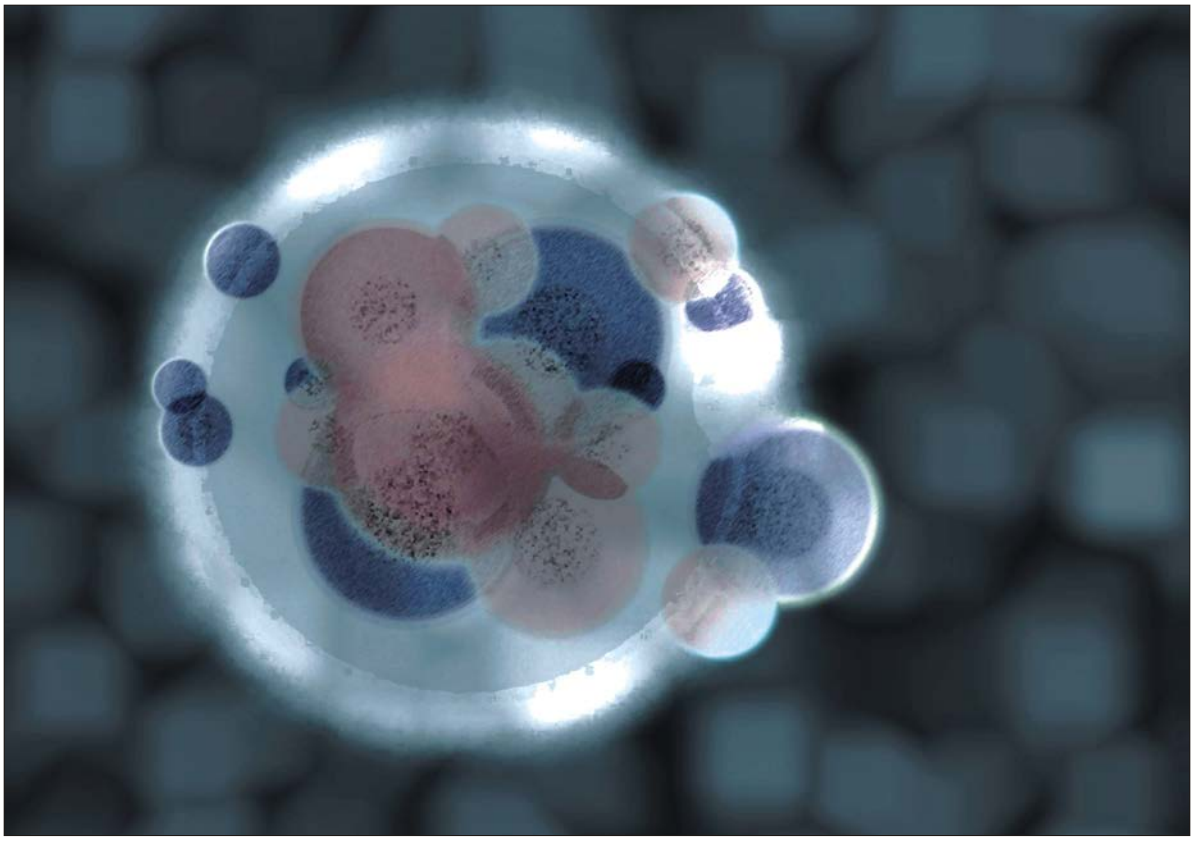

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Efficacy of Vancoplus Against Intra Abdominal Infected Mice: A Novel Fixed Dose Combination of Ceftriaxone Plus Vancomycin

