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Potentox Reduces Biochemical and Inflammatory Response in Osteomyelitis Infection

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ABSTRACT

The aim of present study was to evaluate the effect of levofloxacin and potentox drugs in osteomyelitis -induced rat model. *Staphylococcus aureus* 100 μL (1×10^6 CFU mL^{-1}) was inoculated into the proximal metaphysis of the tibia for induction of osteomyelitis. Body weight and temperature of osteomyelitis-induced rats were monitored every 4th day for three weeks before and after treatment with levofloxacin and potentox. Levofloxacin and potentox were given to animals by intravenously for 21 days. To determine the biochemical parameters (Protein, CRP, Na, Phosphorus, Calcium levels), hepatic enzymes as well as renal function test along with cytokines and malondialdehyde (MDA), myeloperoxidase (MPO) levels in the serum of osteomyelitis-induced untreated and treated groups. Our results showed that the levels of biochemical parameters as well as renal function test along with malondialdehyde, myeloperoxidase levels were significantly altered in the untreated group as compared to levofloxacin and potentox treated groups. When levofloxacin treated group was compared to potentox-treated group, the renal function test and levels of biochemical parameters along with malondialdehyde, myeloperoxidase levels and cytokines were significantly improved in the serum of potentox-treated group. The hepatic enzyme activity was found to be higher in levofloxacin treated group in comparison to potentox-treated group. These study concluded that potentox act as an antioxidant by improving free radical mediated damage and prevents osteomyelitis infection and hepatoxicity in comparison to levofloxacin, caused by *Staphylococcus aureus* microorganism.

Key words: Levofloxacin, potentox, osteomyelitis, biochemical parameters, MDA, MPO

INTRODUCTION

Osteomyelitis is an infective process in bone and bone marrow. Osteomyelitis, or bone infection, is a major worldwide cause of morbidity. Two predisposing factors are required for the development of osteomyelitis. The first is trauma and second is the introduction of bacteria, which usually occurs via the systematic circulation or directly from open fracture wounds (Nicolau *et al*., 1998; Bramlage and Dvm, 1998). The most common bacterial causes of osteomyelitis are *Staphylococcus aureus* and *Staphylococcus epidermidis*. Acute osteomyelitis presents as a suppurative, or pus-producing, infection accompanied by edema, vascular congestion and small-vessel thrombosis. Necrosis of bone tissue is also an important feature of osteomyelitis. Inflammatory response occurs during osteomyelitis.

Mostly in bacterial infections, proinflammatory cytokines plays a significant role in host defense system against infection (Remick and Friedland, 1997). Several *in vivo* studies of systemic bacterial

infection have demonstrated that tumor necrosis factor (Tracey *et al.*, 1987; Mathison *et al.*, 1988) interleukin-6 (Calandra *et al.*, 1991) and the chemokine, interlukin (IL-8) (Friedland *et al.*, 1992; Hack *et al.*, 1992; Marie *et al.*, 1997) all are important components of the proinflammatory response.

Many antibiotics have been used as empirical therapy for osteomyelitis. Potentox is a fixed dose combination of two antibiotics i.e., cefepime and amikacin. Cefepime is a new parenteral cephalosporin with significant potential advantages over other new cephalosporins and nontraditional β-lactam antibiotics with respect to its antimicrobial activity. Cefepime offers a promising therapeutic action for the treatment either as a component of monotherapy or as fixed dose combination (FDC) therapy. Cefepime shows to have a low affinity for chromosomally derived β-lactamases (Dwivedi *et al.*, 2009). It also shows antimicrobial activity against Gram-positive and Gram-negative pathogens including *Staphylococcus*. It has been reported in several studies that cefepime is useful for the treatment of osteomyelitis infection (Jauregui *et al.*, 1993). Amikacin is aminoglycosides and vital component for the treatment of bone and joint infections (Perry and Pearson, 1991). There are several reports suggesting that amikacin plays a significant role in management of osteomyelitis infection (Rouse *et al.*, 2001). In our knowledge, there is no data on fixed dose combination of cefepime plus amikacin in osteomyeltis infection. The combination therapy can exert synergistic activity against the invading pathogens and can also delay the emergence of resistance strains of bacteria. The standard approach has been used for antibiotic combination, usually a β-lactam and aminoglycosides antibiotic. The present study, tried to determine the safety and efficacy of a fixed dose combination drug (Potentox) in osteomyelitis induced rat model and its comparison with levofloxacin drug.

