

ARE STATINS NEPHROPROTECTIVE?: A DOSE DEPENDENT STUDY IN ALBINO RATS**¹RAJITHA PANONNUMMAL, ²JOYAMMA VARKEY, ³DINOOP D R****¹College of Pharmaceutical Sciences, Medical College, Thiruvananthapuram, Kerala, ²College of Pharmaceutical Sciences, Medical College, Thiruvananthapuram, Kerala, ³KTN College of Pharmacy, Palakkad, Kerala, India. Email: rajithadinoop@yahoo. in***Received: 23 Jan 2013, Revised and Accepted: 01 Apr 2013***ABSTRACT**

Purpose:The purpose of the study was to evaluate the dose dependent effects of atorvastatin against vancomycin induced renal damage. **Methods:**Nephrotoxicity was induced by vancomycin administration at a dose of 200mg/kg twice daily for 7 days. Nephrotoxicity was determined by carrying out renal function tests and is confirmed by renal histopathology. Measurements of antioxidant enzymes were also carried out to determine vancomycin induced alterations in cellular antioxidant status. Atorvastatin at three doses 5mg/kg, 10mg/kg and 20mg/kg was tested for protective effect against renal damage induced by vancomycin due to its antioxidant mechanisms. **Results:**Higher doses of atorvastatin exhibits considerable degree of antioxidant activity and atorvastatin at 10mg/kg provided significant degree of protection. But low dose of atorvastatin was insufficient to provide a protective effect due to inability to restore the altered antioxidant status. **Conclusions:**Atorvastatin at 10mg/kg exhibit significant antioxidant activity and provide optimum level of protection against Vancomycin induced renal damage. But at 5mg/kg it does not show significant antioxidant effect sufficient to provide a protective effect. Atorvastatin at 20mg/kg showed effective antioxidant action but it failed to provide a protective effect in renal damage, may be due to its direct toxic effect on rat kidney.

Keywords: Atorvastatin, Nephrotoxicity, Oxidative stress, Reactive oxygen species**INTRODUCTION**

Nephrotoxicity is the most common and clinically important side effects of a variety of medications that limits their clinical usefulness and is a serious complication of drug therapy or chemical exposure [1]. Renal insufficiency occurs in approximately 1–2% of patients with acetaminophen overdose [2] and the incidence of nephrotoxicity from aminoglycosides has increased from 2 to 3% in 1969 to 20% in the past decades [3]. Thus Nephrotoxicity remains as one of the problematic adverse effects of a various class of medications including anticancer agents like cisplatin, immunosuppressant like cyclosporine, and diagnostic agents like radio contrast media, antibiotics like amino glycosides, vancomycin and amphotericin B and limits their clinical usefulness [4].

The use of Antibiotics can cause renal failure through a variety of mechanisms including direct toxicity to the renal tubules, interstitial nephritis and crystallization of the antibiotics within the renal tubule. Although in most instances the mechanism mediating nephrotoxicity are unclear, susceptibility of the kidney to toxic injury appears to be related at least in part, due to the complexities of renal anatomy and physiology [2]. Renal blood flow is unequally distributed, with cortex receiving a disproportionately high flow compared to medulla and papilla. Therefore a blood borne toxicant will be delivered preferentially to the renal cortex and thereby having a greater potential to influence cortical rather medullary or papillary functions. The process involved in forming concentrated urine, serve to concentrate potential toxicants present in the filtrate. Reabsorptive processes along the neuron may raise the intraluminal concentration of a toxicant from 10–50mM by the end of the proximal tubule, 66mM at the hairpin turn of the loop of Henle, 200mM at the end of the distal tubule, and as high as 2000mM in the collecting duct. Progressive concentration of toxicants may result in intraluminal precipitation of poorly soluble compounds, causing acute renal failure due to mechanical obstructions. Proximal tubular epithelium is leaky than the distal tubular epithelium, this causes passive diffusion of toxicants to the proximal tubular epithelium and selectively injure the proximal tubule. Active transport processes within the proximal tubule may further raise the intracellular concentration of an actively transported toxicant. Certain segment of the nephron has a capacity for metabolic bioactivation of several prototoxicants to active toxicants [5].

Many recent evidence suggest that oxygen derived free radicals and decrease in antioxidant activity may have pathological role in xenobiotics induced nephrotoxicity. ROS have deleterious effects on cellular components; leads to vascular damage and kidney

dysfunctions. A variety of compounds with antioxidant property was found to be effective against nephrotoxicity induced by radiocontrast medias and therapeutic agents such as amino glycosides, cisplatin, cyclosporin and vancomycin [6,7,8,9].

Vancomycin is a glycopeptide antibiotic having potent activity against gram-positive organisms. When the drug was marketed in the 1956, nephrotoxicity was the most prominent side effect of this drug and was partly responsible for its replacement by less toxic semi synthetic anti-staphylococcal penicillins and cephalosporins. The exact mechanism by which vancomycin impairs renal function remains unclear [10]. It is postulated that Vancomycin causes the increased expression of the complement genes (C3 and C4b) and complement activation mediates tissue inflammation. Another postulated mechanism is vancomycin causes increased expression of genes encoding lysosomal proteins such as cathepsins (Ctss, Ctsd, Ctsc, and Ctsl) that degrade the worn out organelles such as mitochondria. Vancomycin treatment reduces mitochondrial respiratory control ratio, stimulate oxygen consumption and increases ATP concentration; support the role of vancomycin as a stimulant of oxidative phosphorylation [11]. Some reports indicate that vancomycin increases the production of ROS and Oxidative stress dysfunction is responsible for the renal damage associated with vancomycin treatment [12].

