

Research Article



Linezolid Therapy and its Associated Complications in Methicillin-Resistant *Staphylococcus aureus* (MRSA) Infected Diabetic Rats

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ABSTRACT

Linezolid is an empirical choice of diabetic methicillin-resistant *Staphylococcus aureus* (MRSA) soft tissue infections. The study focused on linezolid therapy associated risk in type 2 diabetes mellitus. The type 2 diabetes mellitus was induced by a high-fat diet (HFD-58% calories fat) for 2 weeks and rats were intraperitoneally injected with streptozotocin (STZ) 35 mg/kg. The neutropenic rats were injected subcutaneously with 10^6 CFU/ml of MRSA. Linezolid was administered orally at a dose of 50 mg/kg twice daily for 14 days. At the end of the study of hematological, biochemical, enzymatic parameters, antioxidants and histopathological studies were performed. The diabetic rats showed the decreased levels of intestinal alkaline phosphatase (IAP), elevated levels of blood glucose, lipid profile, lactic acid, and alteration in hematological parameters, pancreatic damage, and oxidative stress. Linezolid treated infected rats showed hypoglycemia, myelosuppression, decreased IAP, oxidative stress, increase lactic acid, lactate dehydrogenase (LDH) aspartate transaminase (SGOT) and alanine transaminase (SGPT) Linezolid treated diabetic rats effectively (9.69×10^3 CFU / abscess bacterial count) acts against the diabetic MRSA infections, but it causes anemia, lactic acidosis, hepatotoxicity, oxidative stress, and pancreatic damage. While administering linezolid to diabetic infected persons might be careful and should monitor the blood glucose, lactic acid, liver markers and RBC level.

Keywords: High-fat Diet; Streptozocin; Linezolid; IAP; methicillin-resistant *Staphylococcus aureus*.

INTRODUCTION

Skin and soft tissue infections (SSTI) have principal problems of public health in the world. The most important pathogenic agents are *Staphylococcus*, *Enterococcus*, *Pseudomonas*, and *Streptococcus*. The diabetic soft tissue infections such as foot infections, cellulitis, lymphangitis, urinary tract infections, and surgical site infections are due to methicillin-resistant *Staphylococcus aureus* (MRSA), a predominant pathogen¹⁻³. SSTI-associated complications were more common in diabetes because high blood sugar levels can weaken the patient's immune system defenses. Patients with diabetic infections were requiring prolonged hospitalization and repeated admission. The antibiotics available to treat MRSA infections are limited. Linezolid, tigecycline, clindamycin, vancomycin, and daptomycin showed efficacy, safety, clinical cure and eradication rates in MRSA-caused SSTI⁴. The controlled trials showed linezolid have superior over vancomycin in the eradication of MRSA infections and clinical success⁵⁻⁷.

Linezolid (LZD) is a synthetic oxazolidinone antibiotic. It interferes with bacterial growth by inhibiting bacterial protein synthesis at an early stage. Linezolid has 100% bioavailability, when given orally and allows conversion to oral therapy when the patient is clinically stable. It reduces the length of hospital stay, cost, intravenous infections and increased patient convenience⁸. Lzd has no risk of resistance and adverse outcomes with concomitant use of medicines. Linezolid is a relatively safe antibiotic when given for short periods. It is well tolerated, with myelosuppression, mitochondrial toxicity being the most

serious adverse effect⁹. So, our research elucidated the LZD therapy-associated complications in diabetic MRSA infection.

MATERIALS AND METHODS

Drugs and chemicals

Linezolid was obtained from Sigma-Aldrich Chemical Pvt Limited, India. All other drugs and chemicals used in the study were obtained commercially and were of analytical grade. For estimation of biochemical tests, kits were obtained from Ecoline, Manufactured by Merck Specialties, Private limited, Ambarnath.

Experimental animals

Wistar rats (120-150 g) were maintained in clean, sterile, polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 25 ± 2 °C and relative humidity of 30-60 %. A 12:12 h light and the dark cycle were followed. The animals had free access to food and water ad libitum. All the experimental procedures and protocols used in this study were reviewed by the Institutional animal ethics committee (688/2/CPCSEA) and were in accordance with the guidelines of the IAEC. Animal care is taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Microorganism

The clinical isolate of MRSA with positive in both Pantone-Valentine leukocidin (Pvl) toxin and methicillin-resistance (*mecA*) gene was obtained from the ARM laboratory.



