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

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ABSTRACT
Objective: The current experiment was conducted to investigate the combined effect of levofloxacin and metformin treatment on glucose level in blood, MDA, nitrite, level, anxiety of STZ+NAD induced diabetic rats.
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 exceed 200 mg/dl (verified on the third day). Saline was administered to non-diabetic rats (controls). The diabetic rats were given alone metformin (50 mg/kg, p.o.), levofloxacin (30 mg/kg, i.p.), or metformin + levofloxacin for fourteen days. The blood sample was obtained after the 14th day of therapy, and the rats were given to behavioral parameter to determine locomotor-activity and anxiousness level. Blood plasma sample has been separately and used for determination of nitrite and malondialdehyde (MDA) level.
Results: It was observed that the combined treatment of metformin and levofloxacin significant rise glucose in blood of diabetic rats as compares to diabetic control ($P < 0.05$) and alone metformin treated diabetic rats ($P < 0.001$) at day-3 and day-7. Further, combined treatment of metformin and levofloxacin significantly reduce time spend at center of OFT ($P < 0.001$), significantly reduce time spend and entry made in light chamber of LDT ($P < 0.001$), significantly rise time spend in open arm and reduce the time spent in closed arm of EPM ($P < 0.001$) as compare to alone metformin treated diabetic rats. Further, combined treatment of metformin and levofloxacin significant increase nitrite level, ($P < 0.001$) but reduce the MDA level in plasma as compare to metformin alone treated-diabetic rats ($P < 0.001$).
Conclusion: In conclusion, the present study suggested the combined treatments of levofloxacin and metformin may modulate the glucose level and anxiety-related activity.
Keywords: Diabetes, glucose, malondialdehyde, anxiety, levofloxacin.

1. Introduction

Diabetes is the most common diseases worl-dwide, there is also rise the cases of morbidity and mortality due to this disorder [1]. Diabetics struggle to regulate their fluctuating blood glucose levels [2], which can result in fatalities, permanent strokes, and heart attacks [3], Fluoroquinolones 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 blood sugar levels [4], that 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 hyperglycemic environment; lower production of interleukins; reduced immunity, and urinary dysmotility in diabetes is accompanied by the emergence of various infections (7). The fluoroquinolones drugs (FQs) are commonly used to treat a variety of illnesses and have the pharmacokinetic advantages, good penetration and high oral bioavailability. However, FQs are associated with the increased incidence of tendon rupture, peripheral neuropathy, and fluoroquinolone-associated aortic aneurysm and aortic dissection (AA/AD) (8). While blood sugar abnormalities caused by fluoroquinolones are uncommon, they are a serious and potentially fatal consequence that is more likely to occur in elderly, suffering from diabetes, and kidney failure individuals [9], Beside this FQ may have been associated with hypoglycemia by increase the pancreatic insulin (10). Furthermore, the administration of levofloxacin in the diabetic rats revealed hypoglycemic effects (11). Previous studies have suggested that the high level of hypoglycemia in diabetic patients are associated with the use of levofloxacin (12). FQs possess insulinotropic activity at the clinically relevant concentration and thus enhances the glucose-induced insulin secretion (13). Further, the risk of hypoglycemic emergency increases with the

combination of levofloxacin with insulin or sulfonylurea (14). Also, a recent survey revealed the hazardous interactions between the sulfonylureas and antimicrobials when used together (15). In the present study, authors studied the combined effect of levofloxacin and metformin in rats with diabetes.

Materials and Methods

Animal

Male, body wt. 150-200 g, wistar rats were obtained from disease-free animal house at the *** University of Veterinary and Animal Sciences (LUVAS) in Hisar and kept in the Central Animal House at the *** University in Haryana under controlled lighting and environmental condition, with unrestricted access to nutritious food and water. The rats were given time to adjust to laboratory circumstances before the experiment, which conducted place between the hours of 9:00 and 17:00. The study's protocols were authorized by the Institutional Animal Ethics Committee, *** University, Haryana (Letter number: 1767/RE/S/14/CPCSEA:31.08.2017; Project No.2; Dated: 17.12.2018). Animals were cared properly according to the requirements of 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. L.t.d, India); metformin (Cipla Pvt. Ltd, India); nicotine adenine dinucleotide (NAD) (Central Drug House, India), were used. All treatments were given in an unchanged volume (amount) of 5 ml/kg, i.p., STZ solution was freshly preparation at pH 4.5 in 0.1 M citrate buffer.

