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ORIGINAL ARTICLE

Effect of Combined Treatment with Levofloxacin and Metformin on Diabetes-the Diabetes Related Behavioral and Biochemical Alterations

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ABSTRACT

Objectives: The current experiment was conducted to investigate the combined effect of levofloxacin (LVX) and metformin treatment on blood glucose levels, malondialdehyde (MDA),nitrite levels, and anxiety in streptozotocin (STZ)+ nicotine adenine dinucleotide (NAD)-induced diabetic rats.

Materials and Methods: In this study, Wistar rats have been used. After receiving a single dose of STZ + NAD (45 mg/kg, *i.p.*+ 50 mg/kg, *i.p.*), the rats developed diabetes. Glucose levels in diabetic rats exceeded 200 mg/dL (verified on the third day). Saline was administered to non-diabetic rats (controls). Thediabetic rats were administered metformin (50 mg/kg, *p.o.*), LVX (30 mg/kg, *i.p.*), or metformin + LVX for 14 days. Blood samples were obtained after the 14th day of therapy, and the rats were given behavioral parameters to determine locomotor activity and anxiety level. Blood plasma samples were separately collected for the determination of nitrite and MDA levels.

Results: It was observed that the combined treatment of metformin and LVX significantly increased glucose levels in the blood of diabetic rats compared with diabetic control ($p < 0.05$) and diabetic rats treated with metformin alone ($p < 0.001$) at days 3 and 7. Further, combined treatment of metformin and LVX significantly reduced time spent at the center of the open field test ($p < 0.001$), significantly reduced time spent and entry made in the light chamber of the light-dark test ($p < 0.001$), significantly increased time spent in the open arm and reduced the time spent in the closed arm of the Elevated plus maze ($p < 0.001$) compared with alone metformin-treated diabetic rats. Further, combined treatment with metformin and LVX significantly increased the nitrite level, ($p < 0.001$) but reduced the MDA level in plasma compared with metformin alone-treated diabetic rats ($p < 0.001$).

Conclusion: The present study suggests that combined treatments with levofloxacin and metformin may modulate glucose levels and anxiety-related activity.

Keywords: Diabetes, glucose, malondialdehyde, anxiety, levofloxacin

INTRODUCTION

Diabetes is the most common disease worldwide, and there is also a rise in the incidence of morbidity and mortality due to this disorder.[1] Diabetics struggle to regulate their fluctuating blood glucose levels (BGL),[2] which can result in fatalities, permanent strokes, and heart attacks.[3] Fluoroquinolones (FQ) are crucial as secure, broad-spectrum antibiotics in the treatment of diseases that are resistant to other antibiotic classes, but they may also cause problems with BGL,[4] which could be challenging to regulate, particularly for diabetic patients.[5] Type 2 diabetes (T2D) accounts for almost 85-95% cases of diabetes.[6] It has been reported that a hyperglycemic environment; lower production of interleukins; reduced immunity, and urinary dysmotility in diabetes are accompanied by the emergence of various infections.[7] FQ drugs are commonly used to treat various illnesses and have pharmacokinetic advantages, good penetration, and high oral bioavailability. However, FQs are associated with an increased incidence of tendon rupture, peripheral neuropathy, and FQ-associated aortic aneurysm and

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aortic dissection.[8] Although blood sugar abnormalities caused by FQ are uncommon, they are a serious and potentially fatal consequence that is more likely to occur in the elderly, those suffering from diabetes, and kidney failure individuals.[9] In addition, FQ may have been associated with hypoglycemia by increasing pancreatic insulin.[10] Furthermore, the administration of levofloxacin (LVX) in diabetic rats revealed hypoglycemic effects.[11] Previous studies have suggested that high levels of hypoglycemia in patients with diabetes are associated with the use of levofloxacin.[12] FQs possess insulinotropic activity at clinically relevant concentrations and thus enhance glucose-induced insulin secretion.[13] Furthermore, the risk of a hypoglycemic emergency increases with the combination of levofloxacin with insulin or sulfonylurea.[14] A recent survey revealed hazardous interactions between sulfonylureas and antimicrobials when used together.[15] In the present study, we studied the combined effects of levofloxacin and metformin in rats with diabetes.

