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Effects of Linezolid and Metformin Combination on Vital Biochemical Functions with Special Reference to Lactic Acidosis in Streptozotocin Induced Diabetic Rats

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ABSTRACT

The present work was to investigate and assess the Linezolid and Metformin combination on biochemical functions associated with liver and kidneys and further to study effects on lactic acidosis in diabetic rats, which was not investigated previously. Linezolid and Metformin were administered into Streptozotocin induced diabetic rats at the dose of 100 mg/kg body weight. Each treatment group was received the dose of Metformin or Linezolid two times a day with 6 hours of interval for 14 days. There was significant decrease noticed in ALT and AST in Linezolid and Metformin combination groups, which were abnormally high due to diabetes. Also there was significant decrease in urea nitrogen in Linezolid and Metformin combination against stand alone Linezolid group ($p<0.001$). The significant increase in lactic acid in Linezolid and Metformin combination against stand alone Linezolid or Metformin groups, the finding was not having any biological risk which judged based on previous literature ($p<0.05$). Therefore it can be concluded that Metformin may have beneficial effects by improving the hepatic and renal functions, which impaired due to diabetes. And further there was no risk of lactic acidosis reported due to Linezolid and Metformin combination despite of having mitochondria as a common toxicological target.

Keywords: Metformin, antidiabetic, streptozotocin, lactic acidosis, metabolic acidosis, mitochondria

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INTRODUCTION

The present work investigated the Metformin associated effects on some biochemical parameters to assess the functions of major vital organs like liver and kidneys. And additionally lactic acidosis was also studied to judge safety of Linezolid and Metformin combination. Previously, Metformin associated lactic acidosis (MALA) is not very clearly demonstrated the biological risk when exposed daily for longer use. There are different opinions comes out about the Metformin safety. On one side reports conclude that MALA didn't play any life threatening risk in long term therapy. While few reports suggest that MALA requires dialysis to avoid morbidity or mortality [1, 2]. Further it also suggests that MALA is a rare but classic side effect of Metformin [3]. Two years after the introduction of this drug in the US market, a study showed an incidence of MALA of two to nine cases per 100,000 patients treated with Metformin each year with an associated mortality rate as high as 50% [4]. The physiopathology is complex and mostly unclear about it. However, this side effect seems to be closely related to the anti-hyperglycemic effect of Metformin [5]. It is also known that Metformin impairs lactate clearance of the liver through the inhibition of complex I of the mitochondrial respiratory chain [4, 6]. Intracellular hyperlactatemia was reported in previous study in platelet cells associated with Metformin treatment [7]. Linezolid (Zyvox) was launched in year 2000, and several cases of Linezolid-induced lactic acidosis have been described in the literature since 2003 [2, 8, 9]. Later on it was reported that lactic acidosis was associated with mitochondrial dysfunctions through inhibition of mitochondria protein synthesis [10]. With these findings, the other research work revealed that Linezolid inhibits the mitochondrial protein synthesis inhibition and alters the function of respiratory chain [11].

Thus above scientific investigations and interpretations point the in depth study on the effects of these drugs which are having similar toxicological targets to avoid un-safe practice. This is probably the first report on investigation of combinations effects of these two drugs in diabetic condition. Together with the lactic acidosis it was also thought essential to investigate the other important biochemical parameters to correlate the functioning of the vital organs like liver, kidney in diabetic rats by administering such drugs in combination. Hence attempt has been made to study the effect of Metformin and Linezolid combination on different biochemical parameters including lactic acid in Streptozotocin induced diabetic rats [12].

Rat is a robust experimental model for toxicological evaluation of drug induced lactic acidosis [13]. Diabetic rats were used in this study to resemble the likely patho-physiological condition in human and use of Metformin in diabetic condition will benefit the pharmacodynamic effects of Metformin [14]. A 14 day diabetic rat experimental model was designed on the basis of the report, where Linezolid induces lactic acidosis from first week of therapy [15]. The magnitude of increase in the levels of hepatic parameters like ALT and AST can be very well correlated with abnormal liver function associated with diabetes [15, 16].