Atorvastatin is a most commonly and widely prescribed HMG-CoA reductase inhibitor, used for the treatment of hyperlipidemia. The drug lower cholesterol by inhibiting the enzyme HMG-CoA reductase, which is the rate-limiting enzyme of the mevalonate pathway of cholesterol synthesis [13]. Recent studies provide compelling evidence that the clinical benefits of statin therapy may be attributed to mechanisms independent of cholesterol lowering. These pleiotropic effects of statins include anti-inflammatory activity, antioxidant activity, ability to provide plaque stability and prevent platelet aggregation, normalize the sympathetic outflow and provide a favorable coagulation profile, anti proliferative and immunosuppressive properties [14]. Evidence indicates that dyslipidemia plays a role in initiating and sustaining chronic kidney disease and HMG-CoA reductase inhibitors have noticeably improved cardiovascular outcomes in patients lacking significant kidney disease and show promise of doing as much in renally compromised patients [15]. Statin therapy, beside a proven cardio protective effect, seems to develop a nephroprotective effect also in pre-dialysis chronic kidney diseases and in kidney transplant patients [16]. Even though the efficacy of statin treatment in end stage renal disease population is not well established, the

observational and registry data suggest a benefit [17]. Preclinical studies indicate that statins may play a role in modulating renal impairment following aortic or transplantation surgery and improves renal dynamics and conferring a peculiar histological protection at cellular levels after ischaemia[18]. A very few recent preclinical studies reveal that the pleotropic antioxidant effects of statins may contribute protection against xenobiotics induced nephrotoxicity, which may be partly mediated by Reactive Oxygen Species (ROS) and oxidative stress[19]. But some literature indicates that chronic use of statins in high dose may itself leads to direct renal tubular toxicity[20,21].

So Present study was focused to evaluate the role of ROS and oxidative stress in vancomycin induced renal damage. Study also aimed to evaluate whether the pleotropic antioxidant effect of atorvastatin provide any protection against it and if so, is it depend on the dose of atorvastatin used.

MATERIALS AND METHODS

Animals: Wistar rats of either sex, weighing 150-300g were used as the experimental animals. These animals were housed in groups of 3 in polypropylene cages having a stainless steel lid with provision for keeping food and drinking water. Paddy husk was used as the bedding material. The animals were fed on a standard rodent pellet diet and water ad libitum. The animals were maintained under standard conditions of relative humidity, 12 hours light-dark cycle, adequate ventilation and ambient room temperature. All the experiments were carried out between 9.0am to 4.0pm.

Ethical clearance: The study protocol was approved by the institutional animal ethics committee, Medical College, Thiruvananthapuram (IAEC Approval NO. 05/87/2009/ MCT).

Drugs: For the present study, the vancomycin reference sample was supplied by VHB Medical Sciences Limited, Pant Nagar, Uttarakhand. Atorvastatin Reference sample was obtained from Ranbaxy pharmaceuticals, Pvt, Ltd, Punjab.

Grouping of animals and treatments:

A total of 36 albino rats was used for the study. These animals were divided into 2 main groups; I and II and were further divided into sub-groups and were given various treatments as follows.

Group I (12rats): To study the nephrotoxic potential of vancomycin.

- **Group IA (6 rats):** Control group animals and was received distilled water for 7 days (i. p).
- **Group IB (6 rats):** Received vancomycin twice daily for 7 days 200 mg/Kg (i. p).

Group II (24 rats): To study the renal protection by atorvastatin in vancomycin induced nephrotoxicity.

- **Group IIA (6 rats):** Received atorvastatin 5mg/Kg orally for 5days followed by vancomycin 200mg/Kg i. p 12th hourly for 7 days; in combination with atorvastatin 5 mg/Kg orally, followed by Atorvastatin 5mg/Kg orally for 5 more days.
- **Group IIB (6 rats):** Received atorvastatin 10mg/Kg orally for 5days followed by vancomycin 200mg/Kg i. p 12th hourly for 7 days; in combination with Atorvastatin 10 mg/Kg orally, followed by Atorvastatin 10mg/Kg orally for 5 more days
- **Group IIC (6 rats):** Received atorvastatin 20mg/Kg orally for 5days followed by vancomycin 200mg/Kg i. p 12th hourly for 7 days; in combination with Atorvastatin 20 mg/Kg orally, followed by Atorvastatin 20 mg/Kg orally for 5 more days.
- **Group IID (6 rats):** Received vehicle CMC (0.5%) 0.5ml/100g orally for 5 days followed by vancomycin 200mg/kg i. p 12th hourly for 7 days along with CMC orally followed by vehicle orally for 5 more days.

On the last day of the treatment, animals in all Groups were kept for urine collection in metabolic cages; after loaded with water and 24 hr urine volume was collected and the urine samples were subjected to biochemical investigations. Blood samples were collected from individual rats of all groups by retro-orbital bleeding under ether anaesthesia for biochemical investigations. At the end of the study,

all the animals were sacrificed by cervical dislocation. The abdomen was opened and viscera exposed. Kidneys were dissected out, washed with normal saline to remove the blood completely. Water was blotted out on a filter paper. Weight of each kidney was taken using an analytical balance. Right kidney was used for the biochemical investigations. Body weight of animals was recorded prior to the study and at the end of the study. Change in body weight from initial weight was determined in every group and was expressed as a percentage. Right kidney weight was expressed as an organ to body weight ratio. Left kidney was subjected to histopathological investigations.