MATERIALS AND METHODS

Animal

Male, body weight. 150-200 g, Wistarrats were obtained from a disease-free animal house at the Lala lazpat Rai University of Veterinary and Animal Sciences in Hisar and kept in the Central Animal House at the Maharshi Dayanand University in Haryana under controlled lighting and environmental conditions, with unrestricted access to nutritious food and water. The rats were given time to adjust to the laboratory conditions before the experiment, which was conducted between 9.00 and 17.00. The study protocols were approvedby the Institutional Animal Ethics Committee, Maharshi Dayanand University, Haryana (approval number: 1767/RE/S/14/CPCSEA: 31.08.2017, dated: 14.12.2018). Animals were cared for properly according to the requirements of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, Forest and Climate Change, Government of India.

Drugs and treatments

In the current study, streptozotocin (STZ) (Central Drug House, India); levofloxacin (Cipla Pvt. Ltd., India); metformin (Cipla Pvt. Ltd., India);nicotine adenine dinucleotide (NAD) (Central Drug House, India) were used. All treatments were administered in an unchanged volume (amount) of 5 mL/kg; *i.p.*, STZ solution was freshly prepared at pH 4.5 in 0.1 M citrate buffer.

Induction of diabetes

Induction of diabetes by a single intraperitoneal dose of STZ + NAD (45 + 50 mg/kg. On days 1, 3, 7, and 14, BGL were monitored using a glucometer. Diabetic rats are characterized by BGL exceeding 200 mg/dL.[16,17]

Loco-motor activity

Rats were placed alone in the center of an open field, and their behavioral activity was monitored using a camera (for video making) set up at a height of 100 cm. Over a 5-min period, an observer who was blind to the treatments counted the number of squares crossed no. and time spent at corners and centers by rat.[18,19]

Assessment of anxiety-related behavior

Light-dark test (LDT)

Every rat was placed singly in the exact center of the light chamber, and their behavior was recorded for 5 minutes by aviewer who was blind to treatments using a camera (for making video) held at a height of 100 cm. The time spent and entry in the light and dark chambers were recorded for each rat.[20]

Elevated plus maze (EPM)

Every rat was placed individually in a maze with its face on the open arm, and its behavior activity was captured or recordedusing a camera (for video recording) held at 100cm of height for 5 minutes by a viewer who was blind to treatment. The time taken to enter and exit the open and closed arms were recorded for each rat.[21]

Biochemical estimation

Plasma separation from blood

On day 14th, a blood samplewas drawn, centrifuged at 2500 rpm for 10 min, and plasma was separated for biochemical testing.

Nitrite assay

An equal quantity of plasma was added with an equal quantity of Griess reagent (0.1% N-1-napt naphthyl ethylenediamine dihydrochloride; 1% sulfanilamide; and 2.5% *o*-phosphoric acid), a mixture of solution was incubating at the temp. of the room for 10 min, and absorbance at 540 nm was determined.[22]

Thiobarbituric acid reacting substances assay

To measure lipid peroxidation, 0.2 mL of blood plasma was added to 0.2 mL of sodium dodecyl sulfate; 1.5 mL of acetic acid; and 1.5 mL of thiobarbituric acid. Using water, the volume was increased to 4 mL. The mixture was further heatedfor sixty minutes ina 95 °C water bath before cooling to room temperature. 1 mL of H_2O and 5 mL *n*-butanol/pyridine mixture were added after cooling. The resulting solution was forcefully agitated and centrifuged at 4000 rpm for 10 min. Layers of organic matter were isolated and utilized to calculate absorbance at 532 nm.[23]

Experimental protocol

The current study used Wistar rats and the number of animals=10 in each group. Induction of diabetes via a single intraperitoneal injection of STZ + NAD (45 mg/kg + 50 mg/kg). Rats with diabetes levels >200 mg/dL were considered diabetic. BGL wasmeasured on days 1, 3, 7, and fourteen. The non-diabetic group was given saline. Metformin (50 mg/kg, *p.o.* and LVX (30 mg/kg)on the first day, metformin administered 30 min before STZ-NAD administration, followed by every day for 14 days. After sixty minutes of treatments, blood was drawn from the tail vein on the 14th day, and the rats were then subjected to behavioral tests for levels of anxiety using the OFT, EPM, and LDT tests. Blood plasma was used to determine malondialdehyde (MDA) and nitrite levels.[24]

Animals: Wistar rats were used in the present study.

n= 10 in each group.