Induction of diabetes

Induction of diabetes by given a single intra-peritoneal dose of STZ+NAD (45 mg/kg + 50 mg/kg. On days 1, 3, 7, and 14, blood glucose levels (BGL) were monitored using a glucometer. Diabetic rats are characterized as having glucose in blood more than 200 mg/dl. (16, 17).

Loco-motor activity

Rat were placed alone in center of an open field, its behavior activity was monitored by using a camera (for video making) set up at of 100 cm of height. Over a 5-minute period, an observer who was blind to the treatments counted the squares crossed no. and time spend at corner and center by rat (18, 19).

Assessment of anxiety-related behavior

Light dark test (LDT)

Every rat was put singly in exact center of light chamber, way they behaved was record for 5 minutes by a viewer who was blind to treatments by using a camera (for making vedio) held at of 100 cm of height. The time spend and entry made in light and dark chambers were recording for each and every rat (20).

Elevated plus maze (EPM)

Every rat was put in individually in maze with its face on open arm, and its behavior activity was captured or record by using a camera (for video recording) held at 100cm a of height of for 5 minutes by a viewer who was blind to treatments. The time spend and entry made in open and closed arms were recorded for each rat (21).

Biochemical estimation

Plasma separation from blood

On the day-14th, sample of blood was drawn, centrifuge at 2500 rpm for ten minutes, and plasma was separated for biochemical testing.

Nitrite assay

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

Thiobarbituric acid reacting substances (TBARS) assay

To measure peroxidation of lipids, 0.2 ml of blood plasma was added to with 0.2 ml of SDS; 1.5 ml of acetic acid; and 1.5 mL of TBA. Using water, volume was increased to 4 ml. Mixture was further heating for sixty minutes on 95°C water bath before being cooled to room temperature. 1 mL H2O and 5 ml n-butanol/pyridine mixture were added after cooling. The resulting solution was forcefully agitated and centrifuged at 4000 rpm for ten minutes. Layer of organic matter was isolated and utilized to calculate absorbance at 532 nm (23).

Experimental protocol

The current study used wistar rats and number of animals = 10 in every group). Induction of diabetes by a single intraperitoneal injection of STZ + NAD (45 mg/kg + 50 mg/kg). Diabetes rats levels more than 200 mg/dl were consider as diabetic. Blood glucose levels were measured on days one, three, seven, and fourteen. The non-diabetic group were given saline. Metformin (50 mg/kg, p.o. and LVX (30 mg/kg) on the first day, metformin given 30 minutes before the STZ-NAD administration, it was followed by every day given 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- assessment using this OFT, EPM, LDT tests. Blood plasma was used for determination of MDA and nitrite level (24).
Animals: Wistar rats were used in the present study.
n = 10 in each group.
1: Vehicle-treated rats (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 ± S.E.M. $P < 0.05$ was considered as statistically significant.

Results

Effect of different treatments on blood glucose level of rats.

"One-way ANOVA" suggested that effects of different treatment on the blood glucose at (A) day 1 ($F_{4,45}$=8.909, P<0.001) (B) day 3 ($F_{4,45}$=21.19, P<0.001), (C) 7 day ($F_{4,45}$=17.41, P<0.001) and (D) 14 days ($F_{4,45}$=0.7248, P = 0.5797).

Tukey's post hoc test, suggested that administration of STZ+NAD significant increase glucose level as compare to non-diabetic (control) (P<0.001). Metformin and levofloxacin significantly decrease sugar level of diabetic rats at 7th day (P<0.01, P<0.05). Further, combined treatment of metformin and levofloxacin significant increase BGL of diabetic rats at 3rd day as compare to diabetic rats (P < 0.05), metformin alone treated diabetic rats (P < 0.001) (shown in figure-1).