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MATERIALS AND METHODS

Chemicals and reagents

Linezolid (LZD) and Metformin HCl (MET) were procured from Symed Labs, Hyderabad India, and Jay Pharma-Chem, Ankleshwar India respectively. Both the drugs were procured in the form of active pharmaceutical ingredient (API) for experimental use. Tween 80 was procured from Qualigens and sterile water for injection was used for formulation preparation.

Animals

Healthy male Wistar rats having an age of approximately 9 weeks and weighing in the range of 180-200 g were obtained for the experiment. Throughout the experiment animals were housed in groups of 5 per cage and were maintained at 22 ±2°C on 12:12h light-dark cycle. All animals were given access to food and water *ad libitum*. Throughout the experiment all animals were handled according to the guidelines given under the care and use of animals and the work was approved by the Animal Ethical Committee.

Experimental design

Animals were randomly divided into the following experimental groups (5 animals/each group):

Group I: Non-diabetic healthy animals treated with vehicle (sterilized and apyrogenic water for injection (healthy control).

Group II: Diabetic animals treated with vehicle (sterilized and apyrogenic water for injection (diabetic control).

Group III: Diabetic rats treated with LZD (100 mg/kg bwt two times in a day with 6 hours interval, PO daily for 14 days.

Group IV: Diabetic rats treated with MET (100 mg/kg bwt two times in a day with 6 hours interval, PO daily for 14 days.

Group V: Diabetic rats treated with LZD + MET (each at 100 mg/kg bwt two times in a day with 6 hours interval, PO daily for 14 days.

Diabetes induction in rats

A single dose of Streptozotocin (STZ) at the dose of 45 mg/kg was administered in citrate buffer (pH 4.5). The preparation was injected subcutaneously into 16 hour fasted rats. The animals were allowed 48 hours of rest for blood glucose stabilization, before the inducement, initial blood glucose of each of the rats was measured.

Fasting blood glucose determination

After 48 hours of STZ injection, glucose was measured from plasma on biochemistry auto-analyzer (Siemens Healthcare's Xpand Model) to determine the blood glucose levels of the STZ induced diabetes. Only those rats were selected for the study, which showed fasting glucose levels above 250 mg/dL.

Collection of blood and biochemical parameters estimations

Eighteen hours after the last dose, blood was collected from retro-orbital sinus under light ether anesthesia. Each sample was collected in centrifuge tubes without using anticoagulant and incubated at room temperature for 15 minutes and then centrifuged at 3500 rpm for 15 minutes. The separated serum was used to estimate following parameters. The parameters estimated were Glucose (GLU), Lactic Acid (LA), Aspartate Aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline Phosphatase (ALP), Alpha amylase (AMY), Creatine Kinase (CK), Triglycerides (TG), Cholesterol (CHO), Total Bilirubin (T-BIL), Blood Urea Nitrogen (BUN). Analyses for serum biochemistry parameters were performed on automated biochemistry analyzer Dimension Xpand model from Siemens Healthcare limited, USA.

STATISTICAL ANALYSIS

The results were expressed as the mean value ± SEM. Statistical differences between groups were assessed using Bonferroni's multiple comparison tests was applied to obtain "p" values. A probability level less than 0.05 were accepted as significant.

EXPERIMENTAL RESULTS

Effects on Lactic acidosis (LA)

Overall there were high lactic acid levels reported in animals treated with Linezolid and Metformin combination when compared to healthy as well as diabetic controls. Statistically there was significant increase reported in Linezolid and Metformin combination as compared to stand alone Linezolid and healthy control group ($p<0.05$) ($p<0.001$) respectively. The statistical significance of increase in lactic acid also reported in stand alone Metformin group when compared to healthy control ($p<0.05$). Figure 1 and Table 1.

Effects on Triglycerides (TG)

In Linezolid and Metformin combination treated animals, there was significant increase in triglyceride levels reported as compared to stand alone Linezolid and Metformin groups ($p<0.01$). Also there was significant increase reported in this group as compared to healthy as well as diabetic control animals ($p<0.001$). Figure 2 and Table 1.