1: Rats treated with vehicle (saline)

2: STZ + NAD-treated rats (45 mg/kg, *i.p.* + 50 mg/kg, (*i.p.*)

3: MET-treated rats (50 mg/kg, *p.o.)*

4: LVX-treated rats (30 mg/kg, *i.p.*)

6: MET + LVX-treated rats (50 mg/kg, *p.o.* + 30 mg/kg (*i.p.*)

Statistical analysis

Data were analyzed by "One-Way analysis of variance" (ANOVA) followed by Tukey's post hoc test, by using Graph-Pad Prism software (version 9.4.0).

Values are expressed as mean ± standard error of mean$p < 0.05$ was considered statistically significant.

RESULTS

Effects of different treatments on BGLof rats

"One-Way ANOVA" suggested the effects of different treatments on BGL at (A) day 1 ($F_{4,45} = 8.909$, $p < 0.001$) (B) day 3 ($F_{4,45} = 21.19$, $p < 0.001$), (C) day 7 ($F_{4,45} = 17.41$, $p < 0.001$) and (D) day 14 ($F_{4,45} = 0.7248$, $p = 0.5797$).

Tukey's post hoc test suggested that administration of STZ + NAD significantly increased glucose levels compared with non-diabetes (control) ($p < 0.001$). Metformin and LVX significantly decreased the sugar level of diabetic rats on the 7th day ($p < 0.01$, $p < 0.05$). Further, combined treatment with metformin and LVX significantly increased the BGL of diabetic rats on the 3rd day compared with diabetic rats ($p < 0.05$), and metformin alone treated diabetic rats ($p < 0.001$) (shown in Figure 1).

Effects of different treatments on the performance of rats in OFT

"One-Way ANOVA" suggested the effects of different treatments on no. of squarescrossed in the OFT ($F_{4,45} = 5.433$; $p = 0.0012$); the time spent in the center of the rats in the OFT($F_{4,45} = 3.960$; $p = 0.0077$), and time spent in the periphery of rats in the OFT ($F_{4, 45} = 3.766$; $p = 0.0100$).

Tukey's post hoc test suggested that metformin and levofloxacin treatment significantly reducedthe no. Of square crossed by diabetic rats ($p < 0.05$, $p < 0.01$). Furthermore, levofloxacin treatment significantly increasedthe time spentat the center ($p < 0.05$) and significantly reduced the time spent at the periphery of the open field ($p < 0.05$) as compared to its respective control group. Further, combined treatment with metformin and levofloxacin significantly decreased the time spent at the center compared with metformin alone-treated diabetic rats ($p < 0.001$) (shown in Figure 2).

Effects of different treatments on anxiety-related behavior in rats in the LDT and EPM test

"One-Way ANOVA" suggested the significant effects of different treatments on time spent in the light chamber ($F_{4,45}$=21.33; $p <$0.0001); time spentin the dark chamber ($F_{4,45} = 21.94$; $p < 0.001$), no. of entry in the light chamber ($F_{4,45} = 27.54$; $p < 0.001$) and no.of entry in the dark chamber in LDT ($F_{4,45} = 22.47$; $p < 0.001$).

Tukey's post hoc test suggested that diabetic rats spend significantly less time in the light chamber of LDT than control rats ($p < 0.05$). Metformin treatment significantly increased the time spent in the light chamber ($p < 0.001$), entries made in the light chamber ($p < 0.01$), and dark chamber of LDT ($p < 0.05$) as comparedto its respective control. Levofloxacin alone and in combination with metformin significantly decreased the entry made in the light or dark chamber of LDT as comparedto T2D rats($p < 0.001$; $p < 0.001$; $p < 0.001$; $p < 0.001$). Further, combined treatments of metformin and levofloxacin significantly decreased the time spent in the light chamber and entry into the light and dark chambers compared with metformin alone treated diabetic rats ($p < 0.001$) (shown in Figure 3).

"One-Way ANOVA" suggested a significant effect of different treatments on the time spent by the rats in the open arm ($F_{4,45} = 43.94$; $p < 0.001$), closed arm ($F_{4,45} = 55.94$; $p < 0.001$), and entries made inthe open arm ($F_{4,45} = 55.94$; $p = 0.001$) and closed arm of EPM ($F_{4,45} = 3.443$; $p = 0.0154$).