Determination of MIC using broth microdilution assays

Microbroth dilution assays were performed according to a Clinical and Laboratory Standards Institute guideline (CLSI)¹⁰. A single colony was transferred and incubated overnight at 37°C with Mueller Hinton broth. The diluted culture was measured for the absorbance at OD₆₂₀ to get a concentration of 1×10^6 CFU/ml. A total of 100 of the freshly diluted cultures and 10 of the serially diluted antibiotics, ranging from 1,000 mg/liter to 1 mg/liter, were dispensed into 96-well culture plates. The plates were incubated at 37°C for 16 to 18 h. The last well in the series without any visible growth was read as MIC.

Experimental design

The animals are divided into 7 groups (n=10). Experimental diabetes was induced by rats fed with a high-fat diet (58 % calories as fat) for 2 weeks. Then, rats were treated with single intraperitoneal injection of 35 mg/kg of STZ¹¹. Diabetic rats were selected on 14 days after STZ injection. Diabetic rats were rendered neutropenic by intraperitoneal cyclophosphamide injections given for 4 days and 5th day at a dose of 150 mg/kg and 100 mg/kg, respectively. This neutropenia was maintained for 5 days. The neutropenic rats were injected subcutaneously with 0.2 ml of the MRSA suspension containing approximately 10^6 CFU/ml in 5% hog gastric mucin¹². Animals received treatment of LZD starting 2 hours post infection. LZD was administered orally at a dose of 50 mg/kg twice daily for 14 days¹³.

Group I: Normal control (N)

Group II: Diabetic rats (HFD+ STZ) (D)

Group III: Infected rats (I)

Group IV: Diabetic and infected rats (D+I)

Group V: Infected rats treated with linezolid (I+LZD)

Group VI: Diabetic and infected rats treatment with linezolid (D+I+LZD)

Group VII: Diabetic controlled (glibenclamide 600 µg/kg) and infected rats treatment with linezolid (D(c)+I+LZD)

The group II and IV, VI and VII animals were treated with HFD-STZ after the 33rd day of induction animals were infected with MRSA except for group I and II. Blood samples were collected from the tip of the tail on 0, 4, 8, 12 and 15th day after infection by without sacrificing the animals, from the tail vein by snipping off the tip of the tail and blood glucose was checked by glucometer. On the 48th day, animals were anesthetized and blood samples were collected into EDTA added vials for hematological study and into dry non-heparinised tubes for serum biochemical profile, and the serum was separated by centrifuging at 3000 rpm for 15 min. The abscesses were excised, added to 2 ml of heart infusion broth, and homogenized. The number of viable organisms in the abscess (the number of CFU per abscess) was determined by a standard plate procedure with mannitol salt agar.

Histopathological studies

The pancreas was excised after sacrificing the animals were immediately washed with buffer and fixed in 10 % buffered formalin. They were dehydrated in graded alcohol, cleared in xylene and embedded in paraffin wax. Sections of 5–6 µm thickness were cut using microtome and stained with hematoxylin-eosin. The histopathological changes were examined under the microscope at the magnification of 10 and 40X.

Preparation of liver homogenate

The liver was homogenized in 0.05 M phosphate buffer pH 7.4 by using a Teflon pestle. The homogenate was used for estimation of a protein¹⁴, Malondialdehyde and lipid peroxidation¹⁵. The remaining part of the homogenate was mixed with 10 % trichloroacetic acid in the ratio of 1:1, centrifuged at 5000 g for 10 min and supernatant was used for estimation of Superoxide dismutase (SOD)¹⁶, Catalase (CAT)¹⁷, Glutathione reductase (GSSH)¹⁸, Glutathione peroxidase (GPx)¹⁹, Peroxidase (Px)²⁰.

Preparation of intestinal homogenate

The intestine was removed, flushed with ice-cold saline and washed again. The mucosa was scraped off with a microscopic slide. 10 %W/V of the mucosa was mixed with 5 mM EDTA (pH-7.4) and homogenized with Teflon pestle²¹. The homogenate was centrifuged and the supernatant was used for estimation of intestinal alkaline phosphatase.