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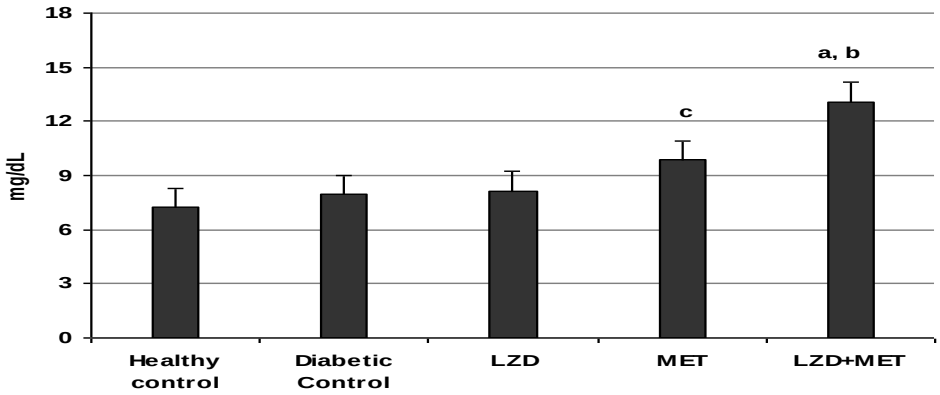
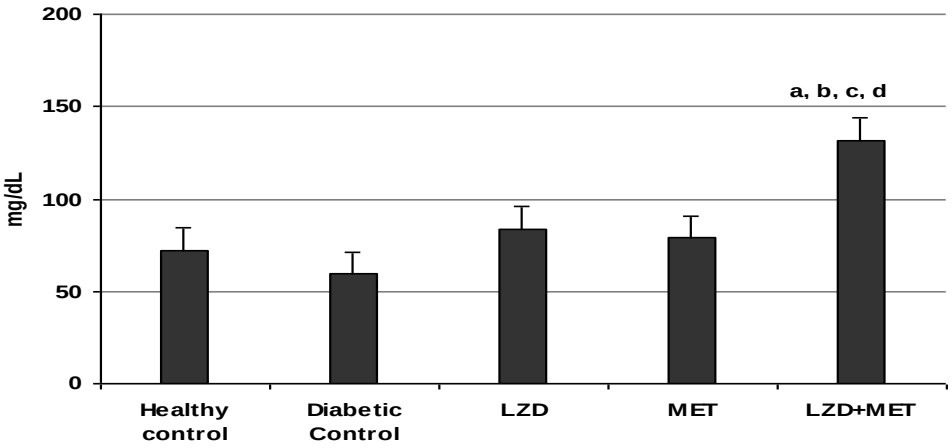
FIGURE 1: Effect of MET and LZD treatment on lactic acid in diabetic rat.



Values are presented as means of the each group ±SEM (n=5).
LZD = Linezolid at 100mg/kg two times a day for 14 days;
MET = Metformin 100 mg/kg two times a day for 14 days. :
a: significantly different from LZD treated group ($p<0.05$),
b: significantly different from healthy control ($p<0.001$)
c: significantly different from healthy control ($p<0.05$)
using the Bonferroni's multiple comparison test.

FIGURE 2: Effect of MET and LZD treatment on Triglyceride in diabetic rat.



Values are presented as means of the each group ±SEM (n=5).
LZD = Linezolid at 100mg/kg two times a day for 14 days;
MET = Metformin 100 mg/kg two times a day for 14 days. :
a: significantly different from diabetic control group ($p<0.001$),
b: significantly different from healthy control group ($p<0.001$)
c: significantly different from MET treated group ($p<0.01$)
d: significantly different from LZD treated group ($p<0.01$)
using the Bonferroni's multiple comparison test.

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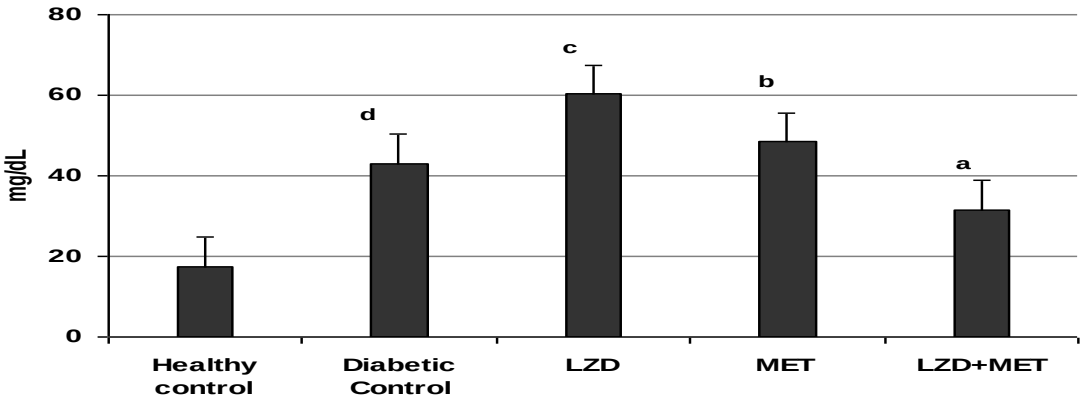
Effects on blood urea nitrogen (BUN)