Biochemical investigations

Preparation of tissue homogenate: 1gm of kidney was sliced, four volumes of a solution containing 140mM potassium phosphate buffer (pH 7.0) were added. The mixture was taken in a clean sterile centrifuge bucket and homogenized to get a 25% tissue homogenate. The homogenate was centrifuged at 15,000 RPM at 4°C for 15 minutes and the supernatant stored at 4°C and used for the antioxidant enzyme assays.

Homogenate protein determination (Biuret Method) [14,22]: Colorimetric determination of the total protein is based on the principle of Biuret reaction (copper salts in an alkaline medium). Protein in plasma or serum forms a blue coloured complex when treated with cupric ions in alkaline solution. The intensity of the blue colour is proportional to the protein concentration. The absorbance of the coloured chelate is measured at 540nm.

Superoxide dismutase assay (Marklund and Marklund1974)[15,16]: SOD catalyzes the dismutation of the superoxide radical into H₂O₂ and elemental oxygen. In this assay, superoxide ions generated by xanthine oxidase during the conversion of xanthine to uric acid and hydrogen peroxide; converts Nitro Blue Tetrazolium (NBT) to NBT-diformazan, which absorbs light at 560 nm. The extent of reduction in the appearance of NBT-diformazan is a measure of SOD activity present in the sample.

Catalase assay (Aebi et al 1984)[17]: Catalase catalyses the decomposition of hydrogen peroxides to water and oxygen, the enzyme activity is dependent on the precise concentration of H₂O₂; in which the decomposition of peroxide is followed spectrophotometrically at 240 nm.

Lipid peroxidation assay (Ohkawa et al 1979)[18]: Thiobarbituric Acid Reactive Substances (TBARS) is a well-established assay for screening and monitoring lipid peroxidation. The unknown MDA containing samples are first reacted with thio-barbituric acid at 95°C for 60 min. The red pigment produced was extracted with n-butanol-pyridine mixture and estimated by the absorbance at 532 nm.

Glutathione content (Modified Beutler et al method)[19]: Reduced glutathione (GSH) interacts with 5-5'-dithiobis 2-nitrobenzoic acid (DTNB) to form the colored product 2-Nitro-5-thiobenzoic acid, which is measured at 412nm.

Histopathological studies: Left Kidney was fixed in 10 % formalin, dehydrated with ascending grades of ethyl alcohol, embedded in paraffin wax, sliced on a rotary microtome, stained with haematoxylin and eosin and histomorphological features were examined under high power.

Statistical analysis

Descriptive statistics had been presented as mean value ± SEM. Statistical analysis was done by using one way ANOVA with post Hoc comparisons using Tukey HSD tests. In one way ANOVA; F test was being the variance ratio test. For significance P value was fixed at < 0.05.

RESULTS

Vancomycin at a dose of 200mg/kg twice daily was used to induce nephrotoxicity in this study. Vancomycin induced nephrotoxicity was evidenced by altered renal function parameters and urine electrolyte measurements. Body weight changes and kidney weight changes also indicate the same. Treatment with vancomycin

significantly increased BUN level to 61.653 ± 2.19 mg/dl. Atorvastatin at 5mg/kg and at 20mg/kg not produce any significant effect on increased BUN. Atorvastatin at 10mg/kg significantly reduced the value to 44.9 ± 1.303 mg/dl ($p=0.0001$). Increased serum creatinine level due to vancomycin treatment was significantly reduced by treatment with atorvastatin at 5mg/kg ($p=0.001$) and 10mg/kg ($p=0.0001$). But atorvastatin at 20mg/kg does not produce any significant effect on increased serum creatinine. Raised serum potassium associated with Vancomycin exposure was reduced by atorvastatin at 5mg/kg ($p=0.277$) and atorvastatin at 10mg/kg ($P=0.005$). But the treatment with atorvastatin at 20mg/kg in Vancomycin exposed animals caused a further elevation of serum potassium level ($p=0.999$). A fall in serum creatinine due to vancomycin use was significantly increased by Atorvastatin at 10mg/kg ($p=0.0001$). But atorvastatin at 5mg/kg and at 20 mg/kg cause no statistically significant effect. Datas are shown in the table 1.

Reduced urine output due to vancomycin nephrotoxicity was improved by atorvastatin at 10mg/kg ($p=0.0001$). But the effect seen with atorvastatin at 5mg/kg and 20mg/kg was not significant.

Elevated urine sodium due to vancomycin use was significantly reduced by atorvastatin at 5mg/kg ($p=0.005$) and at 10mg/kg ($p=0.0001$). But the effect seen with atorvastatin 20mg/kg was not significant. The urine potassium level was not significantly altered by any of the treatments. Datas are shown in table: 2.