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Abstract: The study was planned to monitor antioxidant and extracellular parameters on different days of interval in blood of infected and treated groups of mice during abdominal infection by treatment of ceftriaxone plus vancomycin (vancoplus). Fifty-four young *Mus musculus* mice were divided into three groups as follows: control group (n = 6), infected group (n = 24) and treated with CV group (n = 24). Intraperitonial injection of *Escherichia coli* (1×10^6cfu) was given for intra abdominal infection. FDC of CV was administered intravenously in the dosage of 42.8 mg kg^{-1} body twice daily for 7 days. The activities of antioxidant enzymes such as superoxide dismutase and catalase as well as the concentration of malonaldialdehyde, as an indicator of lipid peroxidation were measured to evaluate oxidative stress in blood. A significant increased in malonaldialdehyde, creatinine and uric acid levels ($p<0.001$) were found in infected group when compared to control and treated groups. Superoxide dismutase ($p<0.01$) and catalase ($p<0.001$) activities were found to be decreased in infected group as compared to control and treated groups. It can be concluded that ceftriaxone-vancomycin shows broad spectrum against *E. coli* and prevent oxidative damage of tissue injury by reducing reactive oxygen species. These finding suggests that, based on their anti-oxidative capability, these agents could provide benefit in anti-infective therapy and reduces safely relief against intra-abdominal infection.

Key words: Ceftriaxone-vancomycin, intra-abdominal infection, antioxidant enzymes, *E. coli*, malonaldialdehyde

INTRODUCTION

Intraabdominal infections are caused mainly by anastomotic leaks and represent a serious complication. Diagnosis is usually made when patients become critically ill (Kørner *et al*., 2009). The microorgamisms that cause intra-abdominal infections are usually from the gastrointestinal flora, mainly *E. coli* and *Bacteroides fragilis* (Coelho *et al*., 2007). Intra-abdominal infection refers to when a perforation of the intestine results in leakage of the bacteria from the intestine into the normally sterile peritoneal cavity. This may result from appendicitis, diverticulitis, perforated duodenal ulcer or as a complication of abdominal surgery. The infection generally occurs because enteric microorganisms enter the peritoneal cavity through a defect in the wall of the intestine or other viscus as a result of obstruction, infarction, or direct trauma (Brook, 2008). Intra-abdominal infections are among the most difficult infections to diagnose early and treat effectively. A successful outcome depends on early diagnosis, rapid and appropriate surgical intervention and selection of efficacious antibiotic regimens. Mortality rates associated with intra-abdominal infections range from 3.5% in patients with early infection following penetrating abdominal trauma to more than 60% in patients with well-established infection coupled with resultant multiple organ failure. Serpytis and Ivaskevicius (2005) noted that Intra-abdominal hypertension causes visceral organ hypoperfusion, intestinal ischemia and may also lead to bacterial translocation, release of cytokines and production of free oxygen radicals. One of the most exciting and rewarding microbiological observations of the 20th century was the elucidation in the early 1970s of the role human anaerobic endogenous microflora play in infections, especially in intra-abdominal infections (Nichols, 1995). Muftuoglu *et al*. (2006) evaluated that extent of liver injury after the onset of sepsis plus Abdominal Compartment Syndrome (ACS) is more severe than that resulting from either one independently.

Potential pathogens in intra-abdominal infections include facultative gram-negative bacilli such as *Escherichia coli*, *Morganella morganii*, *Proteus*, *Klebsiella* and *Enterobacter* species. *Obligate anaerobes* that need to be considered in intra-abdominal infections include species such as *Bacteroides*,

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Fusobacterium, *Clostridium*, *Peptococcus*, *Peptostreptococcus* and *Lactobacillus*. Facultative gram-positive cocci that may be involved include *Enterococci*, *Staphylococcus* and *Streptococcus* species. The aerobic gram-negative bacillus *Pseudomonas aeruginosa* should also be considered. The organisms most commonly responsible for intra-abdominal infections are *E. coli*, *Enterobacteriaceae* and *Bacteroides fragilis*. The intra-abdominal administration of antibacterial peptide induced accumulation of neutrophils, increased levels of reactive oxygen species and augmented antibacterial activity in the abdominal cavity (Okuyama-Nishida *et al*., 2009).

