Journal of

Pharmacology and Toxicology

Nephrotoxicity Reduction by Ceftriaxone plus Vancomycin (Vancoplus) Reconstituted with VRP 1020 in Blood *of Mus musculus* Mice

Arvind Soni, Manu Chaudhary and Vivek Kumar Dwivedi
Intellectual Scientific Division, Venus Medicine Research Centre,
Hill Top Industrial Estate, Bhatoli Kalan, Baddi, HP-173205, India

Abstract: The aim of the present study was to evaluated the effect of the VRP 1020 in reconstitution with fixed dose combination of ceftriaxone-vancomycin (Vancoplus). The mice were fed standard pelleted diet and water *ad libitum*. The test room was air conditioned with temperature 23±20°C, humidity 65±5% and with artificial fluorescent light (10-14 h) of light and dark, respectively. Thirty *Mus musculus* mice (weighing 30±5 g) were divided into 5 groups containing 6 mice in each group. Group I: control (normal saline), group II: ceftriaxone (28.57 mg kg^{-1} body weight/day) group III: vancomycin (14.2 mg kg^{-1} body weight/day), group IV: ceftriaxone-vancomycin (42.8 mg kg^{-1} body weight/day) and group V: ceftriaxone-vancomycin+VRP 1020 (42.8 mg kg^{-1} body weight/day). Present finding showed that activities of antioxidant enzymes (superoxide dismutase and catalase) and pyridoxal-5-phosphate level (biologically most active co-enzyme of vitamin B$_6$) were significantly increased along with decreased in lipid peroxidation (malonaldialdehyde) level in vancoplus treated group as compared to ceftriaxone and vancomycin alone and combination of ceftriaxone-vancomycin treated group. Similarly, the levels of extracellular antioxidant (creatinine and uric acid) were found to be significant lowered in vancoplus treated group when compared to ceftriaxone, vancomycin and ceftriaxone-vancomycin treated group. These results indicated that reconstitution of VRP 1020 with fixed dose combination of ceftriaxone-vancomycin protects against ceftriaxone and vancomycin induced nephrotoxicity that improved the activities of free radical scavenging enzymes.

Key words: Vancoplus, free radical, VRP 1020, antioxidant enzymes, malonaldialdehyde

INTRODUCTION

Antibacterials are the primary cause of nephrotoxicity in all the age groups and these agents brings about renal damages by two mechanism viz., direct and immunologically mediated. For some antibacterials (aminoglycosides and vancomycin) nephrotoxicity is very frequent but generally reversible upon discontinuation of the drug. Aminoglycoside and glycopeptides are still frequently used, either alone or in combination therapy. There are numerous factors intervene in bringing about the kidney damage induced by these two classes of antibacterial, such as factors related to the antibacterial itself and other related to the associated pathology as well as pharmacological factors. Nephrotoxicity can be caused by the β-lactams and its related compounds (Fanos and Cataldi, 1999).

Vancomycin hydrochloride is a glycopeptide antibiotic which is used in the treatment of various infections caused by Gram-positive bacteria. It has been traditionally considered as a nephrotoxic and ototoxic drug, based on observations by early investigators of elevated serum levels in renally impaired patients who had experienced ototoxicity and subsequently through case reports in the medical literature (Appel *et al.*, 1986; Farber and Moellering, 1983; Matzke *et al.*, 1986; Rybak *et al.*, 1990).

Corresponding Author: Dr. Vivek Kumar Dwivedi, Intellectual Scientific Division, Venus Medicine Research Centre, Hill Top Industrial Estate, Bhatoli Kalan, Baddi, HP-173205 India

Several studies have been reported that at high doses of vancomycin (VCM) induces oxidative stress in liver of young Wistar rats (Cam *et al.*, 2006; Öktema *et al.*, 2005).

