Possible modulation of the pharmacological effect of metformin by pyridoxine in diabetic rats

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Abstract: The present study was designed to determine the possible modulation of the pharmacological effect of metformin by pyridoxine in diabetic rats. Male wistar rats were made diabetic with a single injection of streptozotocin (45 mg/kg, i.p.). The rats with blood glucose level (BGL) greater than 200 mg/dl were considered as diabetic rats. Rats were divided into five groups: Group-1: Control rats; Group-2: Diabetic rats; Group3: Metformin (10 mg/kg/day p.o for 14 days) treated rats, Group-4: Pyridoxine (50 mg/kg/day i.p. for 14 days) treated rats, Group-5: Pyridoxine (50 mg/kg/day i.p. for 14 days) + Metformin (10 mg/kg/day p.o for 14 days) treated rats. After 14 days of treatment, diabetic rats showed significant increase in BGL, depression-related behavior, MDA level but decreased plasma nitrite level. Metformin treatment significantly abolished BGL, differentially modulated the depression-related behavior in TST and FST, and increased the plasma nitrite and MDA level of diabetic rats. Further, the combined administration of pyridoxine and metformin to the diabetic rats did not affect BGL, depression-

related behavior, plasma nitrite, and MDA level significantly as compared to pyridoxine or metformin alone treated diabetic rats. Thus, it was concluded that pyridoxine administration did not modulate the effect of metformin in diabetic rats.

Keywords: Diabetes, Pyridoxine, Metformin, Glucose, Nitrite

1. Introduction

Depression occurs frequently in patients with diabetes (Petrak et al., 2018). The prevalence rates of depression could be up to three times higher in patients with type 1 diabetes and twice as high in people with type 2 diabetes compared with the general population worldwide (Roy and Lloyd, 2012). The insulin signaling system has been proposed as a novel target for depression (Lyra et al., 2019; Watson et al., 2018; Kan et al., 2013). Various studies have suggested that altered insulin signaling is implicated in the pathophysiology of depression (Woo et al., 2020; Kan et al., 2013). Metformin is the most frequently prescribed hypoglycemic agent (He and Wondisford, 2015; Pernicova and Korbonits, 2014; Ferrannini, 2014) and has been shown to depression in diabetic patients (Papachristou and Papanas, 2021) through several mechanisms including by (Fang et al., 2020). Metformin also modulates brain noradrenaline and serotonin level (Shivavedi et al., 2017) and exerts anti-inflammatory and antioxidant activities (Keshavarzi et al., 2019). Previous studies revealed that diabetes is often accompanied by a reduced plasma level of pyridoxine (Okada et al., 1999). Besides this the deficiency of pyridoxine is also responsible for the emergence of depression and related behavioral alterations (Stover and Field, 2015) and the supplementation of vitamin B6 lowers the incidence of depression (Mozaffari et al., 2021). Pyridoxine treatment also potentiates insulin secretion and insulin sensitivity (Toyota et al., 1981; Karalis et al., 2020) and modulates the neurotransmitters (i.e. nitric oxide, 5-HT, noradrenaline, etc) implicated in the pathogenesis of depression and related behavior (Walia et al., 2018; Maratha et al., 2022) of which NO influence both influence the depression-related behavior and insulin secretion (Dhir and Kulkarni, 2011; Nystrom et al., 2012; Bahadoran et al., 2020). Further, the increased intake of pyridoxine improves glycemic control, and depression-related behavioral alterations (Okada et al., 1999). However, its effect in combination with oral hypoglycemic agents is not investigated. The present study was designed to determine the possible modulation of the pharmacological effect of metformin by the pyridoxine in diabetic rats.

2. Materials and Methods

2.1. Animal

In the present study, wistar rats (male, 150-200 g) were used and procured from disease free small animal house, Lala Lajpat Rai University of Veterinary and Animal Sciences (LLRUVAS), Hisar. All the rats were kept under controlled light and environmental conditions and had free access to food and water. Rats were allowed to acclimatize to laboratory conditions before the experiment. All the experiments were carried out between 9:00 and 17:00 h. The experimental protocols were approved by Institutional Animal Ethics Committee (1767/RE/S/14/CPCSEA:31.08.2017; Protocol No.4; Approval Date: 14.12.2018). Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, Ministry of Environment and Forest Government of India, were followed for animal care.