The early use broad-spectrum antimicrobial like carbapenems, meropenem and imipenem/cilastatin for treatment of intra-abdominal infections reduce mortality and morbidity (Tellado and Wilson, 2005). Khan *et al*. (2005) reported that ceftriaxone, metronidazole and aminoglycoside is less toxic and expensive than ceftriaxone and metronidazole. Fixed dose combination of ceftriaxone-vancomycin protects against ceftriaxone and vancomycin induced nephrotoxicity that improved the activities of free radical scavenging enzymes (Soni *et al*., 2009). Combination of Ceftriaxone-vancomycin was reported for the treatment of various bacterial infections such as serious respiratory tract disease, bronchitis, gastro-intestinal tract infections, urinary tract infection, cellulites and meningitis (Chaudhary *et al*., 2008b). The purpose of antimicrobial agents is to limit persistent or residual peritoneal infection following drainage, prevent wound infection and limit adverse effects of the infection on the host (Mazuski *et al*., 2002). They are able to attack bacterial cells without having an adverse effect on host cells. This property is made possible by biochemical differences between the cells of the host and the infecting organism therefore, the present study was focused on to determine the efficacy and safety profile of ceftriaxone-vancomycin (Vancoplus) on antioxidant enzymes and extracellular oxidant level in *Mus musculus* mice infected by *E. coli* bacterial strain.

MATERIALS AND METHODS

Study conduct: The study was carried out from 10th July to 5th October 2008 in pre-clinical unit of Venus Medicine Research Centre, Venus Remedies Ltd. Baddi (India).

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 and its ceftriaxone-vancomycin reconstituted with chemical vector were obtained from Venus Remedies Ltd. India.

Infection and clinical evaluation of disease: The *Escherichia coli* strain (ATCC 8739) was grown on MacConkey agar (Venus Medicine Research Center, Baddi, HP, INDIA) for 24 h at 35°C. After it was grown, 1×10^{6} *Escherichia coli* bacteria (in a 1 mL volume of saline solution) were injected into the abdominal region of mice.

Animals and treatments: Fifty four *Mus musculus* mice (weighing 25-30 g) were used in the experiment. The mice were fed standard pelleted diet and water *ad libitum*. All mice were housed in a filtered-air environment maintained at 20±2°C. Control (normal saline), infected (by *E. coli*) and vancoplus treated mice were kept in separate cages for the infectivity experiments. This study was approved by Institutional Animal Ethics Committee. The mice were divided into three main groups. Out of which, group II and III further divided according to the days interval given below:

- **Group-I (n = 6) :** Control (normal saline)
- **Group-II (n = 24) :** Infected with *E. coli*
  - **Group II/0 day (n = 6):** Infected with *E. coli*
  - **Group II/3 day (n = 6):** Infected with *E. coli*
  - **Group II/5 day (n = 6):** Infected with *E. coli*
  - **Group II/7 day (n = 6):** Infected with *E. coli*
- **Group-III (n = 24):** CV (Vancoplus) (42.8 mg kg^{-1} b.wt. day^{-1})
  - **Group III/0 day (n = 6):** Infected + treated with CV
  - **Group III/3 day (n = 6):** Infected + treated with CV
  - **Group III/5 day (n = 6):** Infected + treated with CV
  - **Group III/7 day (n = 6):** Infected + treated with CV

The respective drugs were administered intravenous for 7 days according to body weight after 8 h interval twice a day. 0.7 mL blood samples were drawn on 3rd, 5th and 7th days 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: SOD activity was determined by the method of Misra and Fradovich (1972). 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 one minute at 25°C. 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.

Catalase assay: Catalase activity was measured by the method of Luck (1965). The reaction mixture consisted of 0.3 mL phosphate buffer (0.2 M pH 6.8), 0.1 mL H_2O_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_2O_2 in 100 sec at 25°C.

Measurement of lipid peroxidation: Free radical mediated damage was assessed by the measurement of the extent of lipid peroxidation in the term of malonaldialdehyde (Ohkawa *et al*., 1979). It was determined by thiobarbituric reaction. The reaction mixture consisted of 100 µL of 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% Thio Barbituric Acid (TBA) and water to make up the volume to 4.0 mL. The tubes were boiled in water bath at 95°C for one hour 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.