Statistical analysis

Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Dunnett's test. P values < 0.05 were considered significant.

RESULTS

In vitro antimicrobial activity

The clinical and laboratory standards institute clinical breakpoints were considered for interpretation of linezolid MIC (sensitive ≤ 4 µg/ml and resistant ≥ 8 µg/ml) *Staphylococcus aureus* ATCC 29213 was used as a control strain for MIC detection. MRSA isolates tested, was sensitive to LZD ranged from 0.5 µg/ml - 2 µg/ml. LZD was showed well *in vitro* activity against MRSA isolates and can be considered for the treatment of these infections.

Bacterial count

The number of viable organisms in the abscess (number of CFU per abscess) showed in table 1. The infected animals showed 4.20×10^6 CFU/abscess. Infected diabetic animals showed 8.68×10^6 CFU/abscess. The infected diabetic animals treated with LZD showed 9.69×10^3 CFU/abscess.



Table 1: MRSA count of infected and treatment groups

Group	MRSA CFU/Abscess
Infected (I)	4.20 x10 ⁶ ±0.02
D+I	8.68 x10 ⁶ ±0.04
I+ LZD	7.22 x10 ³ ±0.01
D+I+LZD	9.69 x10 ³ ±0.06
D(controlled)+I+LZD	9.06 x10 ³ ±0.02

Values are mean ± S.D; n=10 in each group

Blood Glucose and Lipid level

Blood glucose level of different experimental groups was shown in Fig.1. The glucose level was significantly (p < 0.01) decreased after 8th dose of LZD in group V and VI when compared to control. The LZD treated infected rats showed 72 mg/dl of glucose. The LZD treatment in soft tissue infected diabetic rats showed 201 gm/dl glucose. The glibenclamide and LZD treated infected diabetic rats showed normal blood glucose level (82 mg/dl). The cholesterol, triglycerides were significantly increased in all groups except normal and infected animals. LZD treatment does not alter in circulatory lipid level.

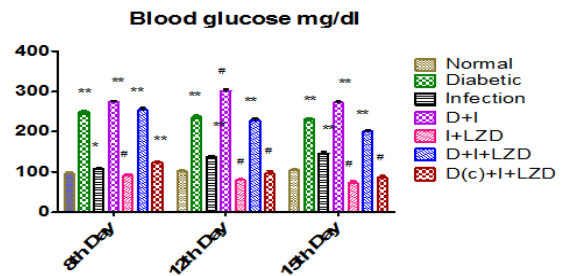


Figure 1: Effect of LZD on blood glucose in different experimental groups

Values are mean ± S.D; n=10 in each group; # P>0.05, * P<0.05 and ** P<0.01, when compared to normal control (one way ANOVA followed by Dunnett’s test).

Hematological parameters

The diabetic rats showed a significant reduction (p < 0.05) in RBC, Hb, elevation of WBC, no significant difference (p > 0.05) in platelets (PLT) and prothrombin time (PT) when compared to normal animals (Table. 2). LZD treated infected diabetic rats showed a significant reduction (p < 0.01) in RBC, WBC, PLT, Hb, and PT when compared to control.

Table 2: Hematological parameters of normal and different experimental groups

Group	RBC (x10 ⁶ /μl)	WBC (x10 ³ / μl)	PLT (x10 ⁵ / μl)	Hb (gm/dl)	PT (Sec)
Normal	6.24±0.2	12.38±0.6	8.28±0.1	12.18±0.3	11.28±0.06
Diabetic (D)	5.32±0.1*	12.86±0.4*	8.22±0.2 ^{ns}	11.84±0.1*	11.24±0.08 ^{ns}
Infected	6.14±0.3 ^{ns}	13.02±0.1**	7.94±0.3 ^{ns}	12.10±0.2 ^{ns}	11.32±0.09 ^{ns}
D+I	5.59±0.4 ^{ns}	13.17±0.4**	7.86±0.4*	10.57±0.3**	11.30±0.08 ^{ns}
I+LZD	4.93±0.1**	10.32±0.2**	4.96±0.2**	10.03±0.1**	11.46±0.1**
D+I+LZD	4.72±0.2**	10.96±0.2**	4.42±0.2**	9.62±0.4**	11.69±0.06**
D(c)+I+LZD	4.96±0.1**	10.62±0.5**	4.68±0.1**	9.84±0.2**	11.53±0.04*

Values are mean ± S.D; n=10 in each group; ^{ns} P>0.05, * P<0.05 and ** P<0.01, when compared to normal control (one way ANOVA followed by Dunnett’s test).