2.2. Drugs and treatments

Pyridoxine (B) (LobaChemie, Maharashtra, India); streptozotocin (STZ) (Central Drug House, India), and metformin (Sigma Aldrich, India) were used in the present study. All the treatments were administered in a constant volume of 5 ml/kg, i.p. except insulin (1 unit/200g rat, s.c. daily for 14 days).

2.3. Induction of DM and measurement of blood glucose

DM was induced by a single i.p. injection of STZ (45 mg/kg), prepared freshly in the 0.1 M citrate buffer (pH 4.5). The STZ-treated rats were received 5% glucose solution to prevent the risk of hypoglycemia (Sunmonu and Afolayan, 2013). On day 14, blood glucose level was determined using a glucometer (Arokiyaraj et al., 2011). The rats were considered diabetic if blood glucose level was greater than 200 mg/dl (Sunmonu and Afolayan, 2013; Arokiyaraj et al., 2011).

2.4. Determination of behavioral alterations in rats

2.4.1. Assessment of Locomotor Activity using Open Field Test (OFT)

Rats were individually placed at the center of open field to record their behavior with the help of a video camera (placed at a height of 100 cm) for 5 min. The number of squares crossed, time spent at center and the corner by the rat was determined by an observer blind to the treatments.

2.4.2. Tail Suspension Test (TST)

Rats were suspended individually using adhesive tape placed approximately 1 cm from the tip of the tail at a height of 50 cm from the ground. A video camera placed at a height of 100 cm was used for recording. The total immobility period was then determined over a period of 6 min. The observer was blind to treatments. Immobility is considered when the rats hung passively without any movements (Steru et al., 1985).

2.4.3. Forced Swim Test (FST)

Rats were placed individually in a vessel filled with water at 25±30C and a video camera was placed at a height of 100 cm to record the behavior. The total immobility period was then determined for last 4 min over a total observation period of 6 min. The observer was blind to treatments. Rats were considered immobile when they float passively without any movement (Porsolt et al., 1977).

2.5 Biochemical estimation

Following the continuous treatments for 14 days, the blood sample was collected for the collection of plasma. The blood collected from the rats was centrifuged at 2500 rpm for 10 min. Plasma was separated and used for the biochemical assays.

2.5.2. Nitrite assay

In brief, an equal volume of plasma was mixed with an equal volume of Griess reagent (0.1% of N-1-napththyl ethylenediamine dihydrochloride, 1% sulphanilamide, and 2.5% o-phosphoric acid), the mixture was incubated for 10 min at room temperature and absorbance was measured at 540 nm (Green et al., 1982).

2.5.3. Malondialdehyde (MDA) assay

In brief, 0.2 mL of plasma was mixed with 0.2 mL SDS, 1.5 mL acetic acid, and 1.5 mL TBA. The volume was made up to 4 ml using water. The solution was then heated at 95°C for 60 min in a water bath followed by cooling at room temperature. After cooling, 1 mL water and 5 mL n-butanol/pyridine mixture was added. The resultant solution was shaken vigorously and centrifuged at 4000 rpm for 10 min. The organic layer was separated and used for the determination of absorbance at 532 nm (Ohkawa et al., 1979).

2.6. Experimental protocol

Male wistar rats were used in the present study. The rats were divided into different groups and each group contains an equal number of rats (*n* = 10 in each group). The rats in the control group received saline and were considered non-diabetic rats. Selected dose includes metformin (10 mg/kg/day p.o. ×14 days) (Kamboj et al., 2013), and pyridoxine (50 mg/kg, i.p × 14 days) (Maratha et al., 2022). The control group received saline and Diabetic rats received metformin (10 mg/kg/day p.o. for 14 days) alone and in combination with pyridoxine (50 mg/kg, i.p for 14 days). Following administration on the 14th day, the blood sample was collected, and plasma was separated and used for nitrite and malondialdehyde (MDA) assays. The rats were subjected to behavioral assays for the determination of locomotor activity and depression-related behavioral alterations. The behavioral alterations were determined using OFT, TST, and FST (Steru et al., 1985; Porsolt et al., 1977) on the 14th day.