Ceftriaxone is a broad-spectrum cephalosporin class of antibiotic. As with other β-lactams, the antibacterial activity of ceftriaxone is due to inhibition of mucopeptide synthesis in the cell wall (Beam, 1985; Kojima *et al.*, 1989; Luft *et al.*, 1975). Ceftriaxone possesses a broad spectrum antimicrobial activity including aerobic Gram-positive and Gram-negative bacteria and also against some anaerobic bacteria (Nahata and Barson, 1985; Richards *et al.*, 1984; Anghehrn, 1983). The drug is widely used in the treatment of infections caused by microorganisms resistant to conventional therapy or as an alternative to antibiotics with a low therapeutic index. In combination with vancomycin, ceftriaxone has been shown to be additive or synergistic against several Gram-negative pathogens (Chaudhary *et al.*, 2005; Chaudhary and Shrivastava, 2005; Tripta *et al.*, 2007). The efficacy and safety study of fixed dose combination of ceftriaxone and vancomycin have already been evaluated in experimental models of infections (Francioli and Glauser, 1993; Chaudhary *et al.*, 2008a, b).

Clinical studies with combined cephalosporins and aminoglycosides therapy suggest that a synergistic nephrotoxic interaction was possible between agents in these two classes of antimicrobial drugs. For example, the most compelling evidence supports a synergistic interaction between other cephalothin and gentamicin (Dellinger *et al.*, 1979). The interaction between other cephalosporins-aminoglycosides combination is not of substantial as with cephalothin, but numerous case reports and clinical studies have been suggested the possibility of an enhanced nephrotoxic interaction with such regimens. Numerous factors may increase the likelihood of an apparent synergistic nephrotoxic aminoglycosides-cephalosporidine interaction (Abrutyn *et al.*, 1978). Thus, the present study was planned to reduce the nephrotoxicity and oxidative stress caused by ceftriaxone and vancomycin antibiotics by VRP 1020 (an amino acid) that reduces or eliminate toxicity in blood of *Mus musculus* mice.

MATERIALS AND METHODS

Chemicals

All the biochemicals used in the present study were procured from Sigma, St. Louis, MO, USA. Other chemicals purchased locally were of analytical grade. All the antibiotics such as ceftriaxone, vancomycin, its fixed dose combination vancoplus and VRP 1020 (an amino acid) were obtained from Venus Remedies Ltd. India. The study was carried out from 3rd April to 21st September 2008 in pre-clinical unit of Venus Medicine Research Centre, Venus Remedies Ltd. Baddi (India). The ratio of fixed dose combination of ceftriaxone+vancomycin in vancoplus was 2:1.

Animals and Treatments

Thirty *Mus musculus* mice (weighing 30±5 g) were used in the experiment. The mice were fed standard pelleted diet and water *ad libitum*. The test room was air conditioned with temperature 23±20°C, humidity 65±5% and with artificial fluorescent light (10-14 h of light and dark, respectively). This study was approved by Institutional Animal ethics committee. The mice were divided into 5 groups of 6 mice each as given below:

- **Group-I:** Control (normal saline)
- **Group-II:** Ceftriaxone (28.57 mg kg^{-1} body weight/day)
- **Group-III:** Vancomycin (14.20 mg kg^{-1} body weight/day)
- **Group-IV:** Ceftriaxone-vancomycin (CV) (42.80 mg kg^{-1} body weight/day)
- **Group-V:** Ceftriaxone-vancomycin+VRP 1020 (vancoplus) (42.8 mg kg^{-1} body weight/day)

Dose of VRP 1020, reconstituted with vancoplus was 1 mg kg^{-1} body weight/day. The respective drugs were administered intravenously for 7 days according to body weight after 8 h interval in twice a day. At the end of treatment, 1 mL blood samples were drawn in heparinized vials from the heart by cardiac puncture under the light ether anesthesia. Blood samples were diluted 10 times with chilled distilled water, left for at least 1 h at 0-4°C before the estimation of enzyme assay.