Effect of different treatments on performance of rats in OFT.

"One way ANOVA" suggested that effects of different treatment on no. of square crossed in OFT ($F_{4, 45}$ =5.433; P = 0.0012); the time spend in center of rats in OFT ($F_{4, 45}$ =3.960; P = 0.0077) time spend in periphery of rats in OFT ($F_{4, 45}$ = 3.766; P = 0.0100).

Tukey's post hoc test, suggested the metformin and levofloxacin treatment significantly reduce the no. of square crossed by the diabetic rats (P<0.05, P<0.01). Further, levofloxacin treatment significant increase time spend at center (P<0.05) and significantly reduce time spend at periphery of open field (P<0.05) as compared to its respective control group. Further, combined treatment of metformin and levofloxacin significantly decreased the time spend at the center as compare to metformin alone treated diabetic rats (P < 0.001) (shown in figure-2).

3.3 Effect of different treatment on anxiety related behavior of rats in LDT and EPM test.

"One-way ANOVA" suggest the significant effects of different treatments on time spend in light chamber (F4,45=21.33; P<0.0001); time spend in the dark chamber (F4,45=21.94; P<0.001), no. of entry in the light chamber (F4,45=27.54; P<0.001) and no. of entry in dark chamber in LDT (F4,45=22.47; P<0.001).

The Tukey's post hoc test, suggested that diabetic rats spend significantly less time in light chamber of LDT as compared to control rats (P<0.05). Metformin treatment significantly increase the time spent in the light chamber (P<0.001), entries made in light chamber (P<0.01) and dark chamber of LDT (P<0.05) as compare to its respective control. Levofloxacin alone and in combination with metformin significant decrease the entry made in light chamber or dark chamber of LDT as compare to T2D rat (P<0.001; P<0.001; P<0.001; P<0.001). Further, combined treatments of metformin and levofloxacin significantly decreased time spend in light chamber, entry made in light chamber and dark chamber as compare to metformin alone treated diabetic rats (P < 0.001) (shown in figure-3).

"One -way ANOVA" suggested the significant effect of different treatments on the time spend by the rats in open arm ($F_{4,45}$=43.94; P<0.001), closed arm ($F_{4,45}$=55.94; P<0.001), entries made in 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 T2D rats spend significant less time in open arm and significant more time in closed arm as compare to control (P<0.01). Administration of levofloxacin significant rise time spend in open arm and reduce time spend in closed arm of EPM as compares to diabetic rats (P<0.001, P<0.001). Further, combined treatment of metformin and levofloxacin significant rise the time spend in open arm and reduce time spend in closed arm of EPM as compare to metformin alone treated diabetic rats (P < 0.001) (shown in figure-4).

Effect of different treatments on plasma nitrite and malondialdehyde levels of rats

"One way ANOVA" suggested that significantly effects of different treatments on nitrite levels in plasma (F45,45=3.801; P=0.0096) and plasma malondialdehyde levels (F4,45=7.198; P<0.001) of rats.

Tukey's post hoc test, suggested the plasma nitrite level of T2D rats was significantly lesser than the control (P<0.05). Levofloxacin treatment significantly reduce MDA level of T2D rats (P<0.05). Furthermore, combined

treatment of metformin and levofloxacin significantly increased the nitrite level ($P < 0.001$) but reduce MDA level as compares to alone metformin treated diabetic rats ($P < 0.001$) (shown in figure-5).