Tukey's post hoc test suggested that T2D rats spend significantly less time on the open arm and significantly more time on the closed arm as compared to the control ($p < 0.01$). Administration of levofloxacin significantly increased the time spent in the open arm and reduced the time spent in the closed arm of EPM compared with diabetic rats ($p < 0.001$, $p < 0.001$). Further, combined treatment with metformin and levofloxacin significantly increased the time spent in the open arm and reduced the time spent in the closed arm of EPM compared with metformin alone treated diabetic rats ($p < 0.001$) (shown in Figure 4).

Effects of different treatments on plasma nitrite and malondialdehyde levels in rats

"One-Way ANOVA" suggested significant effects of different treatments on nitrite levels in the plasma ($F_{45,45} = 3.801$; $p = 0.0096$) and plasma malondialdehyde levels ($F_{4,45} = 7.198$; $p < 0.001$) of rats.

Tukey's post hoc test suggested that the plasma nitrite level of T2D rats was significantly lower than that of the control ($p < 0.05$). Levofloxacin treatment significantly reduced the MDA level of T2D rats ($p < 0.05$). Furthermore, combined treatment with metformin and levofloxacin significantly increased the nitrite level ($p < 0.001$) but reduced the MDA level compared with metformin alone in diabetic rats ($p < 0.001$) (shown in Figure 5).

DISCUSSION

Diabetes mellitus is a metabolic disorder characterized by persistent increases in BGL due to abnormalities in either insulin secretion or action or both.[25] STZ is commonly used to induce experimental diabetes in experimental rodents.[26] In the present study, the administration of a single dose of STZ-induced diabetes in rats. The diabetic rats did not show any significant alterations in OFT performance compared with the

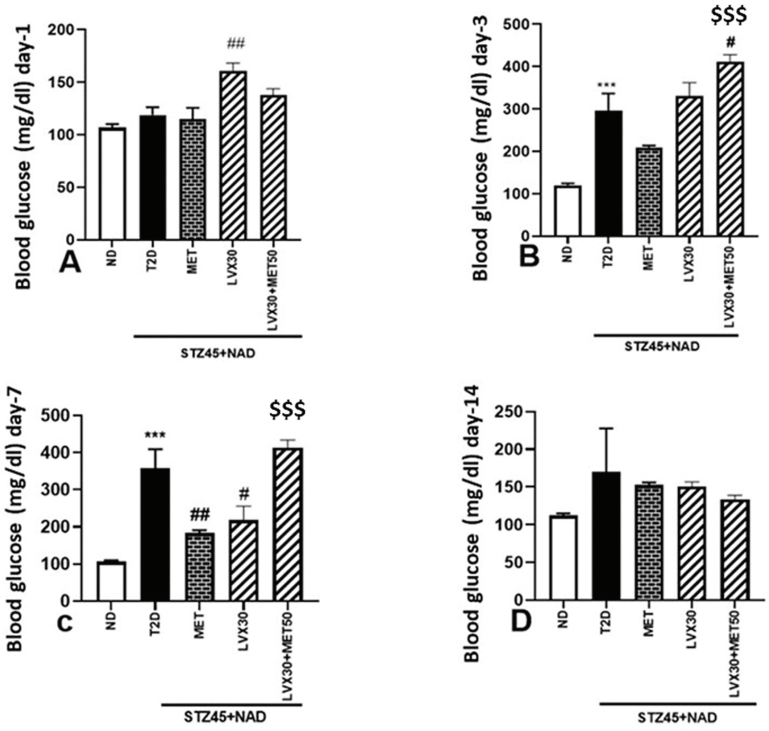

Figure 1. Effect of various treatments on the blood glucose level of rats.Values were expressed as mean ± SEM, n=10 in each group

$^{**}p < 0.01$, $^{***}p < 0.001$ significant difference from the non-diabetic rat. $^{\#}p < 0.05$,$^{\#\#}p < 0.01$ significant difference from the diabetic rat. $^{\$\$}p < 0.001$ significant difference from the metformin-treated diabetic rat