Biochemical parameters

The LZD treated diabetic rats showed (Table.3) significant (p < 0.01) elevation of SGOT, SGPT, ALP, LDH, and lactic acid when compared to normal animals. The diabetic rats showed the significant (p < 0.01) reduction in IAP (2.82

mU/ mg of tissue) levels when compared to control. The LZD treated infected diabetic animals showed the significant reduction (1.84 mU/ mg of tissue) in the level of IAP and elevation of SGOT, SGPT, ALP, LDH, and lactic acid.

Table 3: Biochemical parameters in control and experimental groups

Group	SGOT U/L	SGPT U/L	ALP U/L	LDH U/L	Lactic acid mg/dl	IAP mU/mg of tissue
Normal	62.8±2.6	46.2±1.3	151.2±5.2	389.2±10.6	12.6±0.4	4.49±0.08
Diabetic (D)	86.2±6.8**	82.0±2.4**	236.4±7.6**	582.8±13.5**	15.3±0.6**	2.82±0.04**
Infected (I)	63.1±2.5 ^{ns}	48.6±3.6*	154.8±5.8 ^{ns}	396.1±9.2 ^{ns}	12.8±0.2 ^{ns}	4.54±0.07 ^{ns}
D+I	84.2±6.4**	86.6±5.2**	251.0±8.2**	549.2±9.2**	17.1±0.4**	2.46±0.04**
I+LZD	82.9±7.2**	89.7±7.2**	392.8±6.8**	659.2±10.8**	21.6±0.6**	2.19±0.06**
D+I+LZD	90.4±4.7**	94.2±3.4**	402.4±9.9**	708.6±14.5**	22.3±0.5**	1.84±0.02**
D(c)+I+LZD	85.8±6.1**	90.4±4.6**	362.6±7.6**	640.9±8.9**	20.4±0.6**	1.99±0.06**

Values are mean ± SD; n=10 in each group; ^{ns} P>0.05, * P<0.05 and ** P<0.01, when compared to normal control (one way ANOVA followed by Dunnett’s test).



Lipid peroxidation

Diabetic rats, LZD treated rats and LZD treated infected diabetic rats showed (Table.4) a significant ($P < 0.01$) decrease in the level of total protein and an increase in the level of lipid peroxidation markers malondialdehyde (MDA) and lipid hydroperoxides (LH) when compared to normal control.

Table 4: Liver protein, MDA, and LH in control and experimental animals

Group	Protein (mmole/g wet issue)	MDA (nmoles/mg protein)	LH (nmoles/mg protein)
Normal	191.4±6.2	0.78±0.02	0.62±0.01
Diabetic	122.4±4.6**	1.16±0.04**	0.96±0.02**
Infected	184.8±6.3 ^{ns}	0.91±0.03**	0.68±0.04 ^{ns}
D+I	116.8±5.6**	1.24±0.04**	1.02±0.03**
I+L	152.1±8.4**	0.96±0.02**	0.84±0.02**
D+I+Lzd	104.4±6.2**	1.32±0.03**	1.14±0.02**
D(c)+I+Lzd	148.7±7.8**	1.14±0.02**	0.94±0.04**

Values are mean ± S.D; n=10 in each group; ^{ns} $P > 0.05$ and ** $P < 0.01$, when compared to normal control (one way ANOVA followed by Dunnett's test).