The combination treatment of LZD and MET has shown the decreasing levels of BUN in blood. Statistically there was significant decrease in BUN reported in LZD+MET treated group when compared to stand alone LZD treated group ($p<0.001$). High levels of BUN were reported in stand alone LZD, MET groups as well as in diabetic control animals when compared to healthy controls ($p<0.001$). Figure 3 and Table 1.

FIGURE 3: Effect of MET and LZD treatment on Blood Urea Nitrogen in diabetic rat.



Values are presented as means of the each group ±SEM (n=5).
LZD = Linezolid at 100 mg/kg two times a day for 14 days;
MET = Metformin 100 mg/kg two times a day for 14 days. :
a: significantly different from LZD treated group ($p<0.001$),
b: significantly different from untreated control ($p<0.001$)
c: significantly different from healthy control ($p<0.001$)
d: significantly different from healthy control ($p<0.01$)
using the Bonferroni's multiple comparison test.

Effects on alpha amylase (AMY)

Overall there was significant decrease in AMY reported in all treated groups including diabetic control when compared to healthy control. The decrease in AMY in serum was considered due to diabetic condition. The reduction in AMY was statistically significant as compared to healthy control ($p<0.001$). Figure 4 and Table 1.

Effects on hepatic parameters

Alkaline phosphatase (ALP): There was significant increase in level of ALP in diabetes control, stand alone Linezolid and Metformin treated animals. Though there was no significant, but decrease in ALP reported in Linezolid and Metformin combination group when compared to diabetic control. Figure 4 and Table 1.

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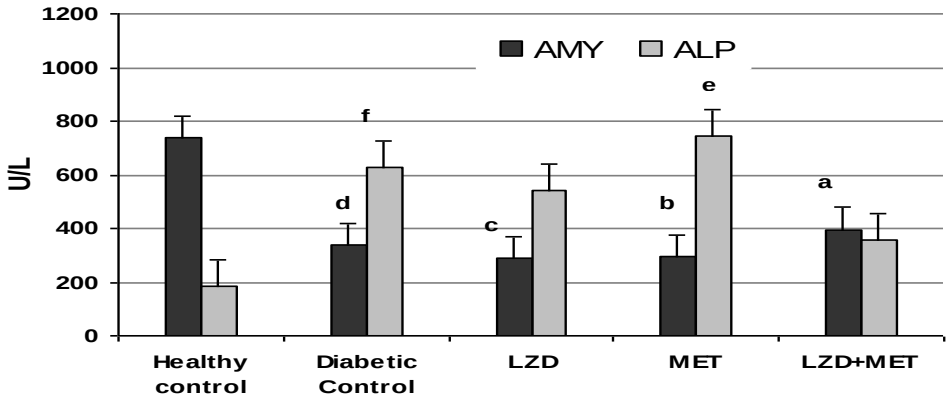
FIGURE 4: Effect of MET and LZD treatment on AMY and ALP in diabetic rat.



Values are presented as means of the each group ±SEM (n=5).
LZD = Linezolid at 100mg/kg two times a day for 14 days;
MET = Metformin 100 mg/kg two times a day for 14 days. :

AMY:
a: significantly different from healthy control ($p<0.001$),
b: significantly different from healthy control ($p<0.001$)
c: significantly different from healthy control ($p<0.001$)
d: significantly different from healthy control ($p<0.001$)
using the Bonferroni's multiple comparison test.

ALP:
e: significantly different from healthy control ($p<0.01$),
f: significantly different from healthy control ($p<0.05$)
using the Bonferroni's multiple comparison test.