Creatinine, Uric acid, Serum Glutamyl Oxaloacetic Transaminase (SGOT) and Serum Glutamyl Pyruvic Transaminase (SGPT) measurement: These biochemical parameters were estimated by using commercially available standard kit (Bayer Diagnostics India Ltd., Baroda, Gujrat India).

Statistical analysis: The resulting data was analyzed statistically. All values are expressed in 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. p-values <0.05 were considered statistically significant.

RESULTS

Clinical signs of abdominal infection: Although, mortality due to intra-abdominal infection was low, the animal appeared ill. Infected mice showed significant weight loss for several days during infection, which become normalized after treated with vancoplus (Fig. 1a). Body temperature dropped at 1-3 days p. i., then returned to normal base line measure (Fig. 1b).

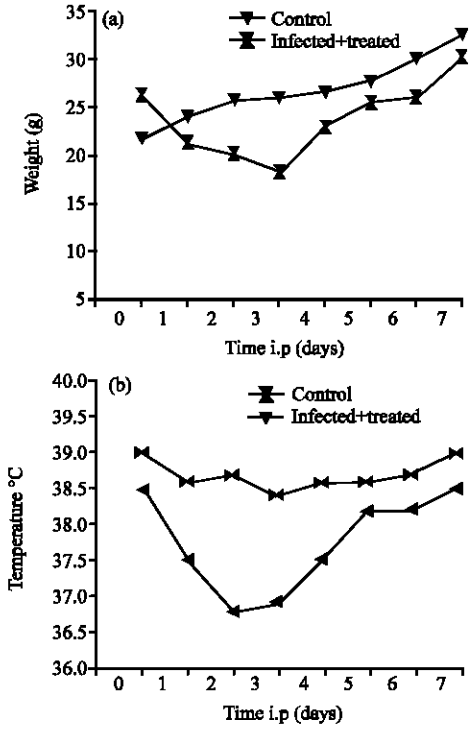

Fig. 1: *Mus musculus* mice infected with *E. coli* bacterial strain experience weight loss and decreased temperature. Serial measurement of (a) weight and (b) temperature were made on days 0-7 after infection and treatment with ceftriaxone-vancomycin in comparison to control group

Antioxidant and extracellular level of infected and treated mice: A significant ($p<0.001$) decreased in superoxide dismutase and catalase activities was found in infected groups as compared to control group in blood. These activities were found to be increased significantly on 3rd, 5th and 7th days of interval after administration in vancoplus group ($p<0.01$) as compared to infected group respectively (Fig. 2, 3).

A significant increased in MDA level in blood was found in infected group ($p<0.001$) as compared to control group. While in case of vancoplus, the MDA level was lowered significantly ($p<0.001$) on 3rd, 5th and 7th days of interval as comparison to day intervals of infected group and almost come near to normal level when compared to control group (Fig. 4).

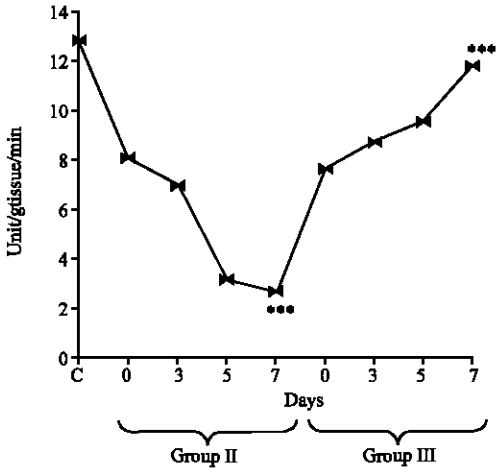

Fig. 2: Effect of vancoplus on superoxidase dismutase activity in blood of mice. Values are expressed in Mean±SD. C = Control group I, group II = Infected and group III = Infected plus treated with vancoplus drug. ***p<0.001 highly significant, **p<0.01 significant, *p<0.05 considered significant and p>0.05 not significant (ns)