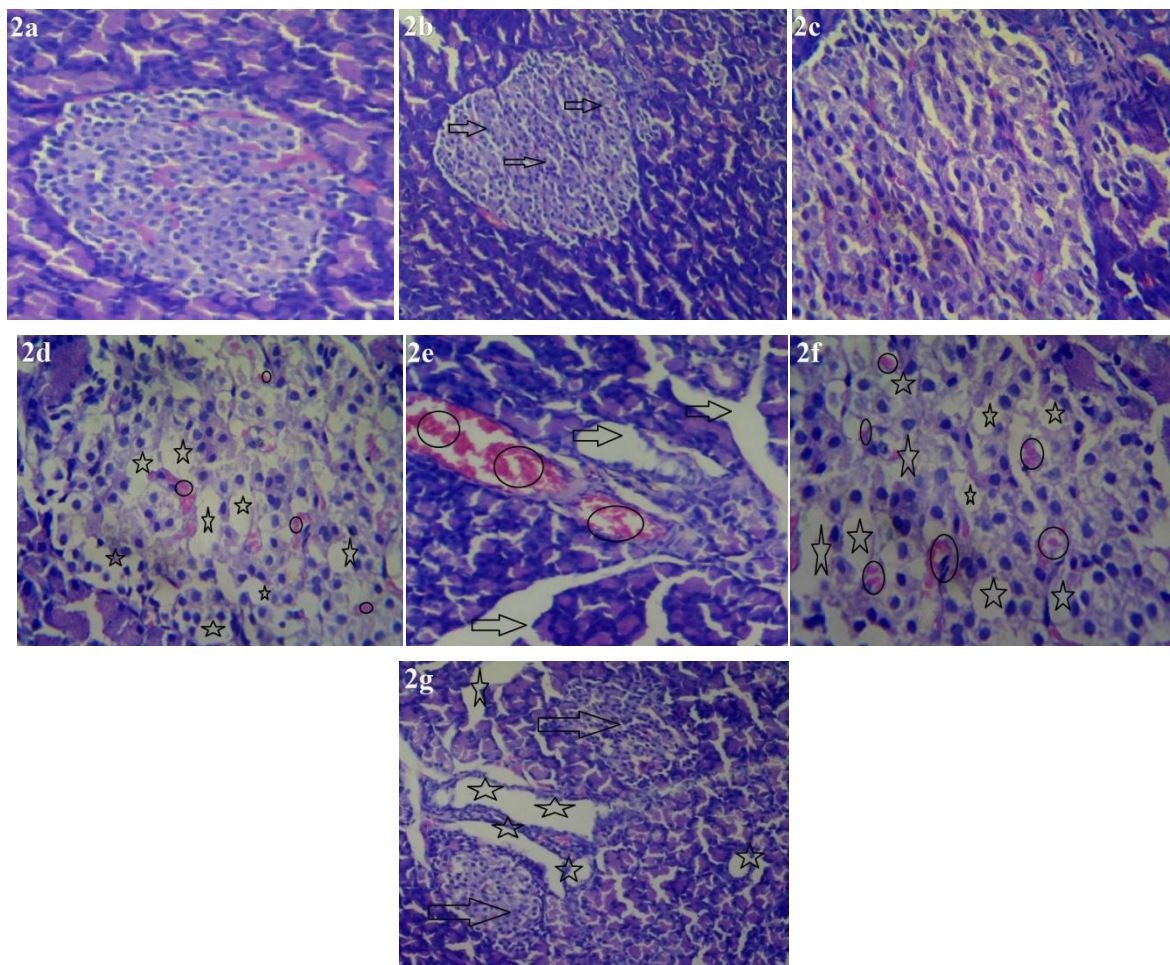


Figure 2: Histopathology of pancreas

Fig. 2 (a-g) showed histopathology of the pancreas. Fig. 2a control rats showed normal islets of Langerhans. Fig. 2b diabetic rats showed necrosis and shrinkage of islets of Langerhans (arrow). Fig. 2c Infected rats showed normal islets. Fig. 2d infected diabetic rats showed cytoplasmic vacuolation (star) and blood vessel congestion (round).

Fig. 2e Infected rats treated with LZD showed inflammatory accumulation in the Langerhans Fig. 2f infected diabetic rats treated with LZD showed blood vessel congestion and cytoplasmic vacuolation. Fig. 2g LZD treated infected diabetic controlled rats showed shrinkage of islets of cytoplasmic vacuolation

Enzymatic antioxidants level

The changes in the levels of enzymatic antioxidants SOD, CAT, GSSH, GPx and Px in the liver of control and experimental animals are shown in Fig. 2. A significant