Inorder to reveal the role of oxidative stress in vancomycin induced nephrotoxicity and to study the dose dependent antioxidant effect of atorvastatin against it, measurement of antioxidant enzymes and lipid peroxidation product MDA was carried out. The level of antioxidant enzymes SOD(Fig:1), GSH(Fig:2) and catalase(Fig:3) was significantly decreased by vancomycin treatment but lipid peroxidation product MDA(Fig:4) was significantly elevated in this group. Atorvastatin at 10mg/kg and 20mg/kg significantly increases the level of all antioxidant enzymes and reduces the level of MDA significantly. The effect seen with atorvastatin at 5mg/kg in these parameters was not significant.

Vancomycin treatment causes significant changes in bodyweight and kidney weight of animals and atorvastatin at 10mg/kg ameliorate all these changes. Datas are shown in table 3.

Table 1: Blood chemistry of albino rats after various treatments (N=6).

Groupings	TREATMENTS Drug/Dose/route	BUN in mg/dl (values are mean \pm SEM)	Serum creatinine in mg/dl (values are mean \pm SEM)	Creatinine clearance in ml/min (values are mean \pm SEM)	Serum potassium in meq/L (values are mean \pm SEM)
Group 1 _A	Distilled Water 0.08ml/100gi. p	40.467 \pm 0.715	0.467 \pm 0.033	0.4154 \pm 0.0309	4.67 \pm 0.16
Group I _B	vancomycin 200mg/kg i. p	61.653 \pm 2.19**	1.4 \pm 0.0516**	0.0278 \pm 0.002**	10.7 \pm 1.65***
Group II _A	vancomycin 200mg/kgi. p +atorvastatin 5mg/kg p. o	57.433 \pm 1.991	1.083 \pm 0.03 [^]	0.041 \pm 0.02	7.6 \pm 0.5
Group II _B	Vancomycin200mg/kgi. p+atorvastatin 10mg/kg p. o	44.9 \pm 1.303 [#]	0.53 \pm 0.093 [#]	0.2629 \pm 0.032 [#]	5.07 \pm 0.19 [*]
Group II _C	Vancomycin200mg/kgi. p +atorvastatin 20mg/kg p. o	61.277 \pm 1.818	1.147 \pm 0.049	0.0255 \pm 0.003	11.27 \pm 1.21
Group II _D	Vancomycin200mg/kgi. p+CMC0.5ml/100g p. o	64.793 \pm 1.733	1.383 \pm 0.031	0.026 \pm 0.006	14.82 \pm 1.26

#p=0.0001 as compared to Vancomycin treatment Group

*p=0.005 as compared to Vancomycin treatment Group

[^]p=0.001 as compared to Vancomycin treatment Group

**p=0.0001 as compared to control Group

***p=0.002 as compared to control Group

Table 2: Urine chemistry of albino rats after various treatments (N=6)

Groups	Treatments Drug/Dose/route	24 hr urine Volume in ml (values are mean \pm SEM)	urine Sodium in meq/L (values are mean \pm SEM)	urine Potassium meq/L (values are mean \pm SEM)
Group 1 _A	Distilled Water 0.08ml/100gi. p	6.43 \pm 0.23	42.1 \pm 1.53	75.15 \pm 2.02
Group I _B	vancomycin 200mg/kgi. p	3.65 \pm 0.23 [^]	97.93 \pm 2.04**	71.93 \pm 0.47
Group II _A	vancomycin 200mg/kg i. p +atorvastatin 5mg/kg p. o	5.03 \pm 0.14	90.52 \pm 0.58 [*]	70.65 \pm 1.46
Group II _B	vancomycin200mg/kg i. p +atorvastatin 10mg/kg p. o	8.25 \pm 0.96 [#]	62.18 \pm 0.18 [#]	73.83 \pm 1.23
Group II _C	vancomycin200mg/kg i. p +atorvastatin 20mg/kg p. o	3.02 \pm 0.25ml	98.56 \pm 1.23	74.15 \pm 2.0
Group II _D	vancomycin200mg/kg i. p+ CMC0.5ml/100gp. o	3.43 \pm 0.086	99 \pm 1.16	72 \pm 1.0

#p=0.0001 as compared to vancomycin treatment group.

*p=0.005 as compared to vancomycin treatment group.

**p=0.0001 as compared to control Group

[^] p=0.016 as compared to control Group.

Table 3: Body weight and kidney weight changes of albino rats (N=6)

Groups	Treatments Drug/Dose/route	% changes in Bodyweight (values are mean ± SEM)	Kidney weight to body weight ratio in % (values are mean ± SEM)
Group 1A	Distilled Water 0.08ml/100g i. p	3.713 ±0.445	0.314 ±0.009
Group IB	vancomycin 200mg/kg(i. p)	9.787 ±0.490**	0.576 ± 0.054***
Group IIA	vancomycin 200mg/kg(i. p) + atorvastatin 5mg/kg(p. o)	8.282±0.477	0.416 ± 0.031
Group IIB	vancomycin200 mg/kg(i. p)+atorvastatin 10mg/kg(p. o)	5.225±0.342#	0.374±0.023*
Group IIC	vancomycin200mg/kg(i. p)+atorvastatin 20mg/kg(p. o)	10.039±0.595	0.516±0.079
Group IID	vancomycin200mg/kg(i. p) +CMC0.5ml/100g(p. o)	13.43±0.086	0.588±0.035