Group-1. Vehicle-treated rats (non-diabetic rats)

Group-2. STZ (45 mg/kg, i.p.) treated rats (diabetic rats)

Group-3. Metformin (10 mg/kg, po for 14 days) treated diabetic rats

Group-4. Pyridoxine (50 mg/kg, ip for 14 days) treated diabetic rats

Group-5. Metformin + Pyridoxine treated diabetic rats

2.8. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test by using GraphPad Prism software (version 9.3.4). Values are expressed as Mean ± S.E.M. $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Effect of metformin treatment on the various behavioral and biochemical parameters of STZ-induced diabetic rats

Administration of STZ-induced diabetes in the experimental rats. Administration of metformin alleviated the STZ-induced behavioral and biochemical alterations in diabetic rats (*shown in Figure-1, 2, and 3*). "One way ANOVA" revealed the significant effect of various treatments on the blood glucose level ($F_{2,27} = 23$, $P<0.001$); performance of rats in OFT (number

of squares crossed: $F_{2,27}$ = 29.36, P<0.001; time spent at the center: $F_{2,27}$ = 1.610, P=0.2185; and time spent at the peripheral squares: $F_{2,27}$ = 0.282, P=0.7563); immobility period of mice (in TST: $F_{2,27}$ = 209.6, P<0.001 and in FST: $F_{2,27}$ = 33.16, P<0.001) and biochemical parameters (plasma nitrite: $F_{2,27}$ = 18.54, P<0.001 and plasma MDA level: $F_{2,27}$ = 9.064, P=0.0010). Tukey's test suggested that the diabetic rats displayed significantly increased blood glucose level (P<0.001), decreased plasma nitrite level (P<0.001), increased MDA level (P<0.01), decreased performance in OFT (P<0.001) and immobility period in TST (P<0.001) and FST (P<0.001). Administration of metformin to the diabetic rats significantly decreased the blood glucose (P<0.01), increased plasma nitrite (P<0.01) and MDA level (P<0.01), and decreased the immobility period of diabetic rats in TST (P<0.001) and increased the immobility period of diabetic rats in FST (P<0.001).

3.2 Effect of combined administration of pyridoxine and metformin on behavioral and biochemical parameters of diabetic rats

Two-way ANOVA revealed the significant effect of pyridoxine alone ($F_{1,36}$ = 9.904; P=0.0033) and MET alone ($F_{1,36}$ = 0.1688; P=0.6836) but no significant effect of pyridoxine × MET interaction ($F_{1,36}$ = 0.3785; P=0.5423) at day 3 on the blood glucose of diabetic rats. Two-way ANOVA revealed the significant effect of pyridoxine alone ($F_{1,36}$ = 3.626; P=0.0649) but no significant effect of MET alone ($F_{1,36}$ = 0.03689; P=0.8488) and Pyridoxine × MET interaction ($F_{1,36}$ = 2.176; P=0.1489) at day 7 on the blood glucose of diabetic rats. Two-way ANOVA revealed no significant effect of pyridoxine alone ($F_{1,36}$ = 0.9541; P=0.3352) and MET alone ($F_{1,36}$ = 0.001214; P= 0.9724) but a significant effect for the pyridoxine × MET interaction ($F_{1,36}$ = 11.66; P= 0.0016) on the blood glucose level of diabetic rats at day-14. Tukey's *post hoc* test suggested that the combined administration of pyridoxine and metformin did not affect the blood glucose level of diabetic rats significantly as compared to pyridoxine alone treated diabetic rats (*shown in Figure-4*).

In the open field test, two-way ANOVA revealed the significant effect of MET alone ($F_{1,36}$ = 9.276; P=0.0043) but no significant effect of Pyridoxine alone ($F_{1,36}$ = 3.296; P=0.0778) and Pyridoxine × MET interaction ($F_{1,36}$ = 3.631; P=0.0647) on the number of squares crossed by the diabetic rats. Two-way ANOVA revealed no significant effect of MET alone ($F_{1,36}$ = 1.304; P=0.2610), but a significant effect of pyridoxine alone ($F_{1,36}$ = 7.498; P=0.0095) and Pyridoxine × MET interaction ($F_{1,36}$ = 6.066; P=0.0187) on the time spent at the center. Further, the two-way

ANOVA revealed no significant effect of pyridoxine alone ($F_{1,36}$ = 0.6755; P=0.4165); MET alone ($F_{1,36}$ = 1.859; P=0.1812) and Pyridoxine × MET interaction ($F_{1,36}$ = 0.1131; P=0.7386) on the time spent at the periphery. Tukey's *post hoc* test suggested that the combined administration of pyridoxine and metformin did not increase the time spent at the center of the open field significantly as compared to pyridoxine alone treated diabetic rats (*shown in Figure-5*).