Enzymatic antioxidants

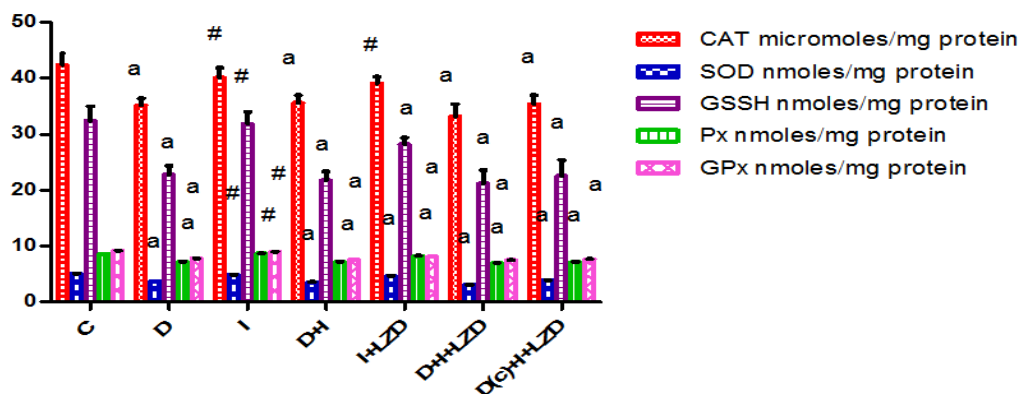


Figure 3: Effect of LZD on enzymatic antioxidants in different experimental groups

Values are mean \pm SD; n=10 in each group; # $P > 0.05$ and a $P < 0.01$ when compared to normal control (one way ANOVA followed by Dunnett's test)

DISCUSSION

Hyperglycemia causes immunosuppression and increases the susceptibility to bacterial infections. The poor glycemic control in diabetes may be a considerable risk factor for infections and infections themselves may also cause hyperglycemia²². The epidemiological and microbial pathogenicity analysis showed that MRSA seems to be the predominant pathogen to induce infections. LZD is a relatively safe antibiotic when given for short periods. In diabetic infections require prolonging treatment of LZD at least 2 weeks and cause serious side effect such as bone marrow suppression, hepatotoxicity, and mitochondrial toxicity. As chronic hyperglycemia cause alterations in biochemical, metabolic, and vascular abnormalities in diabetes.

Our study indicates the LZD treated infected rats showed hypoglycemia because, LZD has weak reversible monoamine oxidase (MAO) inhibitory properties, contribute to hypoglycemia^{23,24}. LZD treated infected diabetic animals showed hyperglycemia, But LZD and antidiabetic drug-treated infected diabetic rats showed normal sugar level. So clinician should monitor blood glucose level while prescribing LZD to diabetic patients to avoid the dysglycemic complications.

HFD and STZ induced obesity model mimics to type 2 diabetes mellitus; alter the lipid profiles, increased lipid peroxidation, increased incidence of atherosclerosis, a major complication of diabetes mellitus. An enhanced oxidative stress has been observed by increased free radical production, lipid peroxidation, and diminished antioxidant status²⁵. In our study, there is a significant rise in serum cholesterol and triglycerides levels in diabetic rats because of high-fat diet.

decrease in the levels of these radical scavengers was noted in diabetic, LZD treated, diabetic and LZD treated infected rats.

Diabetes causes anemia because it decreases the production of erythropoietin hormone, which leads to decreased RBC count in the body²⁶. The high WBC count in the T2DM group increased oxidative stress triggered by the high levels of hyperglycemia. LZD treated infected diabetic rats showed a reduction in blood cells count due to myelosuppression.

HFD and LZD treatment induce severe hepatic damage and alters their membrane permeability and transport, cause leakage of enzymes from the cells. So, the increased release of SGOT, SGPT, and ALP from the liver into circulation indicates severe hepatic damage. LZD administration to T2DM infections increases the liver damage.

T2DM cause development of lactic acidosis by tissue hypoxia leads to alterations in pyruvate metabolism and increase the blood lactic acid level. LZD causes hyperlactatemia by inhibiting mitochondrial DNA polymorphisms²⁷. The hyperlactatemia induced by both diabetes and LZD could damage the organs such as liver, kidney, and pancreas.