SEM: Standard error of mean

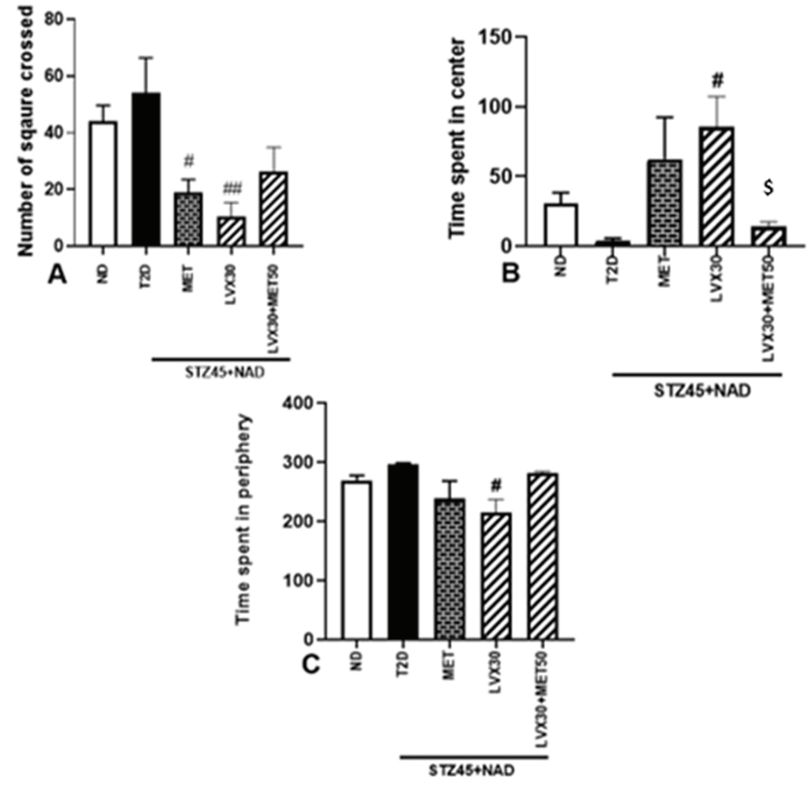

Figure 2. Effect of various treatments on the performance of diabetic rats in OFT. Values were expressed as mean ± SEM, n=10 in each group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ significant difference from the diabetic rat. $^{\$}p < 0.05$ significant difference from the metformin-treated diabetic rat

SEM: Standard error of mean

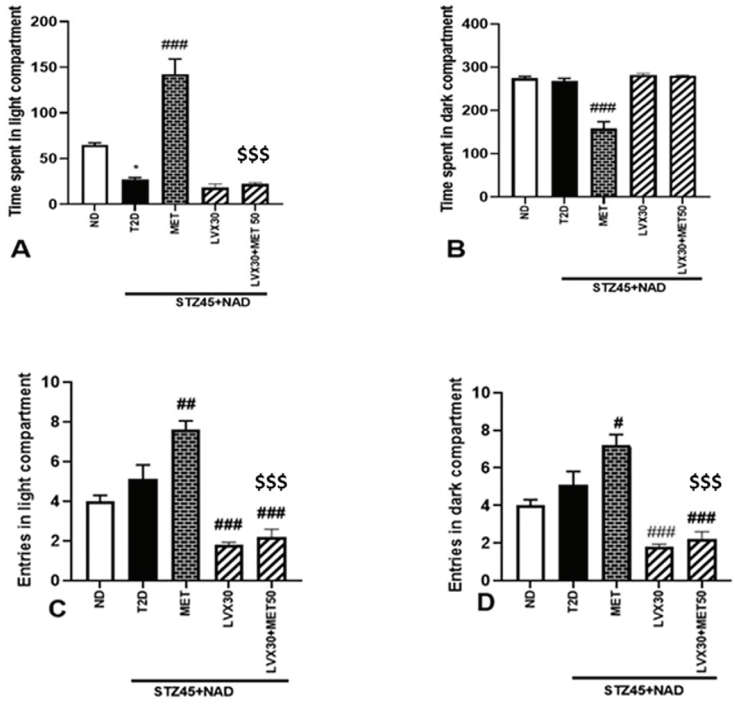

Figure 3. Effect of various treatments on the anxiety related behavior of rats in light-dark test. Values were expressed as mean ± SEM, n=10 in each group. $^{*}p < 0.05$ significant difference from the non-diabetic rat. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$significant difference from the diabetic rat. $^{\$\$\$}p < 0.001$significant difference from the metformin-treated diabetic rat