#p=0.0001 as compared to vancomycin treatment group

*p=0.035 as compared to vancomycin treatment group

**p=0.0001 as compared to control group

***p=0.003 as compared to control group.

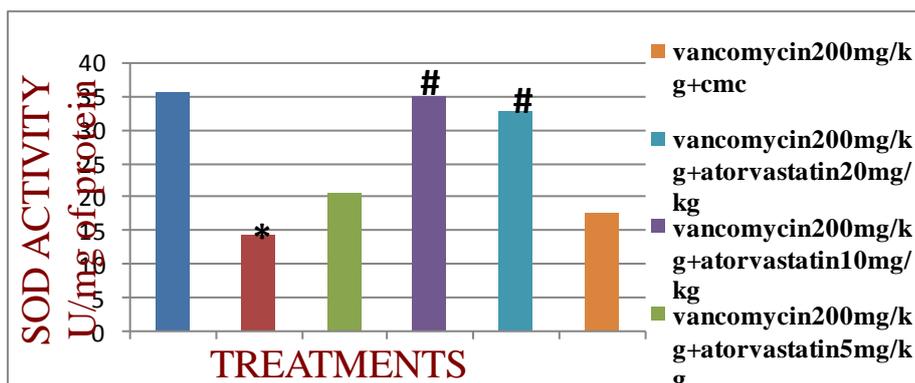


Fig. 1: SOD activity of albino rats given various treatments (values are mean ± SEM).

*p=0.0001 as compared to control group.

#p=0.0001 as compared to vancomycin treated group.

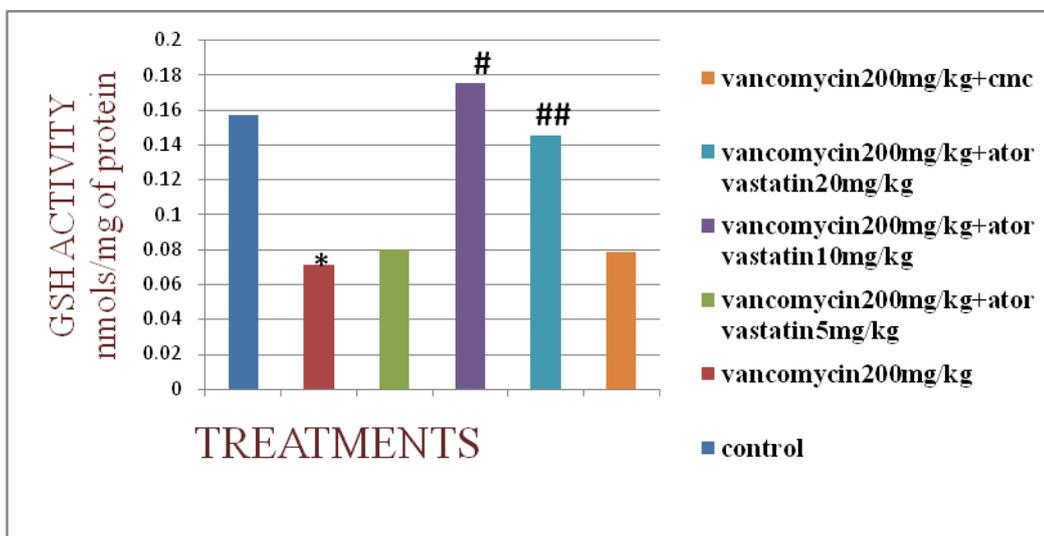


Fig. 2: GSH activity of albino rats given various treatments (values are mean ± SEM)

#p=0.0001 as compared to vancomycin treated group.

##P=0.001 as compared to vancomycin treated group.

*P=0.0001 as compared to control group.

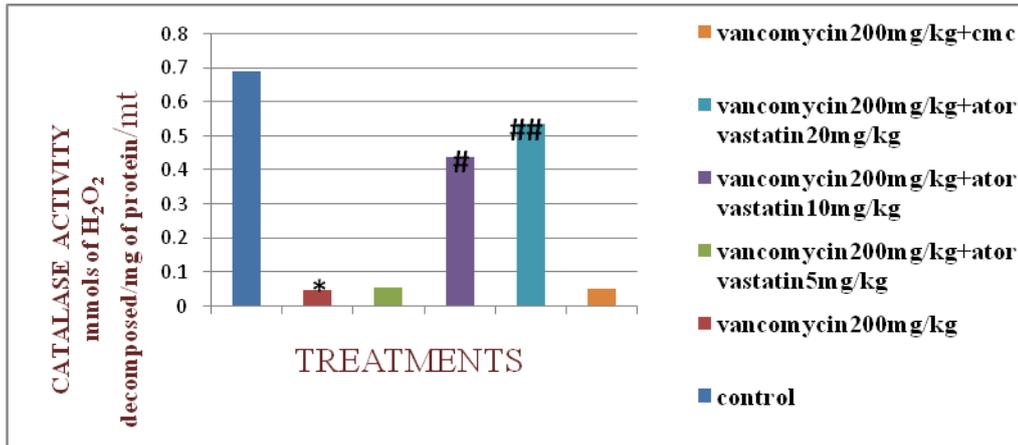


Fig. 3: catalase activity of albino rats given various treatments (values are mean ± SEM).

*p=0.0001 as compared to control group.

#p=0.006 as compared to vancomycin treated group.