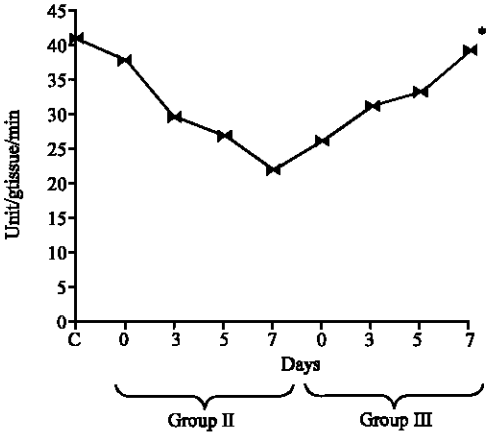

Fig. 3: Effect of vancoplus on catalase activity in blood of mice. Values are expressed in Mean±SD. C = Control group I, group II = Infected and group III = Infected plus treated with vancoplus drug. ***p<0.001 highly significant, **p<0.01 significant, *p<0.05 considered significant and p>0.05 not significant (ns)

Serum creatinine levels were significantly increased (p<0.01; p<0.001) in case of infected group as compared to control group. Serum creatinine level was significantly decreased on 3rd, 5th and 7th days of interval in vancoplus treated group (p<0.01) as compared to days interval of infected group. No significant (p<0.05) change

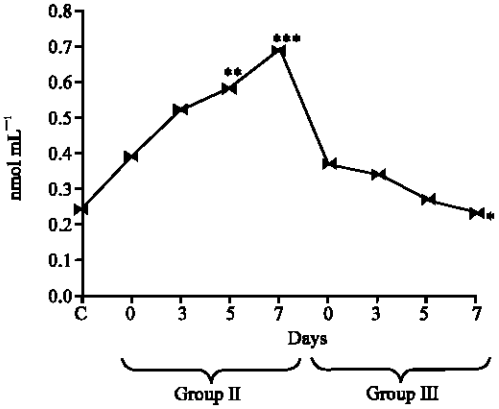

Fig. 4: Effect of vancoplus on MDA level in blood of mice. Values are expressed in Mean±SD. C = Control group I, group II = Infected and group III = Infected plus treated with vancoplus drug. ***p<0.001 highly significant, **p<0.01 significant, *p<0.05 considered significant and p>0.05 not significant (ns)

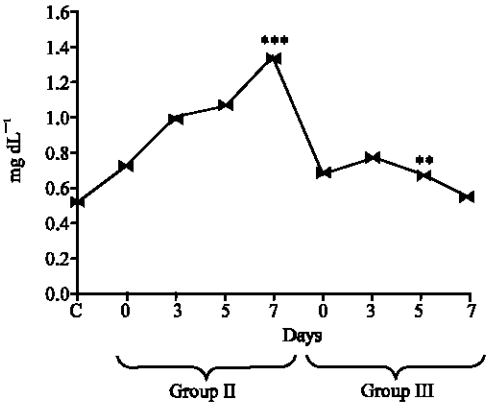

Fig. 5: Effect of vancoplus on creatinine level in blood of mice. Values are expressed in Mean±SD. C = Control group I, group II = Infected and group III = Infected plus treated with vancoplus drug. ***p<0.001 highly significant, **p<0.01 significant, *p<0.05 considered significant and p>0.05 not significant (ns)

was observed in serum creatinine level of vancoplus treated group compared to control group (Fig. 5).

Uric acid level in blood was increased significantly (p<0.01) in infected groups as compared to control group. The level of uric acid was reduced significantly (p<0.001) on 3rd, 5th and 7th days of interval in case of vancoplus (p<0.01) as compared to days interval of infected group. A significantly decreased uric acid level was found in

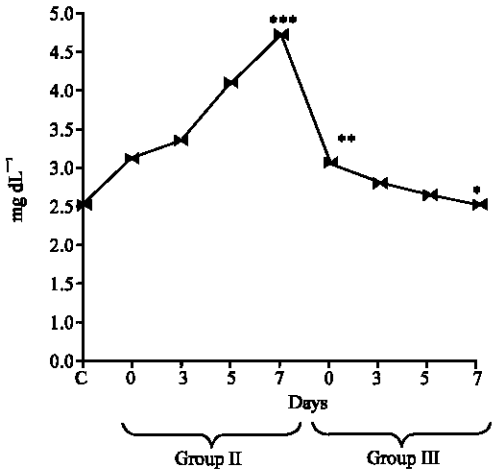

Fig. 6: Effect of vancoplus on uric acid level in blood of mice. Values are expressed in Mean±SD. C = Control group I, group II = Infected and group III = Infected plus treated with vancoplus drug. ***p<0.001 highly significant,**p<0.01 significant, *p<0.05 considered significant and p>0.05 not significant (ns)