MATERIAL AND METHODS

Study conduct: The study was carried out in Preclinical division of Venus Medicine Research Centre Baddi, H.P. India from 15th July 2009 to 20th Oct 2009.

Chemicals: All of the biochemical reagents used in the present study were procured from Sigma, St. Louis, MO (USA). Other chemicals purchased locally were of analytical grade. All of the antibiotics such as levofloxacin, cefepime plus amikacin (Potentox) were obtained from Venus Remedies Ltd. India.

Bacterial strain: The *Staphylococcus aureus* strain (MTCC 737) was used for the preparation of osteomyelitis-induced rats. *Staphylococcus aureus* bacterial strain inoculated on nutrient agar slant were grown in septic culture in nutrient broth at 37°C for 24 h. Organisms were harvested and centrifuged at 2348 g for 15 min, washed three times with Phosphate buffer saline and suspended into the phosphate buffer saline (0.2 M, pH 7.0) to obtain the concentration of 1×10^6 cfu mL^{-1}. The concentration of *Staphylococcus aureus* used for the induction of osteomyelitis in the study was 1×10^6 cfu mL^{-1} (Yadav *et al.*, 2003).

Drugs: The concentration of levofloxacin was 5 g kg^{-1}. The concentration and ratio of fixed dose combination of cefepime + amikacin was 2.5 g kg^{-1} and 4:1, respectively.

Osteomyelitis model: Osteomyeltis infection was induced in all animals according to the method of Korksuz *et al.* (1993). For the purpose of this investigation, it was necessary to develop a model

of osteomyelitis-induced rats. Eighteen wistar rats were anesthetized intramuscularly with ketamine (10 mg/100 g of b.wt.) (Ketolar; Parke-Davis, Prat de Llobregat, Spain). The left hind legs were shaved and cleaned with 70% ethyl alcohol. The proximal part of the tibia was exposed anteriorly and a hole was drilled through the cortex into the medullary cavity by using a high speed drill with a 0.6 mm diameter bit. One hundred microliter of bacterial strain was injected through the hole and stainless steel implant (4×1×1 mm) was inserted in to the medullary cavity. The hole was covered with bone wax to prevent bacterial leakage in to soft tissues. The skin was sutured and the animals were then allowed free movement in their cage for three weeks, before treatment began.

Animals and treatments: Total eighteen osteomyelitis -induced wistar rats (weighing 240 to 245 g) used in the experiment were housed at controlled temperature and humidity in an alternating 12 h light and dark cycle with free access to food and water. The study was approved by the institutional animal ethical committee (VEN/EC/PRJ/014). The drugs were given to animals intravenously according to their body weight for three weeks treatment. The rats were divided into three groups of six rats each. Group I is infected plus untreated group; whereas group II and group III are infected plus levofloxacin and infected plus potentox treated groups respectively.

- **Group I** : Infected with *S. aureus* (1×10^6 cfu mL^{-1})
- **Group II** : Infected plus potentox treated group (0.0416 mg/gm/b.wt.)
- **Group III** : Infected plus levofloxacin treated group (0.0083 mg/gm/b.wt.)

One bacterial strain *Staphyloccous aureus* was injected to all groups. After three weeks induction of infection, treatment started with respective drugs for three weeks. Body weight and temperature was monitored at every fourth day in infected and treated groups for three weeks. All the animals were sacrificed on 21st day with a lethal dose of ether. Blood samples were collected in polypropylene tubes.

Serum preparation: Two milliliter of citrate free blood samples were collected and centrifuged at 3500 rpm for 20 min. The supernatant was decanted carefully and stored at 0-4°C at least for 1 h prior to the estimation of biochemical and enzymatic parameters.

Measurement of myleoperoxidase level: Myeloperoxidase level was determined by O-dianisidine method with slight modification (Kurutas *et al*., 2005). The assay mixture consisted of 0.3 mL of sodium phosphate buffer (0.1 M; pH 6.0), 0.3 mL of H_2O_2 (0.01 M), 0.2 mL of O-dianisidine (0.02 M) (freshly prepared) in distilled water and made to a final volume of 3.0 mL with water. The reaction was started by the addition of 0.025 mL serum. The change in absorbance was recorded at 460 nm wavelength. All measurements were carried out in duplicate. One unit of enzyme activity is defined as that giving an increase in absorbance of 0.001 min^{-1}.