IAP is an important brush border enzyme expressed highest in the duodenum. IAP binds to intracellular proteins and decreases the movement of the fat droplet across the enterocyte. The IAP plays a crucial role in decreasing the rate of fat absorption²⁸. Studies were reported IAP levels are low in T2DM patients; High IAP levels prevent the development of T2DM even though if the person is obese, deficiency of IAP can increase the incidence of T2DM²⁹. Our study showed decreased IAP levels in diabetic and LZD treated rats. LZD treated diabetic rats showed a low level of IAP and increased to cause changes in the microbiome, intestinal inflammation, and intestinal permeability and increase the risk of disease.

Free radicals are formed in diabetes by glucose autooxidation, polyol pathway, and non-enzymatic



glycation. The high levels of free radicals and the simultaneous decrease of antioxidants cause damage to cells and enzymes, increased lipid peroxidation, mitochondrial dysfunction, and development of diabetic complications³⁰.

The present study states that the LZD treatment also induces oxidative stress. It was confirmed by increase lipid hydroperoxide, MDA and a significant decrease in SOD, CAT, GSSH, Px, and Gpx. Lipid peroxidation causes oxidative deterioration of polyunsaturated fatty acid. The concentration of peroxides increases, it initiates uncontrolled lipid peroxidation, thus cause cellular infiltration and islet cell damage confirmed by histopathological studies. It may be due to free radicals, STZ, high-fat or lactic acid deposition. The oxidative damage was high in LZD treated diabetic animals than diabetic animals.

CONCLUSION

Our research elucidated the LZD therapy may increase the diabetic complications such as oxidative stress, anemia, lactic acidosis, and organ damage. But LZD has better efficacy against the diabetic MRSA infections. So we need some protective agents to avoid such complications while using LZD to diabetic patients.

REFERENCES

- Wilson J, Guy R, Elgohari S, Sheridan E, Davies J, Lamagni T, Pearson A, Trends in sources of methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia: data from the national mandatory surveillance of MRSA bacteremia in England, 2006–2009. *Journal of Hospital Infection*, 79, 2011, 211–217.
- Sreeramoju P, Porbandarwalla NS, Arango J, Latham K, Dent DL, Steart RM, Patterson JE, Recurrent skin and soft tissue infections due to methicillin-resistant *Staphylococcus aureus* requiring operative debridement. *The American Journal of Surgery*, 201, 2011, 216–220.
- Bhavan KP, Marshcall J, Olsen MA, Fraser VJ, Wright NM, Warren DK, The epidemiology of hematogenous vertebral osteomyelitis: a cohort study in a tertiary care hospital. *BMC Infectious Disease*, 10, 2010, 158.
- Christian E, Mathew D, Treatment of complicated skin and soft-tissue infections caused by resistant bacteria: the value of linezolid, tigecycline, daptomycin, and vancomycin. *European Journal of Medical Research*, 15, 2010, 554-563.
- Aisling RC, Haley JM, Laura AP, Kerry LP, Comparative effectiveness of linezolid and vancomycin among a natural veterans affairs cohort with methicillin-resistant *Staphylococcus aureus* pneumonia. *Journal of Human Pharmacology and Drug Therapy*, 34, 2014, 473-480.
- Yogev R, Patterson LE, Kaplan SL, Adler S, Morfin MR, Martin A, Padbury BE, Stehouwar SN, Bruss JB, Linezolid for the treatment of complicated skin and skin structure infections in children. *Pediatrics and Infectious Disease Journal*, 22(9), 2003, 172-177.
- Li M, Xiaojuan X, Xingjin Z, Lujing Z, Yuanyuan Q, Comparison of efficacy of linezolid and vancomycin for treatment of hospital-acquired pneumonia: A meta-analysis, *Biomedical Research*, 28, 2017, 3420-3426.
- Paul WA, Namirah J, John PH, Linezolid: Its role in the treatment of gram-positive, drug-resistant bacterial infections. *American Family Physician*, 65 (4), 2002, 663-671.
- Taeksun S, Myungsun L, Han-Seung J, Yumi P, Lori ED, Veronique D, Dean F, Jing W, Lisa CG, Kennet NO, Yingda X, Laura EV, Sang NC, Clifon B, Ray YC, Linezolid trough concentrations correlate with mitochondrial toxicity-related adverse events in the treatment of chronic extensively drug-resistant tuberculosis. *EBio Medicine*, 47, 2015, 2507-2512.
- Wayne PA, Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard, CLSI document M7-A7, Seventh Ed 2006.
- Srinivasan K, Viswanad B, Lydia A, Kaul CL, Ramarao P, Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. *Pharmacological Research*, 52, 2005, 313-320
- Masakatsu T, Morio T, Hideaki M, Jingoro S, Shogo K, *In Vivo* Antibacterial Activity of S-3578, a New Broad-Spectrum Cephalosporin: Methicillin-Resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* Experimental Infection Models. *Antimicrobial Agents and Chemotherapy*, 47, 2003, 2507-2512.
- Mustafa S, Suzan S, Iknur K, Nural C, Zafer T, Semra TK, Ali A, Faruk OD, Ahmet B, Huseyin TS, Efficacy of linezolid in the treatment of mediastinitis due to methicillin-resistant *Staphylococcus aureus*: an experimental study, *International Journal of Infectious Diseases*, 12, 2008, 396-401.
- Lowry OH, Rosenbourgh NJ, Farr AL, Randall RJ, Protein measurement with Folin phenol reagent. *Journal of Biological Chemistry*, 193, 1951, 265-275.
- Nieshus WG, Samuelsson B, Formation of MDA from phospholipids arachidonate during microsomal lipid peroxidation. *European Journal of Biochemistry*, 6, 1986, 126-130.
- Kakkar P, Das B, Viswanathan PN, A modified spectrophotometric assay of superoxide dismutase. *Indian Journal of Biochemistry and Biophysics*, 2, 1984, 130-132.
- Aebi H, *Methods in enzymatic analysis*. 2nd Ed, Academic Press, New York, 1974, 673-684.
- Racker E, Glutathione reductase from bakers' yeast and beef liver. *Journal of Biological Chemistry*, 217, 1955, 855-866.
- Paglia DE, Valentine WN, Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *Journal of Laboratory and Clinical Medicine*, 70, 1967, 158-159.
- Lobarzewski J, Ginalska G, Industrial use of soluble or immobilized plant peroxidases. *Plant Peroxidase News Letter*, 6, 1995, 3-7.
- Takanori K, Tomoyuki T, Shuhachi K, Kei S, Facile Preparation of Rat Intestinal Mucosa for Assay of Mucosal Enzyme Activity. *Journal of Nutritional Science and Vitaminology*, 39, 1993, 399-403.