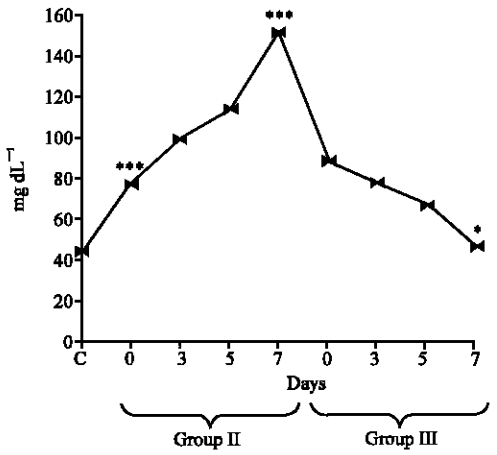

Fig. 7: Effect of vancoplus on SGPT level in blood of mice. Values are expressed in Mean±SD. C = Control group I, group II = Infected and group III = Infected plus treated with vancoplus drug. ***p<0.001 highly significant, **p<0.01 significant, *p<0.05 considered significant and p>0.05 not significant (ns)

vancoplus and almost come near to normal level when compared to control group (Fig. 6).

Serum Glutamyl Oxaloacetic Transaminase (SGOT) and Serum Glutamyl Pyruvic Transaminase (SGPT) levels in liver tissue were significantly increased (p<0.001) on

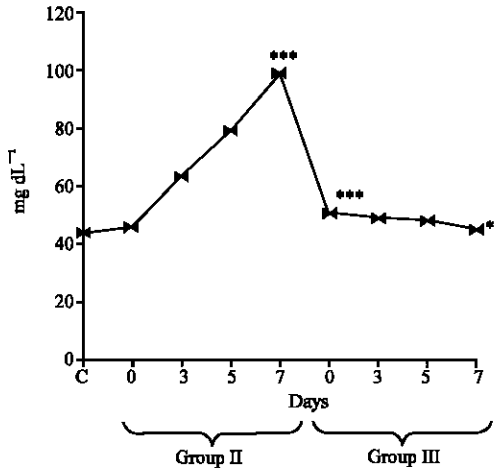

Fig. 8: Effect of vancoplus on SGOT level in blood of mice. Values are expressed in Mean±SD. C = Control group I, group II = Infected and group III = Infected plus treated with vancoplus drug. ***p<0.001 highly significant, **p<0.01 significant, *p<0.05 considered significant and p>0.05 not significant (ns)

3rd, 5th and 7th day of interval in infected group when compared to treated as well as to control group respectively. Alternatively, in case of vancoplus treated group, the level of SGOT and SGPT revert back significantly near to normal level (p<0.05) when compared to control group (Fig. 7, 8).

DISCUSSION

Antibiotics are efficacious because of their property of selective toxicity. They are able to attack bacterial cells without having an adverse effect on host cells. This property is made possible by biochemical differences between the cells of the host and the infecting organism. Combination of ceftriaxone and vancomycin has been recommended as a standard choice for initial treatment of bacterial infection in the mid-1990. Synergistic interaction of this combination was documented in the rabbit model (Klugman *et al*., 1995) and in the CSF of the children with meningitis (Luming *et al*., 1974). It has been reported that fixed dose combination of ceftriaxone-vancomycin decreases the erythrocyte sedimentation rate and total leukocyte count after the treatment (Chaudhary *et al*., 2008b). FDC of ceftriaxone-vancomycin is very effective in treatment of various bacterial infection of different severity.