Estimation of reduced glutathione: The reduced glutathione level was estimated by the method of Hissin and Hilf (1976). The 0.25 mL of serum was mixed with 3.0 mL of 5% (w/v) TCA reagent and the mixture was allowed to stand for 10 min for the precipitation of protein and filtrate out. The precipitated protein was removed by filtration and the filtrate collected for further study. The 1.0 mL of the filtrate was added to 2.0 mL of 0.3 M phosphate buffer (pH 7.4) and 0.5 mL of DTNB

(1% w/v aqueous sodium citrate). A blank was run simultaneously using distilled water in place of the filtrate. An appropriate standard solution of 0.075 mL GSH (10 μmol) was also run simultaneously. The pale yellow colored developed after 5 min and absorbance was recorded immediately at 412 nm wavelength by spectrophotometer.

Lipid peroxidation level: Free radical mediated damage was assessed by the measurement of the extent of lipid peroxidation in the term of malondialdehyde (MDA) formed, essentially according to Ohkawa *et al.* (1979). It was determined by thio barbituric reaction. The reaction mixture consisted of 0.25 mL of serum preparation, 0.20 mL of 8.1% sodium dodecyl sulphate (SDS), 1.5 mL of (20%, pH 3.5) acetic acid, 1.5 mL of 0.8% thio barbituric acid (TBA) and distilled water to make up the final volume of 4.0 mL. The tubes were boiled in water bath at 95°C for 1 h and cooled immediately under running tap water. This was followed by the addition of 1.0 mL of water and 5.0 mL of mixture of n-butanol and pyridine (15:1 v/v) was added and the mixture was vortexed then the tubes were centrifuged at 3500 rpm for 20 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.

Biochemical parameters: All other biochemical parameters (C-reactive protein, protein, calcium, phosphorus, sodium, hepatic and renal function test) were determined by using a commercially available standard kit (Bayer Diagnostics India Ltd.,Baroda, Gujarat, India).

Cytokines assays: Cytokines parameters such as TNFX, IL-6 were assayed by ELISA Reader (Merck, Serial No. 21041098, MIOS-Jounior) according to manufacturer's instruction.

Statistical analysis: The data obtained was 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 infected *vs* treated groups. p-values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Mortality: Mortality was not seen during experimental study. Swelling symptom was observed in infected group after the inoculation of bacterial strain.

Body weight and temperature: In the present study, the body weight was significantly decreased along with significant increased in temperature (°C) in all of the infected groups. After treatment with levofloxacin and potentox drugs for three weeks, the body weight and temperature (°C) were significantly improved which was measured by the difference in the before and after treatment values of the same in both treated group as compared with infected untreated group. Body weight and temperature (°C) were significantly improved in potentox treated group in comparison to levofloxacin treated group (Fig. 1, 2).

Effect on protein and C-reactive protein level: Protein level was found to be decreased along with increased C-reactive protein level in infected group. After treatment with the two drugs for three weeks, the level of protein was found to be significantly ($p<0.001$) increased along with decreased ($p<0.001$) C-reactive protein levels in both treated group as compared with infected

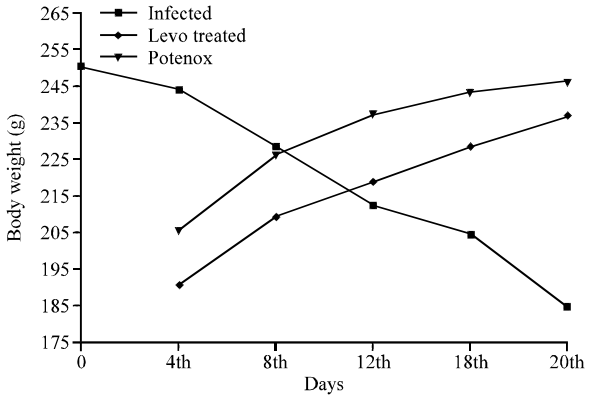

Fig. 1: Values are represented only as mean. Body weight was reduced significantly in osteomyelitis induced group after bacterial infection for 21 days and following treatment of levofloxacin and potentox, body weight increased. Body weight significantly recovered in potentox treated group in comparison to levofloxacin treated group