##p=0.001 as compared to vancomycin treated group

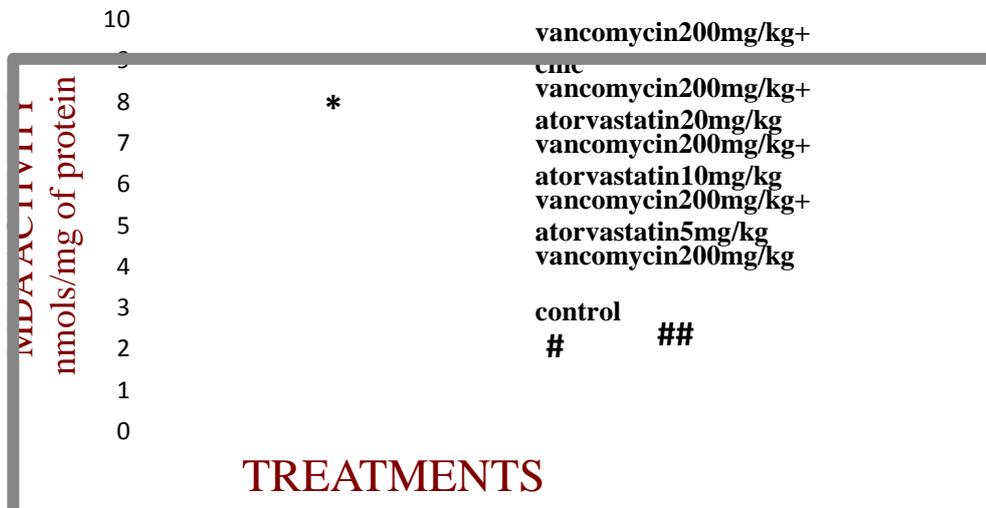


Fig. 4: MDA activity of albino rats given various treatments (values are mean ± SEM).

#p=0.0001 as compared to vancomycin treated group.

##p=0.009 as compared to vancomycin treated group.

*p=0.0001 as compared to control group.

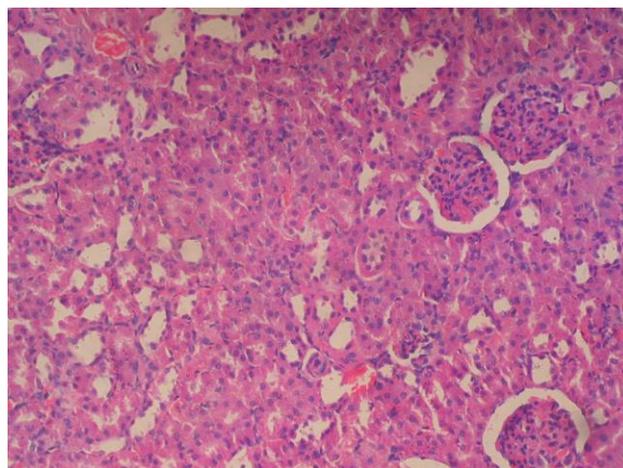


Fig. 5: Normal rat kidney (40 X) showing intact tubules and glomerulai.

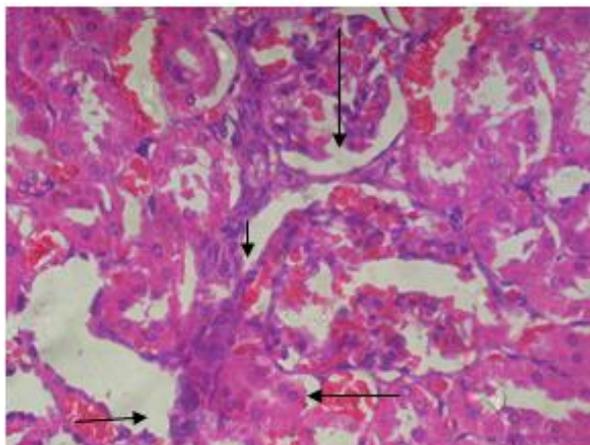


Fig. 6: Rat kidney (40X) after vancomycin treatment showing glomerular congestion, atrophy, nephritis, cloudy swelling, dialation, oedema of tubules.

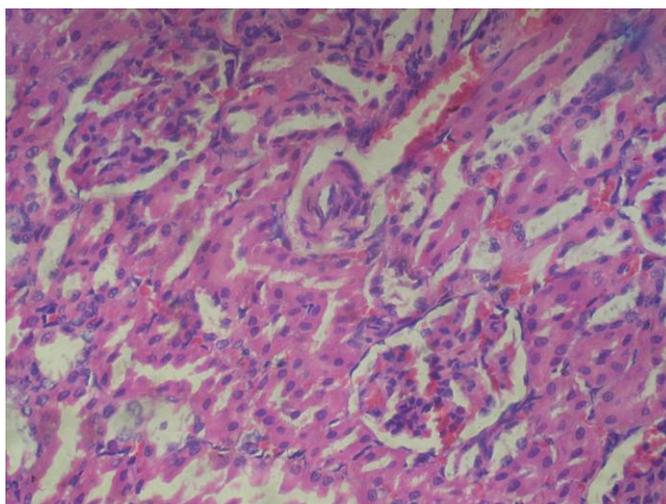


Fig. 8: Rat kidney (40 X) -vancomycin + atorvastatin 10mg/kg treatment showing mild glomerular congestion, no atrophy, and tubules are almost back to normal