In TST, two-way ANOVA revealed the significant effect of pyridoxine alone ($F_{1,36}$ = 9.560; P=0.0038) but no significant effect of MET alone ($F_{1,36}$ = 1.228; P=0.2751) and Pyridoxine × MET interaction ($F_{1,36}$ = 0.4261; P=0.5180) on the immobility period of diabetic rats. In FST, two-way ANOVA revealed the significant effect of pyridoxine alone ($F_{1,36}$ = 0.0035; P=0.9528) but no significant effect of MET alone ($F_{1,36}$ = 93.54; P<0.0001) and Pyridoxine × MET interaction ($F_{1,36}$ = 1.716; P=0.1985) on the immobility period of diabetic rats (*shown in Figure-6*).

In plasma biochemical assay two-way ANOVA revealed the significant effect of pyridoxine alone ($F_{1,36}$ = 7.278; P=0.0106) and MET alone ($F_{1,36}$ = 55.44; P<0.0001) but no significant effect of Pyridoxine × MET interaction ($F_{1,36}$ = 0.7746; P=0.3846) on the plasma nitrite level of diabetic rat. Two-way ANOVA revealed the significant effect of MET alone ($F_{1,36}$ = 8.965; P=0.0050) but no significant effect of pyridoxine alone ($F_{1,36}$ = 0.6747; P=0.4168); and Pyridoxine × MET interaction ($F_{1,36}$ = 0.1275; P=0.7231) on the plasma MDA level of diabetic rats (*shown in Figure-7*).

4. Discussion

Diabetes in experimental animals is mainly induced by the administration of STZ. STZ induces diabetes through the partial destruction of pancreatic beta cells (Mestry et al., 2017; Wu and Yan, 2015; Furman, 2015). In the present study, the administration of a single intraperitoneal injection of STZ (45 mg/kg) induced experimental diabetes in rats. Various studies have reported the induction of diabetes upon the administration of a single dose of STZ (45 mg/kg) in rats (Mestry et al., 2017; Guo et al., 2021; Navale and Paranjape, 2018). STZ-induced diabetic rats displayed increased levels of anxiety and depression-like behaviors (Hirano et al., 2007; Caletti et al., 2012; Tang et al., 2015). In the present study, diabetic rats displayed a significantly lesser number of squares crossed in OFT and increased the immobility period of rats in TST and FST as compared to the control. These findings were in agreement with the previous findings suggesting the development of anxiety and depression-related behavior in diabetic rats (Şahin et

al., 2019). Hyperglycemia increases the production of ROS and oxidative stress (Maritim et al., 2003). Oxidative stress is usually determined by measuring the production of lipid peroxidation i.e., malondialdehyde (MDA) level, and the latter is considered as an index of the level of ROS (Prasad, 2000). In the present study, diabetic rats displayed a significantly increased MDA level as compared to the control. Further, the plasma nitrite was decreased in diabetic rats. The increased level of MDA level in diabetic rats is due to the increase in lipid peroxidation (Senturk et al., 2021).

Metformin has been shown to increase insulin sensitivity, reduce insulin resistance, and increases the peripheral uptake of glucose (Särnblad et al., 2003; Meyer et al., 2002; Jacobsen et al., 2009; Moon et al., 2007). In the present study, daily administration of metformin to diabetic rats significantly decreased the blood glucose level of diabetic rats. The findings were in agreement with the previous studies (Kaur et al., 2020; Saad et al., 2015). In the present study, metformin treatment did not affect the performance of diabetic rats in OFT but decreased the immobility period of diabetic rats in TST while increasing the immobility period of diabetic rats in FST. Metformin treatment thus alleviated the depression-related behavior in TST but produces depressogenic effects in FST. However, in previous studies metformin has been shown to alleviate diabetes-induced anxiety and depression-related behavior (Ai et al., 2020; Li et al., 2019; Chen et al., 2020). Further, the administration of metformin did not affect the plasma MDA level of diabetic rats but increased the plasma nitrite level of diabetic rats. Metformin treatment increased the production of NO by increasing AMP-activated protein kinase-dependent activation of endothelial nitric oxide synthase (eNOS) decreasing the circulating level of endothelin-1, increasing the efficacy and bioavailability of NO (Davis et al., 2006; Orio et al., 2005; Pacher et al., 2007). However, in previous studies metformin administration has been shown to decrease the plasma MDA level (Zhang et al., 2017; Diniz Vilela et al., 2016).