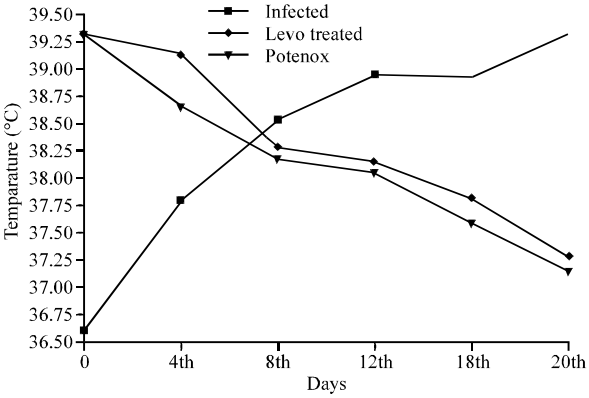

Fig. 2: Values are represented only as mean. Body temperature was increased significantly in osteomyelitis induced group after bacterial infection for 21 days and following treatment of levofloxacin and potentox, body temperature reduced. Temperature recovered significantly in potentox treated group in comparison to levofloxacin treated group

untreated group. When levofloxacin treated group was compared with potentox treated group, the levels of protein and C-reactive protein were improved but did not alter very significantly ($p>0.05$).

Effect on calcium, phosphorus and sodium levels: Calcium and sodium levels were found to be decreased in the serum of infected group as compared to both infected plus treated groups. Whereas the level of phosphorus was increased in the serum of osteomyelitis -induced group in comparison to both treated groups. The level of calcium and sodium was found to be significantly increased ($p<0.001$) in levofloxacin treated group as well as in potentox treated group as compared with infected untreated group after three weeks treatment of respective drugs. However, the level of phosphorus was found to be decreased ($p<0.001$) significantly in both the treated group as compared with infected group. When levofloxacin treated group was compared with potentox treated group, the level of calcium was increased but insignificant in potentox treated group after three weeks treatment of drug. Whereas in case of sodium, the level was significantly increased

Table 1: Status of biochemical parameters and free radical mediate damage (MDA and MPO levels) in osteomyelitis-induced and following treatment of the respective drugs

Parameters	Infected group	Levofloxacin group (L)	Potentox group (P)	L vs. P
	----------(n = 6)----------			
Protein (mg mL^{-1})	7.25±0.25	8.32±0.16^{c}	8.48±0.16^{c}	d
C reactive protein (mg L^{-1})	10.62±0.37	5.22±0.04^{c}	5.18±0.14^{c}	d
Calcium (mg dL^{-1})	7.23±0.11	7.53±0.12^{c}	7.58±0.07^{c}	d
Phosphorus (mg dL^{-1})	6.28±0.2	4.68±0.16^{c}	4.66±0.10^{c}	d
Na level (mg dL^{-1})	159.70±1.69	174.54±2.19^{c}	177.07±2.04^{c}	a
Urea (mg dL^{-1})	57.03±1.35	52.54±2.98^{b}	49.80±1.15^{c}	a
Uric acid (mg dL^{-1})	3.18±0.21	2.07±0.25^{c}	1.96±0.10^{c}	d
Creatinine (mg dL^{-1})	0.56±0.02	0.35±0.02^{c}	0.29±0.03^{c}	c
SGOT level	128.33±17.69	198.67±5.59^{c}	127.83±22.15^{d}	c
SGPT level	48.50±2.5	69.83±4.52^{c}	48.17±4.81^{c}	c
Glutathione (reduced)	0.0128±0.0013	0.0253±0.0009^{c}	0.0299±0.0015^{c}	c
MDA level	1.32±0.24	1.10±0.040^{a}	0.909±0.106^{c}	a
MPO (nmole/min/mL)	10.22±0.67	7.46±0.25^{c}	3.49±0.44^{c}	c

Where N is numbers of animals. All values are expressed as Mean±SD. Significant data are reported between osteomylitis induced group vs. levofloxacin and potentox treated group and levo vs. potentox treated group. Where, $^{d}p>0.05$ (not significant), $^{c}p<0.001$ (highly significant), $^{b}p<0.01$ (significant), $^{a}p<0.05$ (less significant)

($p<0.05$) in potentox treated group. In case of phosphorus, the level was decreased but insignificant in potentox treated group after three weeks treatment when compared with levofloxacin treated group (Table 1).

Effect on urea, uric acid and creatinine: These levels were increased in OM induced groups after inoculation of *Staphyloccous aureus* bacterial strain. When administration of levofloxain and potentox drugs were administered intravenously in infected group for three weeks, the levels of urea, uric acid and creatinine were significantly ($p<0.01$; $p<0.001$) lowered in both treated group as compared to infected untreated group. The levels of urea and creatinine were decreased ($p<0.05$; $p<0.001$) significantly in potentox treated group when compared with levofloxacin treated group after treatment of drugs for 21 days. But in case of uric acid, the level was decreased but insignificant in potentox treated group as compared with levofloxacin treated group (Table 1).