Enzyme Assays
Superoxide Dismutase (SOD) Assay
The reaction mixture composed of 1.0 mL carbonate buffer (0.2 M, pH 10.2), 0.8 mL KCl (0.015 M), 100 µL of blood and water to make the final volume to 3.0 mL. The reaction was started by adding 0.2 mL of epinephrine (0.025 M). The change in absorbance was recorded at 480 nm at 15 sec interval for 1 min at 25°C (UV-1800 SHIMADZU). Suitable control lacking enzyme preparation was run simultaneously. One unit of enzyme activity is defined as the amount of enzyme causing 50% inhibition of auto oxidation of epinephrine (Misra and Fridovich, 1972).

Catalase Assay
The reaction mixture consisted of 0.3 mL phosphate buffer, (0.2 M pH 6.8), 0.1 mL H$_2$O$_2$ (1 M) and water to make the final volume to 3.0 mL. The reaction was started by adding the suitable aliquot of enzyme preparation. The change in the absorbance was recorded at 15 sec interval for 1 min at 240 nm at 25°C. Suitable control was run simultaneously. One unit of enzyme activity was defined as the amount of enzyme that liberates half of the peroxide oxygen from H$_2$O$_2$ in 100 sec at 25°C (Luck, 1965).

Measurement of Lipid Peroxidation
Free radical mediated damage was assessed by the measurement of the extent of lipid peroxidation in the term of malonaldialdehyde (MDA). It was determined by thiobarbituric reaction. The reaction mixture consisted of 100 µL of diluted blood, 0.20 mL of 8.1% sodium dodecyl sulphate (SDS), 1.5 mL of 20% acetic acid (pH 3.5), 1.5 mL of 0.8% thiobarbituric acid (TBA) and water to make up the volume to 4.0 mL. The tubes were boiled in water bath at 95°C for 1 h and cooled immediately under running tap water. Added 1.0 mL of water and 5.0 mL of mixture of n-butanol and pyridine (15:1 v/v) and vortexed. The tubes were centrifuged at 3500 rpm for 30 min. The upper layer was aspirated out and optical density was measured at 532 nm. The reference standard used was 1,1,3,3 tetraethoxypropane (Ohkawa *et al.*, 1979).

Creatinine, Uric Acid, Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT) and Pyrioxdal-5-Phosphate Measurement
These biochemical parameters were estimated by using commercially available standard kit (Bayer Diagnostics India Ltd., Baroda, Gujrat India).

Statistical Analysis
The resulting data were analyzed statistically. All values are expressed as Mean±SD. One-way analysis of variance (ANOVA) with student-Newman-Keuls comparison test was used to determine statistical difference between control and treated groups. A p-value <0.05 was considered statistically significant.

RESULTS

A significant (p<0.001) decreased activity of superoxide dismutase and catalase activities were found in ceftriaxone (21.13, 23.80%) and vancomycin (29.37, 36.49%) treated groups as compared to
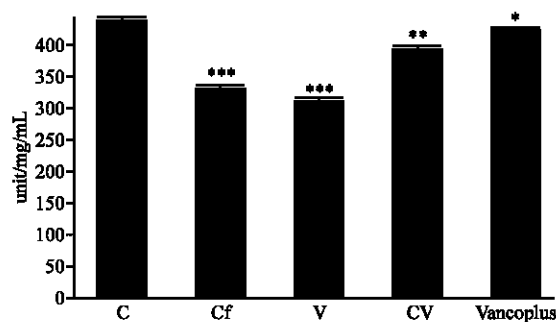
Fig. 1: Effect of ceftriaxone, vancomycin and its FDC on superoxidase dismutase activity in blood of mice. Values are expressed as Mean±SD. C: Control treated group, Cf: Ceftriaxone treated group, V: Vancomycin treated group, CV: Ceftriaxone+vancomycin treated group and Vancoplus: Ceftriaxone-vancomycin+VRP 1020 treated group. ***$p<0.001$ highly significant, **$p<0.01$ significant, *$p<0.05$ considered significant and $p>0.05$ not significant (ns)
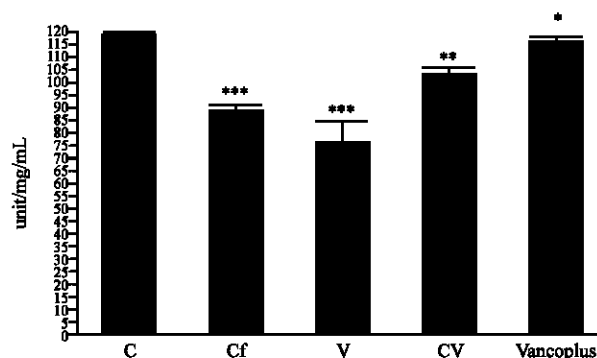