Alanine aminotransferase (ALT): Overall there was decreasing trend seen in stand alone treatment of Linezolid and Metformin and their combinations against the diabetic control animals. The decreasing trend was significant in stand alone Metformin against diabetic control ($p<0.01$). Likely there was also decrease seen in stand alone Linezolid and combination of Linezolid and Metformin when compared to diabetic control ($p<0.05$). Figure 5 and Table 1.

Aspartate aminotransferase (AST)

The decreasing trend in low magnitude was recorded in Linezolid and in combination of Linezolid and Metformin groups, while significant decrease were reported in stand alone Metformin group as compared to diabetic control ($p<0.05$). The significant increase in AST in diabetes control was considered as disease related when compared to healthy control ($p<0.01$). Figure 5 and Table 1. Further there were no changes reported in serum cholesterol (CHO), Total Bilirubin (T-BIL) and creatine kinase (CK) either in diabetic animals or any of the treatment groups. Table 1.

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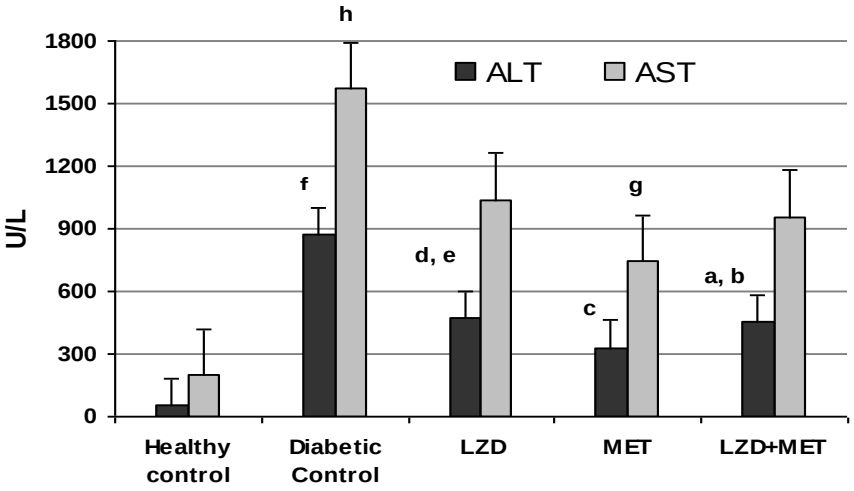
FIGURE 5: Effect of MET and LZD treatment on ALT and AST in diabetic rat.



Values are presented as means of the each group ±SEM (n=5).
LZD = Linezolid at 100mg/kg two times a day for 14 days;
MET = Metformin 100 mg/kg two times a day for 14 days.

ALT:
a, b: significantly different from diabetic and healthy controls ($p<0.05$),
c: significantly different from diabetic control ($p<0.01$)
d, e: significantly different from diabetic and healthy control ($p<0.05$)
f: significantly different from healthy control ($p<0.001$)
using the Bonferroni's multiple comparison test.

AST:
g: significantly different from diabetic control ($p<0.05$)
h: significantly different from healthy control ($p<0.01$),
using the Bonferroni's multiple comparison test.

DISCUSSION

Published reports on repeated dose toxicity study of Metformin revealed some important changes in biochemical functions in healthy rats. The changes demonstrated were increase in the levels of lactic acid, triglycerides, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), while no changes in urea nitrogen [18]. These findings of the study may be important finding in toxicological scenario. But at the same bear certain limitations of evaluating the anti-diabetic drug 'Metformin" in non-diabetic condition of the animals. While in the current work, rats were first induced diabetes and then Metformin treatment was started to mimic the human pathophysiology of diabetes so that changes associated with Metformin, Linezolid or their combinations will be more closure to the changes which can be correlated in humans. The significant increase in lactic acid in diabetic animals which were treated with Metformin and Linezolid combinations demonstrated the additive effects. It gives us an

indication that Linezolid has potential to enhance the lactate levels in Metformin exposed animals probably by altering the mitochondrial pathways. But previous published studies emphasized those biological worsening levels of lactic acids should be at least more than 7 folds of upper normal limit [12]. In the current work, Linezolid and Metformin combination enhanced the lactate levels merely two fold when compared to diabetic controls. Hence conclusions were drawn that Linezolid and Metformin combination doesn't bring any biological risk when administered together despite of having common target mitochondria. Published reports on 13

Table 1: Effect on different biochemical parameters in diabetic rats treated with metformin, linezolid and their combination after completion of the treatment.