Effect on hepatic enzymes (SGOT and SGPT): Serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) were not altered in osteomyelitis-induced group. After administration of levofloxacin drug for three weeks treatment, these levels were ($p<0.001$) statistically significant increased in the levofloxacin treated group in comparison to infected untreated as well as potentox treated group. While in case of potentox treated group, these levels were lowered but did not alter insignificant ($p>0.05$) in comparison to infected untreated group (Table 1).

Effect on reduced glutathione level: Reduced glutathione level was found to be decreased in untreated infected group. After treatment with the respective drugs for 21 days, the GSH level was found to be significantly ($p<0.001$) increased in both the potentox treated group and the levofloxacin treated group. On comparison of both the treated groups, the level was found to be higher in potentox treated group (Table 1).

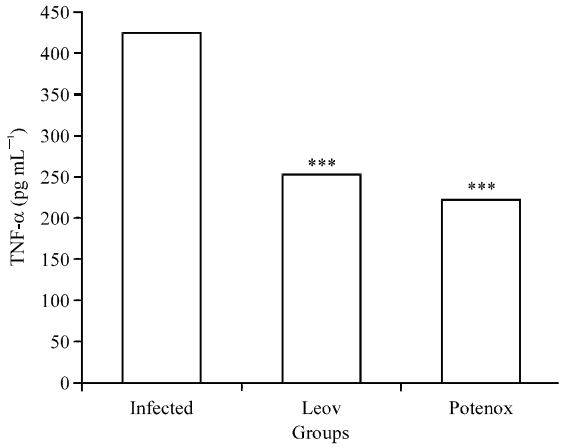

Fig. 3: All values are expressed as Mean±SD. Significant data are reported between infected vs. levofloxacin and potentox treated group and levo vs. potentox treated group. ***p<0.001 (highly significant), **p<0.01 (significant), *p<0.05 (significant) and Ns: p>0.05 (not significant)

Effect on myeloperoxidase level: The level of myeloperoxidase was found to be higher in untreated infected group. This level was found to be decreased significantly in both treated group after treatment of drug for 21 days. When levofloxacin treated group was compared with potentox treated group, the myeloperoxidase level was observed to be highly reduced ($p<0.001$) in potentox treated group (Table 1).

Effect on malondialdehyde level: The level of malondialdehyde (MDA) was increased in osteomyelitis induced group. The level was found to be reduced significantly in both the levofloxacin treated group ($p<0.05$) as well as in potentox treated group ($p<0.001$) after administration of respective drugs for 21 days. When levofloxacin treated group was compared with potentox treated group, the level was found to be significantly reduced in potentox treated group (Table 1).

Effect on TNF-α and IL-6: TNF-α and IL-6 were found to be increased in untreated infected group. The levels of TNF-α and IL-6 decreased significantly in ($p<0.001$; $p<0.001$) levofloxacin treated group as well as in potentox treated group after treatment of 21 days with the respective drugs. When the levels of TNF-α and IL-6 was compared between both treated groups, these levels were found to be significantly ($p<0.01$, $p<0.001$) lowered in potentox treated group (Fig. 3, 4).

Osteomyelitis is an infection of bone and bone marrow with a propensity for progression, usually caused by pyrogenic bacteria or mycobacteria (Kumar *et al.*, 2007). It is an acute or chronic inflammatory process of the bone and bone marrow. Osteomyelitis is a bone infection characterized by progressive inflammatory destruction of the bone, bone necrosis and induction of new bone apposition at the site of infection (Lew and Waldvogel, 1997; Waldvogel *et al.*, 1970).

During osteomyelitis infection, bacterial strain induces local bone destruction (osteolysis) which causes an intense inflammatory response, thrombosis of endosteal and periosteal vessels, bone infarcts with subsequent abscess and sequestrum formation. The pathogenesis of OM is very limited and immune responses in this infection are poorly characterized.