Barclay and Vega (2003) proposed that community-acquired infections, facultative and aerobic gram-negative

organisms are often implicated in infection beyond the proximal small bowel, whereas infections beyond the proximal ileum can also be caused by a variety of anaerobic organisms. No single- or multiple-agent regimen has been consistently shown to be superior or inferior for community-acquired intra-abdominal infection. Because susceptibility profiles for *Bacteroides fragilis* isolates often show significant resistance to clindamycin, cefotetan, cefoxitin and quinolones, these drugs should not be used alone. In this setting, recommended regimens include meropenem, imipenem/cilastatin, third- or fourth-generation cephalosporins (cefotaxime, ceftriaxone, ceftizoxime, ceftazidime and cefepime) plus metronidazole, ciprofloxacin plus metronidazole and piperacillin/tazobactam. In present study, antioxidant enzymes such as SOD and catalase activities were found to be elevated significantly in treated group in comparison to infected groups (Fig. 2, 3). The levels of MDA, creatinine and urea were found to be reduced in treated group when compared to infected group and reached almost near to control group (Fig. 4-6). No significant changes were observed in the levels of serum glutamyl pyruvic transaminase (Fig. 7) and Serum glutamyl oxaloacetic transaminase (Fig. 8). Cephalosporins consisting of a β-lactam antibiotic and a β-lactamase inhibitor or a β-lactam while glycopeptide inhibit the late stages of peptidoglycan synthesis (Reynolds, 1989). Loll and Axelsen (2000) noted that vancomycin is the D-alanyl-D-alamine terminal dipeptide of peptidoglycan precursors, used by bacteria for constructing their cell walls. This prevents the reaction used to link peptidoglycan precursors together from taking place. So that, this invention provides novel glycopeptides cephalosporin compounds having β-lactam and β-lactamase and glycopeptides inhibitor may be a vital influence in the effectiveness of these combinations. It has been found reported that the most effective ratio of ceftriaxone and vancomycin in its fixed dose combination was 2:1 (Chaudhary *et al*., 2008a).

Oxidative stress resulting from bacterial infection has been reported (Kastenbauer *et al*., 2002; Yin *et al*., 2002). The present study is the first report regarding *E. coli* induced oxidative damage. This infection-induced oxidation could further worsen host self-defence systems. In addition, infection elevated oxidation could enhance the development of inflammatory diseases and increase therapy difficulty. Several studies have indicated that fixed dose combination of antibiotics shows antimicrobial activity against various microbial strain (Kastenbauer *et al*., 2002). Thus, it was found that fixed dose combination of ceftriaxone-vancomycin contributed to the observed oxidation alleviation in this present study via their antioxidant and extracellular level protection in renal and liver tissue of mice. This finding suggests that, based on their anti-oxidative capability, these agents could provide benefit in anti-infective therapy and reduces safely relief against intra-abdominal infection. It was concluded that fixed dose combination of ceftriaxone plus vancomycin prevents the antioxidant level and reduces the free radical generations that provide safety and efficacy against severe abdominal infection.

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REFERENCES

Barclay, L. and C.P. Vega, 2003. IDSA guidelines address antibiotic selection for complicated intra-abdominal infections. Clin. Infect. Dis., 37: 997-1005.

Brook, I., 2008. Microbiology and management of abdominal infections. Dig. Dis. Sci., 53: 2585-2591.

Chaudhary, M., A. Soni, V. Dwivedi and S.M. Shrivastava, 2008a. Fixed dose combination of cefepime plus amikacin prevent oxidative stress in liver of *Mus musculuc* mice. Curr. Clin. Pharmacol., 3: 211-214.

Chaudhary, M., S.M. Shrivastava and R. Sehgal, 2008b. Efficacy and safety study of fixed-dose combination of ceftriaxone-vancomycin injection in patients with various infections. Curr. Drug. Saf., 3: 82-85.

Coelho, J.C., G.A. Baretta and L. Okawa, 2007. Selection and use of anti-infective agents for intra-abdominal infections. Arq. Gastroenterol., 44: 85-90.