4. Discussion

Diabetes mellitus is a metabolic disorder characterized by the persistent rise in the blood glucose levels due to the abnormalities in either insulin secretion or action or both (25). The STZ is commonly used in induction of experimental diabetes in experimental rodents (26). In the present study, administration of single dose of STZ induced the diabetes in rats. Diabetic rats did not show any significant alterations in the performance in the OFT as compared to control. However, diabetic rats displayed anxiousness-related behavior parameter in LDT or LDB and EPM test evident by reduce time spend in light chambers of LDT and open arm of EPM. Prior studies have suggested that the diabetic rats displayed anxiousness-related behavior (27). In this current study, diabetes rats shown significant increase in MDA level in blood plasma and significant reduction of nitrite level in blood plasma compare to control. STZ treatment has been showed to influence glucose, NO, and MDA levels. (28, 29, 30). However, several research revealed different effects of STZ on nitrite level, for example, one study suggested a rise in nitrite level in blood plasma after STZ therapy, and other studies suggested a decrease in nitrite after the STZ treatment. STZ therapy has been demonstrated to rise MDA levels in diabetic rats. (31, 32). In the present study, metformin treatment reduces the BGL of diabetic rats. Metformin is mainly used for the treatment of T2DM. Metformin were reducing the blood glucose level without rising insulin secretion but by increasing effects of insulin. Thus, metformin is referred as “insulin sensitizer”. Metformin inhibits hepatic glucose synthesis by reducing the rate of gluconeogenesis and glycogenolysis. Metformin also increases the peripheral glucose disposal by increasing the glucose disposal in skeletal muscle. It normally doesn't cause low blood sugar, which makes it a unique anti-diabetic medicine (33). In the open field test, metformin treatment significant decrease the total no. of square crossed and reversed the anxiogenic effect of STZ in LDT only. Previous studies have suggested the anxiolytic like effect of metformin treatment in diabetic rats (34, 35). It has been established in previous study that metformin treatment displayed a rapid anxiolytic effect, without tolerance due to the up-regulation of GABA-A receptors (36). In the present study, metformin treatment didn’t affect the nitrite and MDA level of diabetic rats. Previous study has suggested that the concertation of nitric oxide (NO) significantly increased following metformin therapy (37). ROS production is directly related to the increase in lipid peroxidation and the insulin resistance is mainly associated with the lipid peroxidation. Further, administration of metformin has been shown to decrease the lipid peroxidation (38, 39, 40).

In this current study, administration of levofloxacin increases the glucose level at 1-day. Further, levofloxacin significantly reduces the glucose-level in diabetic rats at 7- day. It has been reported that the FQs may cause severe low blood glucose by raising the insulin secretion (41). Depending on the dosage, FQs raise insulin levels in the blood via an ATP-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). Wang et al, has suggested that the administration of either gatifloxacin or levofloxacin, was associated with hyperglycemia than hypoglycemia in elderly patients (44). Levofloxacin administration in the diabetic rats significant increase time spend at center and decreased time spent at periphery of open field. Further, in LDT levofloxacin treatment significantly reduce the entry made by diabetic rats in the light and dark box of LDT while in EPM test levofloxacin treatment significantly rise the time spend in the open arm and significantly reduce time spend in closed arm EPM. Levofloxacin treatment significantly decreased the entry made by diabetic rats in open arm of EPM. Thus, levofloxacin treatment exerted anxiolysis in EPM test. Previous studies reported that the administration of Levofloxacin (10-20-40 mg/kg i.p.) did not have a depression-like response in the FST but displayed an anxiety-like response in the EPM test in rats with no change in loco-motor activity (45, 46). It has been reported that the quinolones prevent the binding of GABA and thus raise stimulation of CNS (47). Further, quinolones activate N-methyl-d-aspartate receptors or adenosine receptors and exerts anxiogenic effects (48, 49, 50,51). Levofloxacin treatment didn’t affect the nitrite level in plasma but significant decrease plasma MDA level of diabetic rats.

In the present study, it was observed or research findings that the combined treatment of levofloxacin and metformin rise the glucose-level of diabetic rats at 3rd-day-. Furthermore, the combination of levofloxacin and metformin treatment didn’t affect performance of diabetic rats in OFT. Combined treatment of levofloxacin and metformin significantly decreased the entry in LDT. Combined treatment of levofloxacin and metformin significant increase time spend in the open arm and significantly reduce the time spend in closed arm of EPM. Combined treatment of levofloxacin and metformin didn’t affect the nitrite level but significantly reduce MDA level of diabetic rats. We find out the blood glucose insulin sensitivity and lipid peroxidation to determine possible pathophysiological alterations.