SEM: Standard error of mean

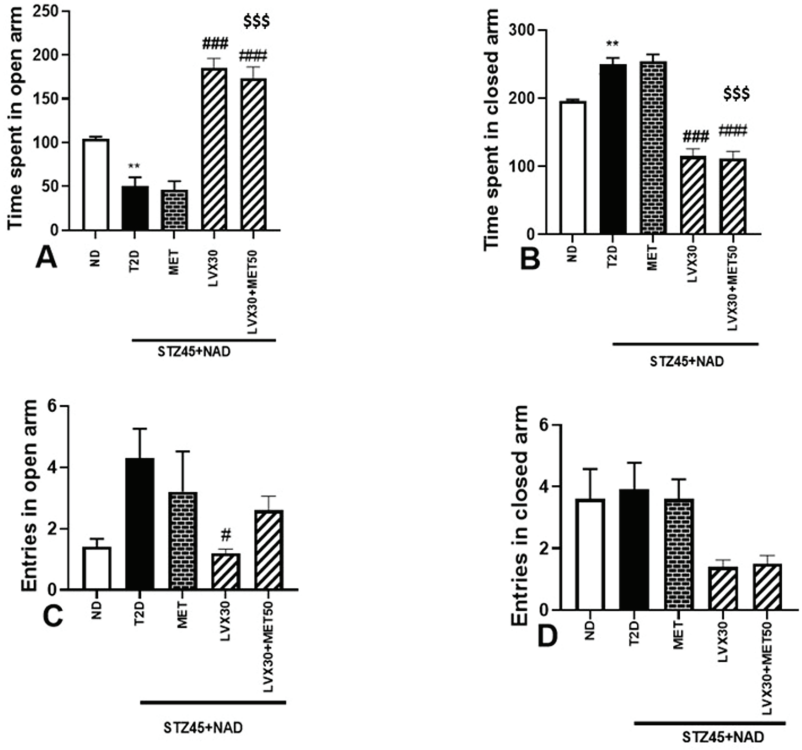

Figure 4. Effect of various treatments on the anxiety-related behavior of rats in EPM test. Values were expressed as mean ± SEM, n=10 in each group. $^{**}p < 0.01$, significant difference from the non-diabetic rat. $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$significant difference from the diabetic rat. $^{\$\$\$}p < 0.001$significant difference from the metformin-treated diabetic rat

SEM: Standard error of mean

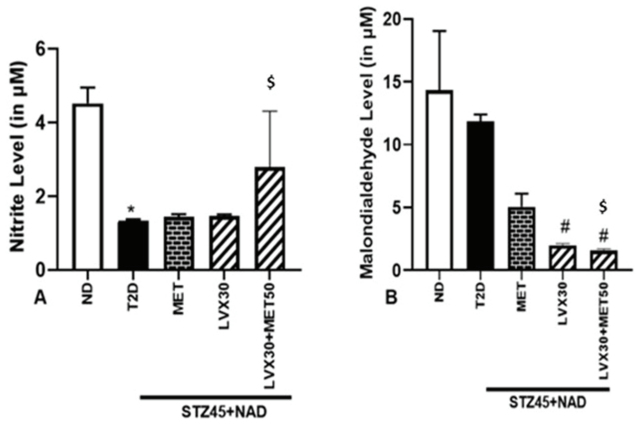

Figure 5. Effect of various treatments on the plasma nitrite and MDA level of rats. Values were expressed as mean ± SEM, n=10 in each group. *p <0.05 significant difference from the non-diabetic rat. #p <0.05 significant difference from the diabetic rat. $$p$ <0.05 significant difference from the metformin-treated diabetic rat

SEM: Standard error of mean

control group. However, diabetic rats displayed anxiety-related behavior parameters in the LDT or light and dark box and EPM tests, which were evident by reducing the time spent in the light chambers of LDT and the open arm of EPM. Prior studies have suggested that diabetic rats exhibit anxiety-related behavior.[27] In this current study, diabetes rats showed a significant increase in MDA level in blood plasma and a significant reduction in nitrite level in blood plasma compared with the control group. STZ treatment has been shown to influence glucose, nitric oxide (NO), and MDA levels.[28-30] However, several studies revealed different effects of STZ on nitrite levels; for example, one study suggested a rise in nitrite levelsin blood plasma after STZ therapy,and other studies suggested a decrease in nitrite after STZ treatment. STZ therapy has been demonstrated to increase MDA levels in diabetic rats.[31,32]

