine and serum urea levels because of nephrotoxicity [17, 18]. Statins represent a class of anti-hyperlipidemic drugs that have many pleotropic effects. In the present study,

pretreatment with Atorvastatin was able to counteract Cisplatin-mediated renal damage. Treatment with Atorvastatin improved renal functions. Atorvastatin significantly improved the lesions induced Cisplatin. This is evident from Histopathological findings and by an improvement in serum creatinine, blood urea nitrogen and serum protein values. Since ROS generation mediates Cisplatin nephrotoxicity, it may be anticipated that antioxidant effect of Atorvastatin may be protecting these conditions. Atorvastatin, in particular at higher dose (10 mg kg<sup>-1</sup>), reverses oxidant-antioxidant imbalance, has good hydroxyl scavenging activity and shows a dose dependent antioxidant effect. These effects are to be primarily mediated by up regulation of antioxidant defense protein Heme Oxygenase-1(HO-1) [19]. From this study it was inferred that antioxidant effects of Atorvastatin plays a significant role in amelioration of Cisplatin induced renal damage. Lipid lowering effect of Atorvastatin may also be involved in this mechanism. The major Nephroprotective effect of Atorvastatin thus, mediated by normalization of ROS production. The main limitation for this study was the animal model since it was not done in tumor bearing animals. Further experimental investigations are needed to estimate the role in various cancer models.

## CONCLUSION

The results indicate that Atorvastatin improve biochemical and histological alterations induced by Cisplatin. However Atorvastatin (3 mg kg<sup>-1</sup>) shows more significant renoprotective effect than Atorvastatin (10 mg kg<sup>-1</sup>). These Nephroprotective effects are beyond the lipid lowering effects of statin. Mechanism of this Nephroprotective effect mainly include amelioration of lipid peroxidation induced by Cisplatin as well as activation of defense mechanisms. Hence Atorvastatin is an adjuvant drug to treat nephrotoxicity associated with Cisplatin therapy.

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