In conclusion, the current study suggested the levofloxacin treatment shown antihyperglycemic effects in diabetic rats. Thus, levofloxacin might be repurposed for the alleviation of diabetes. Further, the combined treatment of levofloxacin and metformin may modulate the glucose level and anxiety-related behavior parameter. Thus, the caution should be taken while administering these drugs together.

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Wrote the main manuscript text: Poonam Singh (Animal experimentation and manuscript writing).
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Conflict of Interest

Please check the following as appropriate:

1. All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.
2. This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.

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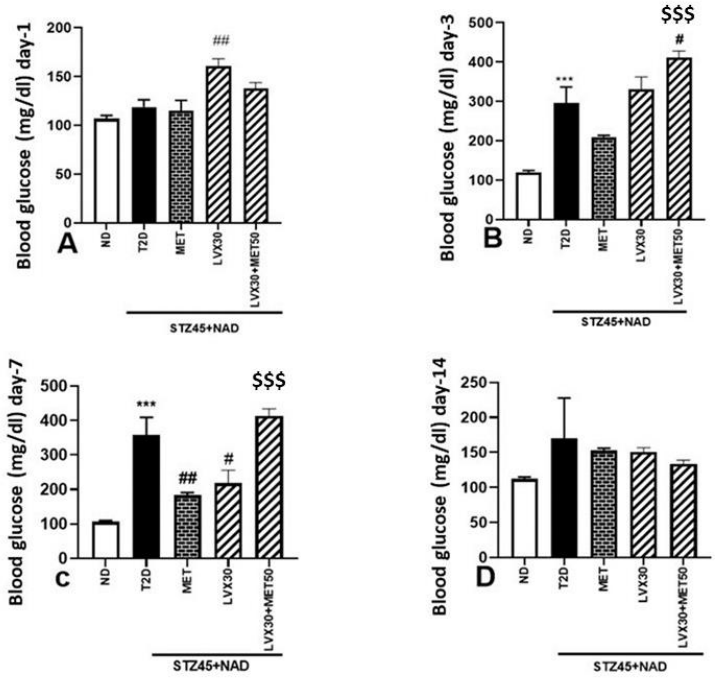

Figure: 1. Effect of various treatments on the blood glucose level of rats.
Values were expressed as mean ± S.E.M. n=10 in each group. **P<0.01, ***P<0.001 significant difference from the non diabetic rat. #P<0.05, ##P<0.01 significant difference from the diabetic rat. $$P<0.001 significant difference from the metformin treated diabetic rat.