Fig. 2: Effect of ceftriaxone, vancomycin and its FDC on catalase activity blood of mice. Values are expressed as Mean±SD. C: Control treated group, Cf: Ceftriaxone treated group, V: Vancomycin treated group, CV: Ceftriaxone+vancomycin treated group and Vancoplus: Ceftriaxone-vancomycin+VRP 1020 treated group. ***$p<0.001$ highly significant, **$p<0.01$ significant, *$p<0.05$ considered significant and $p>0.05$ not significant (ns)

control group, respectively. The activity SOD found significantly increased in CV ($p<0.01$; 14.21, 23.18%) and vancoplus ($p<0.01$, 19.4, 27.9%) treated groups when compared to ceftriaxone and vancomycin alone treated group. Similarly, the activity of catalase also found increased significantly in CV ($p<0.01$, 13.77, 28.16%) and vancoplus ($p<0.001$, 21.58, 34.58%) treated group as compared to ceftriaxone and vancomycin alone treated group, respectively. A significant ($p<0.05$) increased activities of SOD and catalase was observed in vancoplus treated group (6.16, 9.1%) as compared to CV treated group (Fig. 1, 2).

A significant increased MDA level was found in ceftriaxone ($p<0.01$, 46.31%) and vancomycin ($p<0.001$, 58.81%) treated groups as compared to control group. While level of MDA was significantly decreases ($p<0.001$) in case of CV (23.68, 30.95%) and vancoplus (42.63, 48.09%) treated group, in comparison to ceftriaxone and vancomycin alone treated group, respectively. Its level reduced significantly in case of vancoplus (24.82%) treated group was observed when compared to CV treated group and almost come near to normal level (Fig. 3).
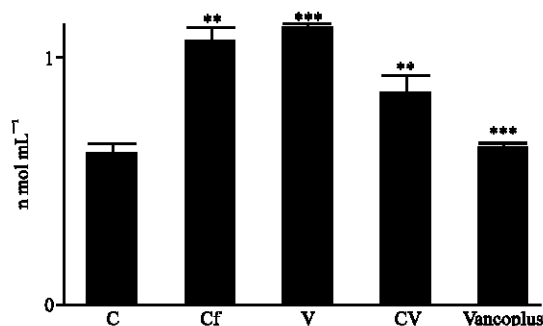
Fig. 3: Effect of ceftriaxone, vancomycin and its FDC on MDA level. Values are expressed as Mean±SD. C: Control treated group, Cf: Ceftriaxone treated group, V: Vancomycin treated group, CV: Ceftriaxone+vancomycin treated group and Vancoplus: Ceftriaxone-vancomycin+VRP 1020 treated group. ***$p<0.001$ highly significant, **$p<0.01$significant, *$p<0.05$ considered significant and $p>0.05$ not significant (ns)
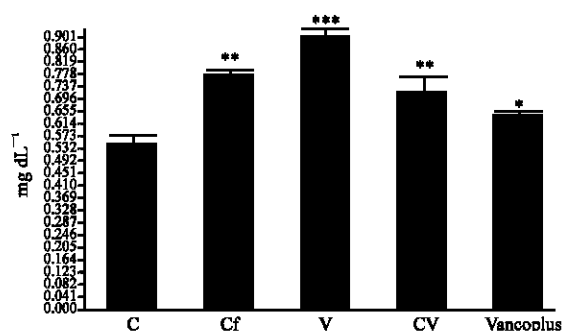