	Normal Control	Diabetic Control	LZD	MET	LZD+MET
GLU (mg/dL)	8360±2.3	421.2±15.8	500.2±39.4	509.2±10.0	430.2±26.1
TG (mg/dL)	72.4±2.2	59.2±3.2	83.8±7.9	78.8±9.34	131.4±8.6
CHO (mg/dL)	95.2±3.8	90.8±4.2	101.4±7.1	80.0±7.2	93.4±2.36
T-BIL (mg/dL)	0.54±.05	0.48±0.02	0.40±0.03	0.46±0.02	0.51±0.01
BUN (mg/dL)	17.4±1.34	43.0±1.17	60.2±3.06	48.4±6.84	31.6±2.01
LA (mg/dL)	7.3±0.3	7.98±0.36	8.16±0.37	9.9±0.39	13.1±0.34
ALP (U/L)	186.8±5.6	628.6±112.9	543.0±87.1	743.6±68.3	354.8±100.8
ALT (U/L)	54.2±3.97	871.0±82.2	470.8±56.9	327.4±60.0	453.4±115.7
AST (U/L)	200.4±6.9	1571.8±198.0	1037.6±103.3	745.0±155.0	955.6±303.6
AMY (U/L)	736.6±27.7	335.8±17.0	287.8±25.7	293.0±50.2	396.6±61.1
CK (U/L)	1107.2±47.6	1212.2±42.84	1119.0±149.3	761.6±178.4	1321.2±106.3

Values are presented as mean ± SEM (*n* = 5).

LZD = linezolid 100mg/kg bwt, two times a day; MET = metformin 100 mg/kg bwt two times a day; ; LZD+MET = Linezolid and Metformin 100 mg/kg bwt each two times a day. GLU = Glucose; TG = Triglycerides; CHO = Cholesterol; T-BIL = Total Bilirubin; BUN = Blood urea nitrogen; LA = Lactic acid; ALP = Alkaline phosphatase; ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; AMY = Amylase; CK = Creatinine kinase. Values (among the five groups) followed by different letters are significantly different ($P \leq 0.05$). a: significantly different from healthy control group ; b: significantly different from the DOX group using the Bonferroni's multiple comparison test.

week Metformin toxicity study in healthy rats revealed no changes in BUN [18]. Another study reported decrease in BUN, but the study was carried out in normal healthy animals despite of diabetic conditions [19, 20]. So, the current strongly supports the findings where decrease in BUN was recorded in groups which received Metformin. The parameter strongly used as marker of renal function. So the results can be positively suggested that use of Metformin may have positive role in diabetic nephro-protection.

In a rabbit study, when Metformin was administered in repeat dose fashion to evaluate its safety, the result revealed that it given an indication of possible hepato-protection. [21]. Another study in mice also showed that Metformin resulted into hepatoprotective action in carbon tetrachloride induced liver injury [22]. In the current work, stand alone Metformin treatment resulted into significant reduction in serum ALT and AST levels when compared to

diabetic control. While other treated groups like stand alone Linezolid and combination with Metformin also showed decreasing trend but not very significant from diabetic control. The decreasing trend in hepatic enzymes can be correlated with potential having for hepato protection [23].

Significant increase in triglyceride levels in Metformin animals which were exposed to Linezolid created condition like hyper triglyceridemia, which can be considered as treatment related effect. The significant reduction in alpha amylase of all groups except healthy controls was considered as irreversible effects of Streptozotocin which induced the pancreatic damage and further either Metformin or Linezolid has not shown any protective effects to restore the pancreatic function [24].

CONCLUSION

Results of the investigations revealed that Linezolid in combination with Metformin enhanced lactic acidosis in diabetic condition. The changes in serum lactic acid in the study were statistically significant, but biologically are less relevant. Therefore the administration of Linezolid in diabetic rat which were exposed to Metformin does not seem to elevate any significant risk of lactic acidosis bearing morbidity or mortality. Additionally, the current experimental model has demonstrated the possible role of Metformin associated nephroprotective and hepato-protective effects in diabetic rats.

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