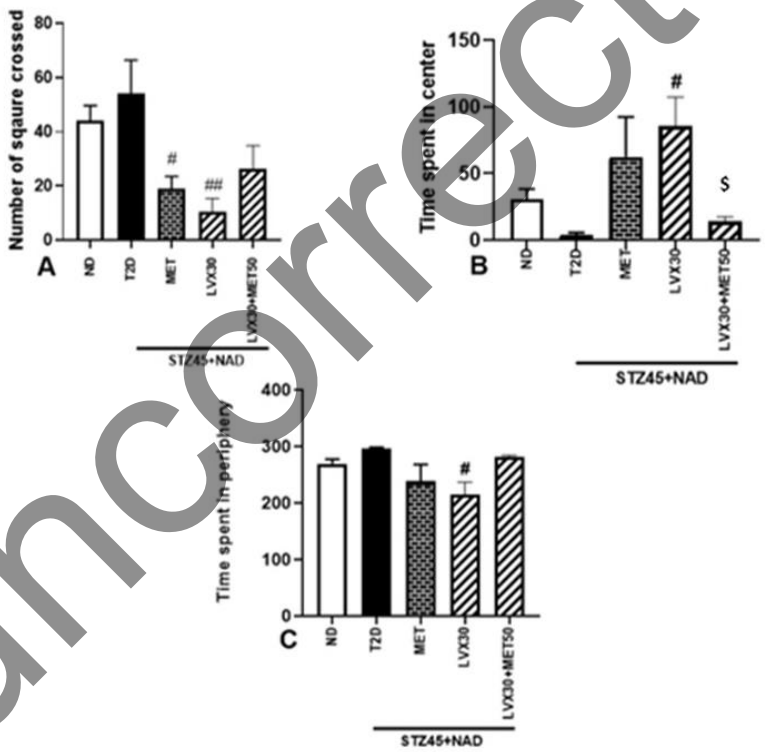

Figure: 2. Effect of various treatment on the performance of diabetic rats in OFT
Values were expressed as mean ± S.E.M. n=10 in each group. #P<0.05, ##P<0.01 significant difference from the diabetic rat. $P<0.05 significant difference from the metformin treated diabetic rat.

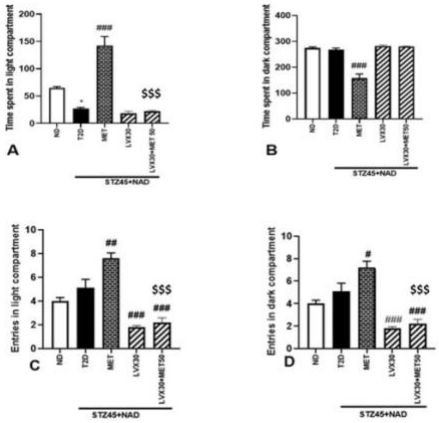

Figure: 3. Effect of various treatment on the anxiety related behavior of rats in light-dark test.
Values were expressed as mean ± S.E.M. n=10 in each group. *P<0.05 significant difference from the non-diabetic rat. #P<0.05, ##P<0.01, ###P<0.001significant difference from the diabetic rat. $$$P<0.001significant difference from the metformin treated diabetic rat.

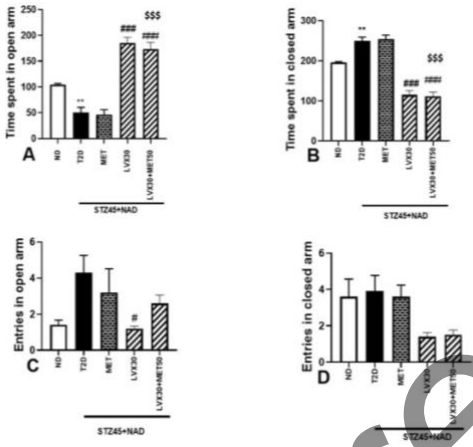

Figure: 4. Effect of various treatment on the anxiety related behavior of rats in EPM test
Values were expressed as mean ± S.E.M. n=10 in each group. **P<0.01, significant difference from the non-diabetic rat. #P<0.05, ###P<0.001significant difference from the diabetic rat. $$$P<0.001significant difference from the metformin treated diabetic rat.

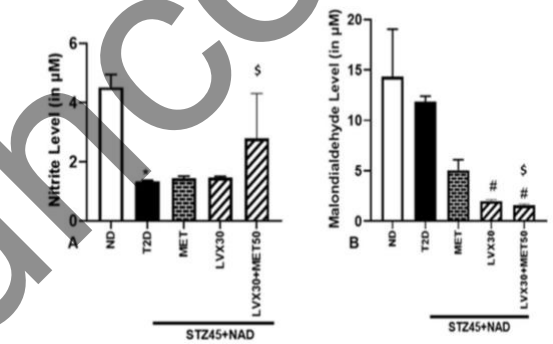

Figure: 5. Effect of various treatments on the plasma nitrite and MDA level of rats
Values were expressed as mean ± S.E.M. n=10 in each group. *P<0.05 significant difference from t -diabetic rat. #P<0.05 significant difference from the diabetic rat. $P<0.05 significant difference from the metformin treated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