Fig. 4: Effect of ceftriaxone, vancomycin and its FDC on Creatinine level. Values are expressed as Mean±SD. C: Control treated group, Cf: Ceftriaxone treated group, V: Vancomycin treated group, CV: Ceftriaxone+vancomycin treated group and Vancoplus: Ceftriaxone-vancomycin+VRP 1020 treated group. ***$p<0.001$ highly significant, **$p<0.01$significant, *$p<0.05$ considered significant and $p>0.05$ not significant (ns)

A significant increased serum creatinine level was found in ceftriaxone ($p<0.01$, 30.19%) and vancomycin ($p<0.001$, 50.32%) when compared to control group. Serum creatinine level was significantly decreased in CV ($p<0.01$, 10.65, 25.16%) and vancoplus ($p<0.01$, 15.91, 29.50%) treated group when compared to ceftriaxone and vancomycin alone treated group. A significant changes was obtained in serum creatinine of vancoplus treated group ($p<0.05$; 5.02%) as compared to CV treated group (Fig. 4).

The SGOT (32.85, 38.99%) and SGPT (22.05, 35.60%) levels were significantly increased ($p<0.001$) in single administration of ceftriaxone and vancomycin treated group as compared to control group, respectively. Alternatively, in case of CV treated group, the level of SGOT (23.94, 30.90%) and SGPT (12.6, 27.82%) were decreased significant ($p<0.01$) as compared to ceftriaxone and vancomycin treated group. Similarly, in case of vancoplus treated group, the level of SGOT (27.72, 34.34%) and SGPT (18.82, 32.92%) were significantly reduced ($p<0.01$, $p<0.001$) when compared to ceftriaxone and vancomycin alone treated group. A significant ($p<0.05$)
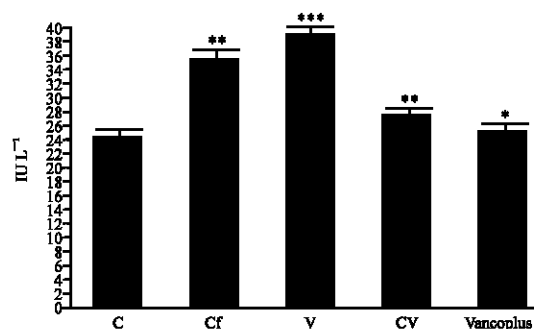
Fig. 5: Effect of ceftriaxone, vancomycin and its FDC on SGOT. Values are expressed as Mean±SD. C: Control treated group, Cf: Ceftriaxone treated group, V: Vancomycin treated group, CV: Ceftriaxone+vancomycin treated group and Vancoplus: Ceftriaxone-vancomycin+VRP 1020 treated group. ***$p<0.001$ highly significant, **$p<0.01$ significant, *$p<0.05$ considered significant and $p>0.05$ not significant (ns)
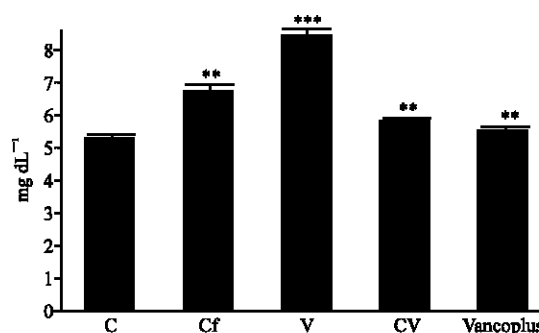

Fig. 6: Effect of ceftriaxone, vancomycin and its FDC on SGPT. Values are expressed as Mean±SD. C: Control treated group, Cf: Ceftriaxone treated group, V: Vancomycin treated group, CV: Ceftriaxone+vancomycin treated group and Vancoplus: Ceftriaxone-vancomycin+VRP 1020 treated group. ***$p<0.001$ highly significant, **$p<0.01$ significant, *$p<0.05$ considered significant and $p>0.05$ not significant (ns)

decreased SGOT (5.0%) SGPT (7.0%) was found in vancoplus treated group as compared to CV treated group and come back near to normal level when compared to control group (Fig. 5, 6).