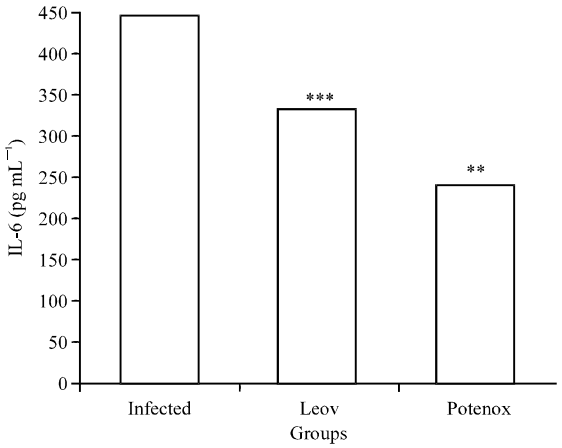

Fig. 4: All values are expressed as Mean±SD. Significant data are reported between infected vs. levofloxacin and potentox treated group and levo vs. potentox treated group. ***p<0.001 (highly significant), **p<0.01 (significant), *p<0.05 (significant) and Ns p>0.05 (not significant)

In the present study, body weight was significantly decreased along with increased body temperature in osteomyelitis-induced group. Lucke *et al.* (2003) reported that there was no alteration in body weight and temperature in osteomyeltis induced rat. Our result showed that there was a significant changes in the body weight and temperature indicating development of bone inflamation due to bacterial infection.

The concentration of TNF-α and IL-6 were elevated in osteomyelitis -induced group. IL-6 is likely to have several actions in osteomyelitis. It has an important role in the acute-phase response (Kopf *et al.*, 1994; Baumann and Gauldie, 1994). Because the acute-phase response in bacterial osteomyelitis, IL-6 cannot be the only mediator but also have a critical role in altering osteoclast and osteoblast function during bone remodeling (Horowitz and Lorenzo, 1996). Bone is remodeled continuously during adulthood through the resorption of old bone by osteoclasts and the subsequent formation of new bone by osteoblasts.

It has been reported that the levels of proinflammatory cytokines, such as IL-6 and TNF- α released by the host in osteomyelitis patients are increased (Evans *et al.*, 1998; Klosterhalfen *et al.*, 1996). Cytokines such as IL-6, TNF- α may be directly involved in bone resorption and osteoclasts 'activity regulation' occurring in osteomyelitis. The levels of protein, calcium and sodium were decreased along with increased phosphorus and C- reactive protein level in osteomylitis-induced group. Calcium, sodium and phosphorus play a significant role in the repair of bone fractures. Due to alteration in calcium and phosphorus levels during bacterial infection, bone becomes fragile. So, calcium and phosphorus levels are essential in regulating the elastic stiffness and tensile strength of bone. Protein level was decreased in bone infection due to loss of bone minerals because cytokine alter the osteoclasts which degraded the bone and its protein components by releasing calcium from bone into circulation. C-Reactive Protein (CRP) is also acute phase protein that causes inflammation during bacterial infection. It has been reported in several studies that the CRP level was increased during bacterial osteomyelitis infection (Evans *et al.*, 1998).

In our study, the levels of urea, creatinine and uric acid were significantly increased in osteomyelitis-induced group. It means that bacterial infection also altered renal enzymes during

osteomyelitis. Various researchers have reported that osteomyelitis due to infection has rarely been associated with glomerular diseases. However, the present study, suggested that glomerulonephritis diseases occur during chronic osteomyeltis caused by *Staphylococcus aureus* infection (Chang *et al.*, 2008). During osteomyelitis infection, the hepatic enzymes (SGPT and SGOT) did not show any alteration. Similar result was reported by Murdoch *et al.* (1996) in vertebral osteomyelitis due to *Staphylococcus lugdunensis*.

Bacterial bone infection causes excessive generation of free radical. It has been reported that oxygen-free radicals play a significant role in the formation and activation of osteoclasts (Garrett *et al.*, 1990). In this study, the level of malondialdehyde and myeloperoxidase levels were significantly increased in infected group in comparison to both the treated groups. It has been reported in several studies that the levels of MDA and myeloperoxidase increase during osteomyelitis (Ersoz *et al.*, 2006).