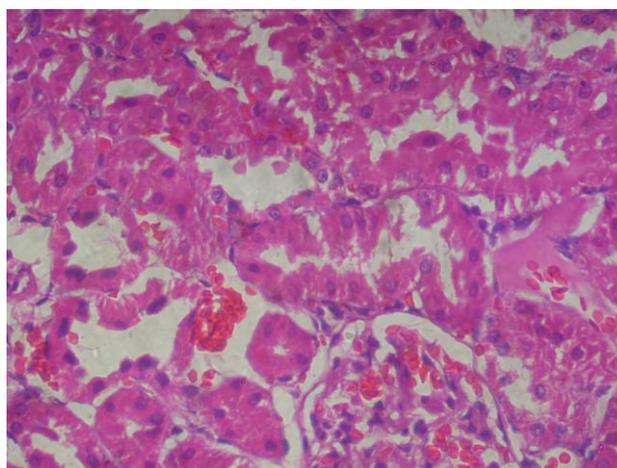


Fig. 9: Rat kidney (40 X) -- vancomycin + atorvastatin 20 mg treatment showing congestion and cloudy swelling of tubules

Vancomycin induced renal damage and effect of different doses of atorvastatin was clearly picturised in the histopathology reports also. Vancomycin treatment results in glomerular congestion, atrophy, nephritis, cloudy swelling, dialation, oedema of tubules of rat kidney (fig:6).

Atorvastatin at 5mg/kg could not produce any significant protection against vancomycin induced pathological changes. (Fig: 7)

Treatment with atorvastatin in a dose of 10mg/kg provided a significant degree of protection by reversing most of the tubular degenerative changes. Tubular cells were almost back to normal. Glomeruli show mild congestion but no atrophy (Fig: 8)

Atorvastatin 20mg/kg failed to provide a protection against vancomycin induced renal damage. Renal histopathology revealed tubular oedema and degeneration, Glomerular congestion and nephritis (Fig: 9,10).

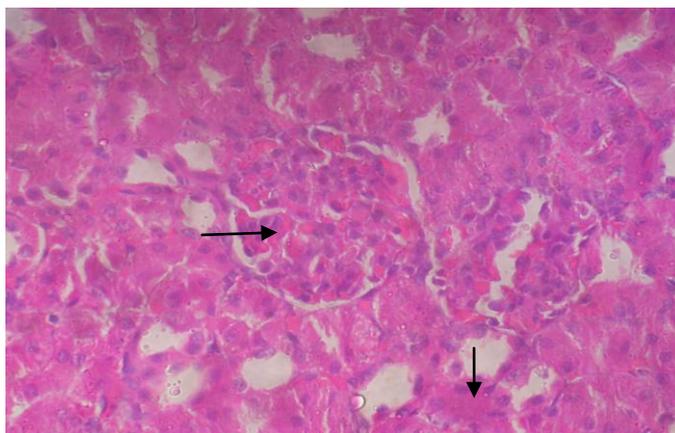


Fig. 10: Rat kidney (40X) --vancomycin + atorvastatin 20mg/kg treatment showing mild glomerular congestion, nephritis and tubular degeneration and oedema

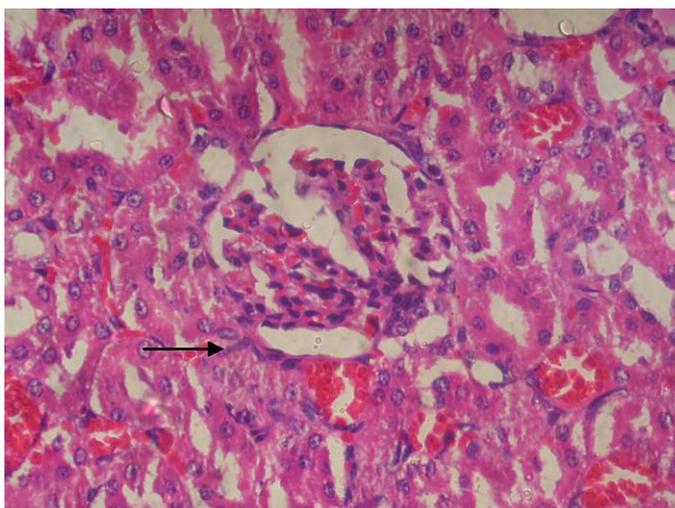
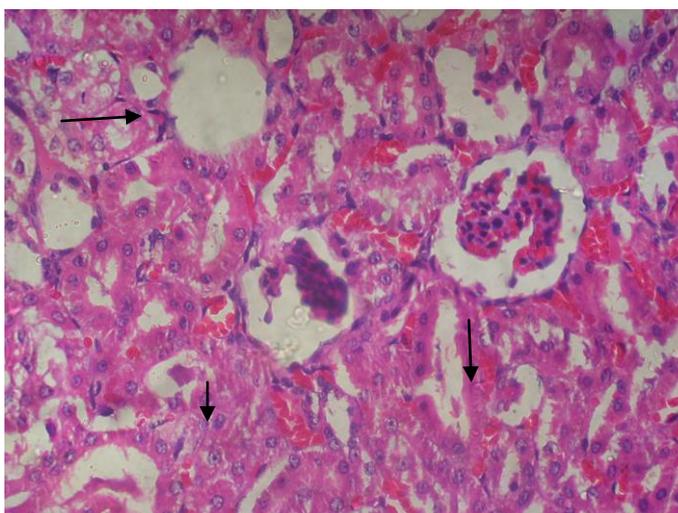


Fig. 11: Rat kidney (40X) -vancomycin + CMC treatment showing glomerular atrophy, tubular vacuolization and necrosis



DISCUSSION

The focus of this study was to reveal the role of vancomycin in ROS production and oxidative stress and subsequent toxicity to rat kidneys and finally the dose dependent effect of atorvastatin in ameliorating vancomycin induced oxidative stress. Experimental studies to explain the protective as well as dose dependent role of atorvastatin in vancomycin induced nephrotoxicity is lacking. Hence the study was undertaken to investigate the role of atorvastatin in

altering the antioxidant enzyme level, urine chemistry and other serum level parameters in vancomycin induced oxidative stress.