Uric acid level was found to increased significantly ($p<0.01$; $p<0.001$) in ceftriaxone (32.30%) and vancomycin (38.02%) treated groups as compared to control group. The level of uric acid was reduced significantly in CV ($p<0.01$, 22.0, 28.64%) and vancoplus ($p<0.001$, 28.17, 27.03%) treated groups as compared to ceftriaxone and vancomycin alone treated group. A significant decreased ($p<0.05$) uric acid level was found in vancoplus treated group (7.8%) as compared to CV treated group and back near to normal level when compared to control group (Fig. 7).

A significant decreased level of pyridoxal-5-phosphate (PLP) was observed in vancomycin ($p<0.001$, 28.51%) and ceftriaxone ($p<0.01$, 22.60%) treated groups as compared to control group. PLP level was significantly elevated in CV ($p<0.01$, 14.21, 20.77%) and vancoplus ($p<0.001$, 21.77, 27.03%) treated groups as compared to ceftriaxone and vancomycin alone treated group. PLP level was significant elevated significantly in vancoplus treated group ($p<0.01$, 8.0%) as compared to CV treated group (Fig. 8).
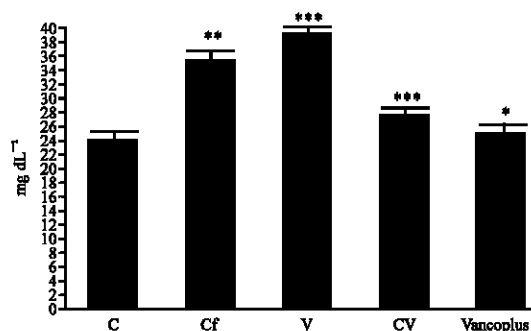
Fig. 7: Effect of ceftriaxone, vancomycin and its FDC on Uric acid level. Values are expressed as Mean±SD. C: Control treated group, Cf: Ceftriaxone treated group, V: Vancomycin treated group, CVbentham publication = Ceftriaxone+vancomycin treated group and Vancoplus: Ceftriaxone-vancomycin+VRP 1020 treated group. ***$p<0.001$ highly significant, **$p<0.01$significant, *$p<0.05$ considered significant and $p>0.05$ not significant (ns)
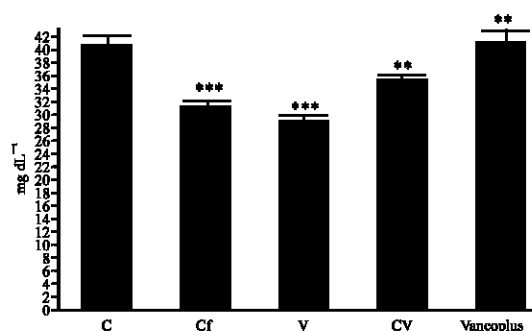

Fig. 8: Effect of ceftriaxone, vancomycin and its FDC on pyridoxal-5-phosphate (PLP) level. Values are expressed as Mean±SD. C: Control treated group, Cf: Ceftriaxone treated group, V: Vancomycin treated group, CV: Ceftriaxone+vancomycin treated group and Vancoplus: Ceftriaxone-vancomycin+VRP 1020 treated group. ***$p<0.001$ highly significant, **$p<0.01$significant, *$p<0.05$ considered significant and $p>0.05$ not significant (ns)

DISCUSSION

Free radical generation and oxidative stress causes nephrotoxicity due to the induction of aminoglycosides. There are several reports on toxic potential of antibiotics. Roy *et al.* (2000) reported that toxic potential of ceftriaxone increases the level of lipid peroxidation in goat (Roy *et al.*, 2000). It has been also reported that single therapy of ceftriaxone antibiotic induces lipid peroxidation level in liver of goat (Chakraborty *et al.*, 2005). Fekety (1990) reported bleeding is probably the most serious side effect of cephalosporins. Ceftriaxone can also causes drug-induced gallstones. The potential of erythromycin and several other macrolides to cause hepatitis is well established (Dellinger *et al.*, 1979).