Many aspects of the antibiotic treatment of osteomyelitis have not been completely investigated. Antibiotics kill bacteria and prevent them from invasive spread, while surgery aims to drain pus, removes necrotic soft and bone tissues and bacterial slime and restores blood supply. Levofloxacin is commonly referred to as a quinolone drug and is a member of the fluoroquinolone class of antibacterials. Potentox is a fixed dose combination of antibiotics i.e., cefepime and amikacin. After treatment of the respective drugs for 21 days, all of the biochemical parameters were improved in levofloxacin-treated as well as potentox-treated groups when compared to infected group. When levofloxacin treated group was compared to potentox treated group, all of the biochemical parameters along with the cytokinin levels and free radical mediated damage (MDA and MPO) levels were improved in potentox treated group after 21 days treatment. For hepatic enzymes, the levels of SGOT and SGPT were significantly increased in levofloxacin treated group. Whereas in potentox treated group, the levels of SGOT, SGPT were decreased but did not alter significantly. This implied that levofloxacin causes major adverse effect in liver during osteomyelitis treatment. Several studies have been reported that levofloxacin causes hepatoxicity and tendon damage during osteomyelitis treatment (Luca *et al.*, 2004). Present result differ from previous study reported that fixed dose combination of cefepime plus amikacin (potentox) prevent hepatoxicity and improved free radical mediated damage during osteomyelitis infection. Potentox have free radical scavenging properties by using chemical vector mediated technology. It has been reported that potentox acts as an antioxidant (Chaudhary *et al.*, 2008). Chemical vector mediated technology is used to provides compatibility of cephalosporins and aminoglycosides without interfering in the pharmacokinetic property of drug component and prevents the oxidation of methionine group and thiazolidine and dihyrothiazine present in antibiotics (Chaudhary and Shrivastava, 2006). On the basis of present findings, it was concluded that potentox has free radical scavenging property which is helpful for the prevention of osteomyelitis caused by *Staphylococcus aureus* infection and also prevent heptotoxicity in comparison to levofloxacin drug.

REFERENCES

Baumann, H. and J. Gauldie, 1994. The acute phase response. Immunol. Today, 15: 74-80.

Bramlage, L.R. and M.S. Dvm, 1998. Infection of bones and joints. AAEP., 44: 148-151.

Calandra, T., J. Gerain, D. Heumann, J. Baumgartner and D.M.P. Glauser, 1991. High circulating levels of interleukin-6 in patients with septic shock evolution during sepsis, prognostic value and interplay with other cytokines. the Swiss-Dutch J5 Immunoglobulin Study Group. Am. J. Med., 91: 23-29.

Chang, I.H., Y.K. Wen and M.L. Chen, 2008. Glomerulonephritis with acute renal failure related to osteomyelitis. J. Chin. Med Assoc., 71: 315-317.

Chaudhary, M. and S.M. Shrivastava, 2006. Minimum inhibitory concentration of ceftriaxone and vancomycin in penicillin resistant *Staphylococcus aureus*, Entrococcus faecalis and Streptococcus pneumoniae. J. Natcon., 18: 177-180.

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

Dwivedi, V.K., A. Soni, M. Chaudhary, C.P. Singh and S.M. Shrivastava, 2009. Fixed dose combination of cefepime plus amikacin (potentox) inhibit pneumonia infection. Exp. Lung. Res., 35: 621-629.

Ersoz, G., B.N. Aytacoglu, N. Sucu, L. Tamer and I. Bayindir *et al.*, 2006. Comparison and evaluation of experimental mediastinitis models precolonized foreign body implants and bacterial suspension inoculation seems promising. BMC Infect Dis., 25: 66-76.

Evans, C.A.W., J. Jellis, S.P.F. Hughes, D.G. Remick and J.S. Friedland, 1998. Tumor necrosis factor-α, interleukin-6 and interleukin-8 secretion and the acute phase response in patients with bacterial and tuberculous osteomyelitis. J. Infect. Dis., 177: 1582-1587.

Friedland, J., S. Suputtamongkol, Y.D.G. Remick, W. Chaowagul and R.M. Strieter *et al.*, 1992. Prolonged elevation of interleukin-8 and interleukin-6 concentrations in plasma and of leukocyte interleukin-8 mRNA levels during septicemic and localized Pseudomonas pseudomallei infection. Infect. Immun., 60: 2402-2408.

Garrett, I.R., B.F. Boyce, R.O. Oreffo, L. Bonewald, J. Poser and G.R. Mundy, 1990. Oxygen-derived free radicals stimulate osteoclastic bone resorption in rodent bone *in vitro* and *in vivo*. J. Clin. Invest., 85: 632-639.

Hack, C.E., M. Hart, R.J. van Schijndel, A.J. Eerenberg, J.H. Nuijens, L.G. Thijs and L.A. Aarden, 1992. Interleukin-8 in sepsis: relation to shock and inflammatory mediators. Infect. Immun., 60: 2835-2842.

Hissin, P.J. and R.A. Hilf, 1976. Flourometric method for determination of oxidized and reduced glutathione in tissue. Anal. Biochem., 74: 214-26.

Horowitz, M.C. and J.A. Lorenzo, 1996. Local Regulators of Bone: IL-1, TNF, Lymphotoxin Interferon-g IL-8, IL-10, IL-4, the LIF/IL-6 Family and Additional Cytokines. In: Principles of Bone Biology, Bilezikian, J.P., L.G. Raisz and G.A. Rodan (Eds.). Academic Press, San Diego, pp: 687-700.