In the present study, metformin treatment reduced the BGL of diabetic rats. Metformin is mainly used for T2D mellitus treatment. Metformin reduced BGL without increasing insulin secretion but by increasing the effects of insulin. Thus, metformin is referred to as "insulin sensitizer". Metformin inhibits hepatic glucose synthesis by reducing the rate of gluconeogenesis and glycogenolysis. Metformin also increases peripheral glucose disposal by promoting glucose disposal in skeletal muscle. It normally does not cause low blood sugar levels, which makes it a unique anti-diabetic medicine.[33] In the open field test, metformin treatment significantly decreased the total no. of square crossed and reversed the anxiogenic effect of STZ in LDT only. Previous studies have suggested an anxiolytic-like effect of metformin treatment in diabetic rats.[34,35] It was established in a previous study that metformin treatment displayed a rapid anxiolytic effect without tolerance due to the upregulation of Gamma-aminobutyric acid (GABA)-Areceptors.[36] In the present study, metformin treatment did not affect the nitrite and MDA levels of diabetic rats. A previous study suggested that the concentration of NO was significantly increased following metformin therapy.[37] Reactive oxygen species production is directly related to the increase in lipid peroxidation, and insulin resistance is mainly associated with lipid peroxidation. Furthermore, metformin administration has been shown to decrease lipid peroxidation.[38-40]

In this study, the administration of levofloxacin increased the glucose level at day 1. Furthermore, levofloxacin significantly reduced the glucose level in diabetic rats for 7 days. It has been reported that FQs may cause severe low BGL by increasing insulin secretion.[41] Depending on the dosage, FQs raise insulin levels in the blood via an adenosine triphosphate-sensitive K^+ blockade pathway.[42] Further, the insulin-tropic effect of FQs developed as a result of the stimulatory effects of beta-cell nutrition rather than the initial production of insulin.[43] Srividhya et al.,[44] suggested that the administration of either gatifloxacin or levofloxacin was associated with hyperglycemia rather than hypoglycemia in elderly patients. Levofloxacin administration in diabetic rats significantly increased the time spent at the center and decreased the time spent at the periphery of an open field. Further, in the LDT, levofloxacin treatment significantly reduced the entry made by diabetic rats in the light and dark boxes of LDT, whereas in the EPM test, levofloxacin treatment significantly increased the time spent in the open arm and significantly reduced the time spent in the closed arm EPM. Levofloxacin treatment significantly decreased the entry made by diabetic rats in the open arm of EPM. Thus, levofloxacin treatment exerted anxiolysis in the EPM test. Previous studies reported that the administration of levofloxacin (10-20-40 mg/kg i.p.) did not induce a depression-like response in the forced swim test but displayed an anxiety-like response in the EPM test in rats with no change in locomotor activity.[45,46] It has been reported that quinolones prevent the binding of GABA and thus increase central nervous system stimulation.[47] Furthermore, quinolones activate N-methyl-D-aspartate receptors or adenosine receptors and exert anxiogenic effects.[48-51] Levofloxacin treatment did not affect the plasma nitrite level but significantly decreased the plasma MDA level of diabetic rats.

In the present study, it was observed or research findings that the combined treatment of levofloxacin and metformin increased the glucose level of diabetic rats at 3rd-day. Furthermore, the combination of levofloxacin and metformin treatment did not affect the OFT performance of diabetic rats. Combined treatment with levofloxacin and metformin significantly decreased the entry in LDT. Combined treatment with levofloxacin and metformin significantly increased the time spent in the open arms and significantly reduced the time spent in the closed arms of EPM. The combined treatment of levofloxacin and metformin did not affect the nitrite level but significantly reduced the MDA level in diabetic rats. We will determine blood glucose insulin sensitivity and lipid peroxidation to determine possible pathophysiological alterations.

CONCLUSION

In conclusion, the current study showed that levofloxacin treatment had antihyperglycemic effects in diabetic rats. Thus,

levofloxacin might be repurposed for diabetes alleviation. Furthermore, combined treatment with levofloxacin and metformin may modulate glucose levels and anxiety-related behavior parameters. Thus, caution should be exercised while administering these drugs together.

Ethics

Ethics Committee Approval: The study protocols were approved by the Institutional Animal Ethics Committee, Maharshi Dayanand University, Haryana (approval number: 1767/RE/S/14/CPCSEA: 31.08.2017, dated: 14.12.2018).

Informed Consent: Not required.

Surgical and Medical Practices: P.S., Concept: P.K.V., Design: V.W., Data Collection or Processing: P.S., Analysis or Interpretation: P.K.V., Literature Search: P.S., Writing: P.S.

Conflict of Interest: The authors have no conflicts of interest to declare.

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