Vancomycin is manifested by vestibular damage and/or cochlear damage, which leads to sensory hearing loss and tinnitus (Duffull and Begg, 1994). Nephrotoxicity is generally reversible and is believed to result from the accumulation of the drug in renal cells (Creekmore *et al.*, 1998). It has been reported that vancomycin-induced nephrotoxicity were generally higher than the accepted therapeutic

range. However, given drug's dependence on renal elimination, vancomycin concentrations would be expected to be elevated in patients with renal dysfunction (Roy *et al.*, 2000).

The results of the present finding indicated that the single administration of vancomycin and ceftriaxone causes nephrotoxicity and induction of oxidative stress in comparison to ceftriaxone-vancomycin and vancoplus. Similarly, several studies have been also reported that fixed dose combination of cephalosporin and aminoglycosides prevent oxidative stress against tissue injury (Chaudhary *et al.*, 2008a, b). Combination of ceftriaxone and vancomycin has been recommended as a standard choice for initial treatment of presumed pneumococcal meningitis since the mid-1990. Synergistic interaction of this combination was documented in the rabbit model (Friedland *et al.*, 1993) and in the CSF of the children with meningitis (Klugman *et al.*, 1995). FDC of ceftriaxone-vancomycin decreases the erythrocyte sedimentation rate and total leukocyte count after the treatment. FDC of ceftriaxone-vancomycin is very effective in treatment of various bacterial infection of different severity (Chaudhary *et al.*, 2008a, b). Similarly, It has been also reported that fixed dose combination of cephalosporins with aminoglycosides using chemical vector mediated technology acts as an antioxidant and prevents nephrotoxicity induced by aminoglycosides (Dwivedi *et al.*, 2009).

In present study, antioxidant enzymes such as SOD (19.5, 27.91, 6.16%) and catalase (21.6, 34.58, 9.1%) activities were found to be elevated significantly in vancoplus treated group in comparison to ceftriaxone, vancomycin and its ceftriaxone-vancomycin treated groups (Fig. 1, 2). There was significant decreased level in serum aminotransferases namely SGOT (27.7, 34.34, 4.97%) SGPT (19.1, 32.92, 7.0%) in vancoplus treated group as compared to ceftriaxone, vancomycin and its combined therapy of ceftriaxone-vancomycin treated groups (Fig.5, 6). The levels of MDA (42.63, 48.09%) serum creatinine (15.91, 29.56%) and uric acid (22.05, 34.24%) were found to be reduced in vancoplus treated g roup when compared to ceftriaxone and vancomycin alone treated group and reached almost near to control group (Fig. 3, 4, 7). It has been reported that level of pyridoxal-5-phosphate decreases by the administration of aminoglycoside induces nephrotoxicity (Luming *et al.*, 1974).

Yasin *et al.* (2003, 2002) reported that administration of vitamin B6 significantly reduces aminoglycosides induced nephrotoxicity and increases the level of renal pyridoxal-5 phosphate level in rat. Present finding showed that level of pyridoxal-5-phosphate was found to increased in vancoplus treated group (21.6, 27.0, 8.0%) as compared to ceftriaxone, vancomycin alone and CV treated groups (Fig. 8). This increased level of PLP (biologically active co-enzyme of vitamin B_6) prevents renal toxicity. Therefore, reconstitution of VRP 1020 with ceftriaxone-vancomycin increases antioxidant enzyme activity along with decrease level of MDA, uric acid and creatinine levels protects against ceftriaxone and vancomycin induced nephrotoxicity.

Therefore, it is concluded that administration of ceftriaxone-vancomycin reconstituted when with VRP1020 for 7 days, protects and scavenge the free radical generation against single administration of antibiotics induced nephrotoxicity.

ACKNOWLEDGMENT

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