Jauregui, L., D. Matzke, M. Scott, P. Minnsa and G. Hageage, 1993. Cefepime as treatment for osteomyelitis and other severe bacterial infections. J. Antimicrobial Chemother, 32: 141-149.

Klosterhalfen, B., M. Peters, C. Tons, S. Hauptmann, C.L. Klein and C.J. Kirkpatrick, 1996. Local and systemic inflammatory mediator release in patients with acute and chronic posttraumatic osteomyelitis. J. Trauma, 40: 372-378.

Kopf, M., H. Baumann, G. Freer, M. Freudenberg and M. Lamers *et al.*, 1994. Impaired immune and acute-phase responses in interleukin 6 deficient mice. Nature, 368: 339-342.

Korksuz, F., A. Uchida, Y. Shinto, N. Araki, K. Iaouf and K. Ono, 1993. Experimental implant-related osteomyelitis treated by antibiotic-calciu hydroxyapatite ceramic composites. J. Bone Joint Surg., 25: 111-114.

Kumar, V., A.K. Abbas, N. Fausto and R.N. Mitchell, 2007. Robbins Basic Pathology. 8th Edn., Saunders Elsevier, UK., pp: 810-811.

Kurutas, E.B., O. Arican and S. Sasmaz, 2005. Superoxide dismutase and myeloperoxidase activities in polymorphonuclear leukocytes in acne vulgaris. Acta Dermatoven, 14: 39-42.

Lew, D.P. and F.A. Waldvogel, 1997. Osteomyelitis. N. Engl. J. Med., 336: 999-1007.

Luca, L.J., T. Mader and. J.H. Calhoun, 2004. Osteomyelitis in long bones. J. Bone Joint Surg Am., 86: 2305-2318.

Lucke, M., G. Schmidmaiera, S.S. Wildemanna, B. Wildemann, R. Schiller, N.P. Haas and M. Raschke 2003. Gentamicin coating of metallic implants reduces implant-related osteomyelitis in rats. Bone, 32: 521-531.

Marie, C., C. Fitting, C. Cheval, M.R. Losser and J. Carlet *et al*., 1997. Presence of high levels of leukocyte- associated interleukin-8 upon cell activation and in patients with sepsis syndrome. Infect. Immun., 65: 865-871.

Mathison, J., E.C. Wolfson and J. Ulevitch, 1988. Participation of tumor necrosis factor in the mediation of gram-negative bacterial lipopolysaccharide-induced injury in rabbits. J. Clin. Invest., 81: 1925-1937.

Murdoch, D.R., R.J. Everts, S.T. Chambers and I.A. Cowan, 1996. Vertebral osteomyelitis due to *Staphylococcus lugdunensis*. J. Clin. Microbiol., 34: 993-994.

Nicolau, D.P., L. Nie, P.R. Tessier and C.H. Nightingale, 1998. Prophylaxis of Acute osteomyelitis with absorbable ofloxacin-impregnated beads. Antimicrob. Agents. Chemother., 42: 840-842.

Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.

Perry, C.R. and R.L. Pearson, 1991. Local antibiotic delivery in the treatment of bone and joint infections. Clin. Orthop. Relat. Res., 263: 215-226.

Remick, D. and J.S. Friedland, 1997. Cytokines in Health and Disease. 2nd Edn., Marcel Dekker, New York.

Rouse, M.S., K.E. Piper, Y. Khaliq, J.M. Steckelberg and R. Patel, 2001. Treatment of *S. aureus* experimental osteomyelitis with matrix III delivered amikacin or clindamycin. Antimicrob Agents Chemother, 41: 1145-1145.

Tracey, K.J., Y. Fong, D.G. Hesse, K.R. Manogue and A.T. Lee *et al*., 1987. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. Nature, 330: 662-664.

Waldvogel, F.A., G. Medoff and M.N. Swartz, 1970. Osteomyelitis a review of clinical features therapeutic considerations and unusual aspects. 3. Osteomyelitis associated with vascular insufficiency. N. Engl. J. Med., 282: 316-322.

Yadav, V., S. Sharma, K. Harjai, H. Mohan and S. Chhibber, 2003. Induction and resolution of lobar pneumonia following intranasal instillation with *Klebsiella pneumoniae*in mice. Ind. J. Med. Res., 118: 47-52.