Kastenbauer, S., U. Koedel, B.F. Becker and H.W. Pfister, 2002. Oxidative stress in bacterial meningitis in humans. Neurol., 58: 186-191.

Khan, S., D.K. Gupta and D.N. Khan, 2005. Comparative study of three antimicrobial drugs protocol (Ceftriaxone, Gentamicin/Amikacin and Metronidazole) versus two antimicrobial drugs protocol (Ceftriaxone and Metronidazole) in cases of intra-abdominal sepsis. Kathmandu. Univ. Med. J., 3: 55-63.

Klugman, K.P., I.R. Friedland and J.S. Bradley, 1995. Bactericidal activity against cephalosporin-resistant *Streptococcus pneumoniae* in cerebrospinal fluid of children with acute bacterial meningitis. Antimicrob. Agents Chemother., 39: 1988-1992.

Kørner, H., H.J. Nielsen, J.A. Søreide, B.S. Nedrebø, K. Søreide and J.C. Knapp, 2009. Diagnostic accuracy of c-reactive protein for intraabdominal infections after colorectal resections. J. Gastrointest. Surg., (In Press). http://www.ncbi.nlm.nih.gov/pubmed/19479312.

Loll, P.J. and P.H. Axelsen, 2000. The structural biology of molecular recognition by vancomycin. Annu. Rev. Biophys. Biomol. Struct., 29: 265-289.

Luck, H., 1965. Catalase. In: Method in Enzymatic Analysis, Bergmeyer, H.U. (Ed.). Academic Press, New Yark, pp: 885-894.

Luming, L., R.E. Brashear and K.L. Ting, 1974. Pyridoxal 5 phosphate in plasma. Source, protein binding and cellular transport. J. Lab. Clin. Med., 84: 339-348.

Mazuski, J.E., R.G. Sawyer, A.B. Nathens, J.T. DiPiro and M. Schein *et al*., 2002. The surgical infection society guidelines on antimicrobial therapy for intra-abdominal infections: An executive summary. Surg. Infect. (Larchmt)., 3: 161-173.

Misra, H.P. and I. Fridovich, 1972. The role of superoxide anion in the auto-oxidation of epinephrine and a sample assay for Super-oxide dismutase. J. Biol. Chem., 247: 3170-3175.

Muftuoglu, M.A., A. Aktekin., N.C. Ozdemir and A. Saglam, 2006. Liver injury in sepsis and abdominal compartment syndrome in rats. Surg. Today., 36: 519-524.

Nichols, R.L., 1995. Surgical antibiotic prophylaxis. Med. Clin. North Am., 79: 509-522.

Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay of lipid per-oxidation in animal tissue by thio barbutric acid reaction. Anal. Biochem., 95: 351-358.

Okuyama-Nishida, Y., N. Akiyama, G. Sugimori, K. Nomura and K. Ogawa, 2009. Prevention of death in bacterium-infected mice by a synthetic antimicrobial peptide, L5, through activation of host immunity. Antimicrob Agents Chemother., 53: 2510-2516.

Reynolds, P.E., 1989. Structure, biochemistry and mechanism of action of glycopeptide antibiotics. Eur. J. Clin. Microbiol. Infect. Dis., 8: 943-950.

Serpytis, M. and J. Ivaskevicius, 2005. Intra-abdominal hypertension and multiple organ dysfunction syndrome. Medicina (Kaunas)., 41: 903-909.

Soni, A., M. Chaudhary and V.K. Dwivedi, 2009. Nephrotoxicity reduction by ceftriaxone plus vancomycin (Vancoplus) reconstituted with VRP 1020 in blood of *Mus musculus* Mice. J. Pharmacol. Toxicol., 4: 107-116.

Tellado, J.M. and S.E. Wilson, 2005. Empiric treatment of nosocomial intra-abdominal infections: A focus on the carbapenems. Surg. Infect. (Larchmt), 6: 329-343.

Yin, M.C., S.W. Hwang and K.C. Chan, 2002. Nonenzymatic antioxidant activity of four organosulfur compounds derived from garlic. J. Agric. Food Chem., 50: 6143-6147.