Nephrotoxicity induced by vancomycin was evident by raised BUN and serum creatinine and diminished creatinine clearance. A significant decrease in urine output also indicates the nephrotoxicity induced by vancomycin. Urine electrolyte measurements confirm the same. Increased urinary sodium excretion may be the result of impaired sodium reabsorption and inappropriate sodium wasting due to

tubular damage; which may be the result of vancomycin induced acute interstitial nephritis and tubular necrosis. Raised serum potassium level may be the result of acute interstitial nephritis induced by vancomycin use. Marked cloudy swelling of tubular epithelial cells and inflammatory process associated with vancomycin use resulted in increased kidney weight. In our study decreased body weight may be the result of metabolic disturbances associated with nephrotoxicity induced by vancomycin. Damage to the kidneys by vancomycin lead to the accumulation of metabolic wastes such as urea, ammonia which results in generalized fatigue, unpleasant taste in mouth and decreased food intake and severe weight loss.

The Vancomycin mediated renal damage was clearly demonstrated in histopathology reports also. In the present study dialation, vacuolization, degeneration and cloudy swelling of epithelial cells in the tubules, tubular necrosis and congestion, atrophy of glomeruli, glomerular destruction and nephritis was observed in vancomycin treated rats.

Several reports indicate that oxidative stress might be responsible for vancomycin mediated nephrotoxicity. Oxidative stress is an imbalance between free radical production and antioxidant activity. So the measurement of lipid peroxidation products eg: MDA and free radical scavenging antioxidant enzymes like superoxide dismutase (SOD) and catalase, antioxidant protein glutathione (GSH) are good markers for studying the effect of oxidative stress [23,24,25]. SOD catalyzes the conversion of superoxide radicals to hydrogen peroxide (H_2O_2) and H_2O_2 produced by SOD is removed by GSH and catalase.

Vancomycin induced oxidative stress was evident by decreased activity of antioxidant enzyme levels such as SOD, GSH, catalase and by increased production of lipid peroxidation product MDA. The free radicals produced by vancomycin may inactivate SOD and catalase in renal cortex in vancomycin treated rats; as it is well known that peroxynitrite radicals impair SOD activity (MacMillan-Crown and Thompson, 1999) and superoxide radicals inactivate catalase (Rister and Baehner, 1976) [26]. Impaired functioning of antioxidant enzymes by vancomycin would result in unopposed production of free radicals. This ROS causes destructive peroxidation of cell membrane lipids, leads to cell membrane damage and yields a wide variety of lipid peroxidation end products, including MDA (Vardi et al., 2005) [27,28].

Thus the decline in renal functions together with oxidative stress marker measurements indicates that vancomycin treatment at a dose of 200mg/kg twice daily induced kidney injury and ROS play a major role in its development.

In the present study atorvastatin at the dose 5mg/kg just modified the altered renal function parameters and histopathological changes induced by vancomycin. This dose of atorvastatin failed to provide any significant improvement in oxidative stress and antioxidant parameters which is altered by vancomycin use. It is confirmed that atorvastatin at 5mg/kg was failed to provide a significant level of protection on vancomycin induced pathological changes. Histopathological reports also comply with the same. But atorvastatin at 10mg/kg cause significant improvement in antioxidant enzymes SOD, catalase, GSH and thus provides significant level of protection in vancomycin mediated renal damage by restoring the altered antioxidant enzymes and proteins. But the higher dose of atorvastatin (20mg/kg) failed to provide a protection even though it exhibit a significant degree of antioxidant activity. This may be because of the fact that this dose itself exhibits some degree of toxicity to rat kidneys regardless of its antioxidant activity. At higher dose of atorvastatin there occurs a complex interplay between the nephrotoxicity induced by vancomycin and high dose of atorvastatin.

CONCLUSION

In view of the experimental result it may be concluded that oxidative stress may underlie the pathogenesis of vancomycin-induced nephrotoxicity and the protective effect offered by atorvastatin against it was dose dependent. A low dose of 5mg/kg was insufficient to provide a protective effect. A dose of 10mg/kg provides the optimum degree of protection by restoring the antioxidant status and a high dose of 20mg/kg exhibit considerable

degree of antioxidant activity, but failed to provide a protective effect, may be due to direct renal damage associated with this dose.

Further studies using other biomarkers may be needed to confirm the presence of ROS and oxidative stress in vancomycin induced renal damage and to reveal the beneficial role of atorvastatin in it. Studies are also awaited to confirm the mechanism behind direct renal damage associated with high dose of atorvastatin and to reveal the complex interaction between a high dose of atorvastatin with xenobiotics having severe nephrotoxic potential.

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