22. Kao LS, Knight MT, Lally KP, Mercer DW, The impact of diabetes in patients with necrotizing soft tissue infections. *Surgical Infections*, 6, 2005, 427–438.
23. Bodnar T, Sttarr K, Halter JB, Linezolid-associated hypoglycemia in a 64-year-old man with type 2 diabetes. *American Journal of Geriatrics and Pharmacotherapy*, 9 (1), 2011, 88-92.
24. Viswanathan P, Iarikov D, Wassel R, Davidson A, Nambiar S, Hypoglycemia in patients treated with linezolid. *Clinical Infectious Disease*, 59(8) 2014, e93–e95.
25. Stephanie D, Severine S, The protective effect of antioxidants consumption on diabetes and vascular complications. *Disease*, 4, 2016, 1-51.
26. Cawood TJ, Buckley U, Murray A, Corbett M, Dillon D, Goodwin B, Sreenan S, Prevalence of anemia in patients with diabetes mellitus, *Iranian Journal of Medical Sciences*, 175, 2006, 25-27.
27. Apodaca AA, Rakita RM, Linezolid-induced lactic acidosis. *New England Journal of Medicine*, 348, 2003, 86-87.
28. Jason F, David G, Intestinal Alkaline Phosphatase: A summary of its role in Clinical Diseases. *Journal of Surgical Research*, 202, 2016, 225-234.
29. Madhu SM, A High Level of Intestinal Alkaline Phosphatase Is Protective Against Type2 Diabetes Mellitus Irrespective of Obesity. *EBio Medicine*, 2, 201, 2016-23.
30. Maritim AC, Sanders RA, Watkins JB, Diabetes, oxidative stress and antioxidants: a review. *Journal of Biochemical and Molecular Toxicology*, 17, 2003, 24–38.

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