Diabetic rats often displayed reduced levels of pyridoxal L- phosphate (PLP) an isoform of pyridoxine. Pyridoxine has been shown to ameliorate oral glucose tolerance, decrease the blood glucose level and normalize insulin function (Spellacy et al., 1977). Pyridoxine supplementation lowers blood glucose levels (Nair et al., 1998), alleviated anxiety and depression-related behavior (Abraham et al., 2010), improves oxidative stress, and reduces the MDA level (Taş et al., 2014) in diabetic rats. Therefore, the effect of combined administration of metformin and pyridoxine was studied. It was observed that the combined administration of

metformin and pyridoxine to diabetic rats increased the blood glucose level. Further, the combined administration of metformin and pyridoxine did not affect the performance of diabetic rats in OFT, and depression-related behavior in TST and FST did not affect the plasma nitrite and MDA level as compared to metformin alone treated diabetic rats.

In conclusion, the co-administration of pyridoxine did not affect the effect of metformin in diabetic rats.

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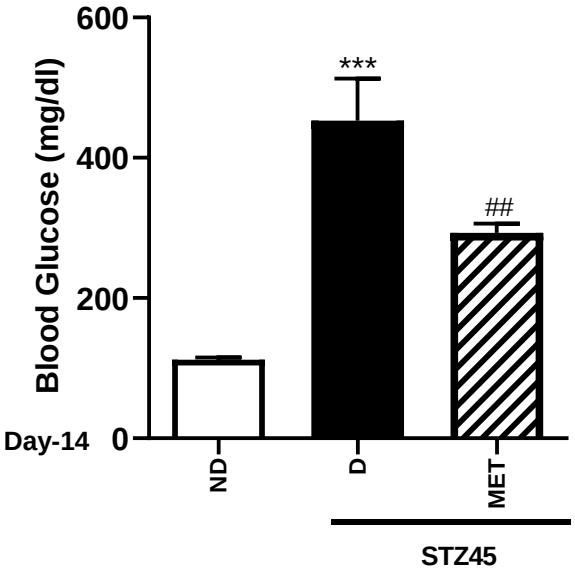

Figure: 1. Effect of various treatments on the blood glucose of diabetic rats. Values are expressed as mean±S.E.M. n=10 in each group. ***P<0.001 significant difference from the non-diabetic group. ##P<0.01 significant difference from the diabetic group.

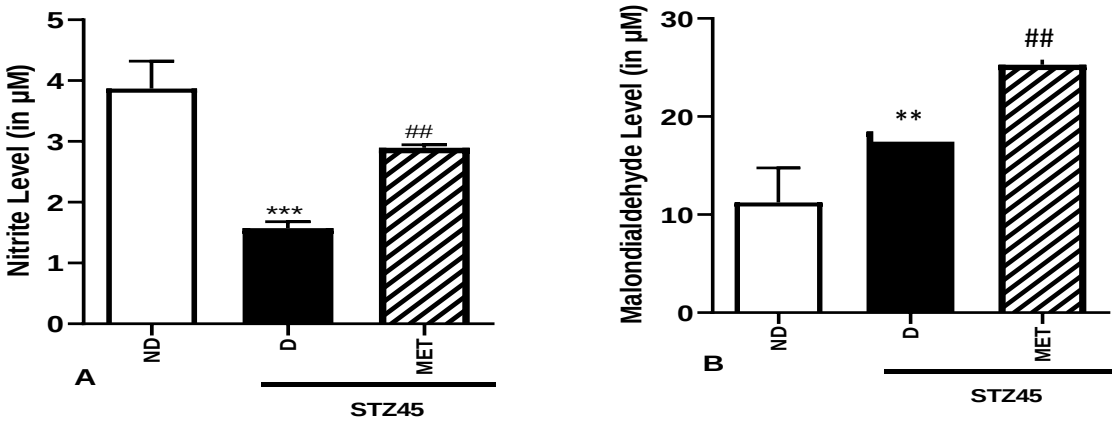

Figure: 2. Effect of various treatments on the nitrite level and malondialdehyde level.

Values are expressed as mean±S.E.M. n=10 in each group. ***P<0.001, **P<0.01 significant difference from the non-diabetic group. ##P<0.01 significant difference from the diabetic group.

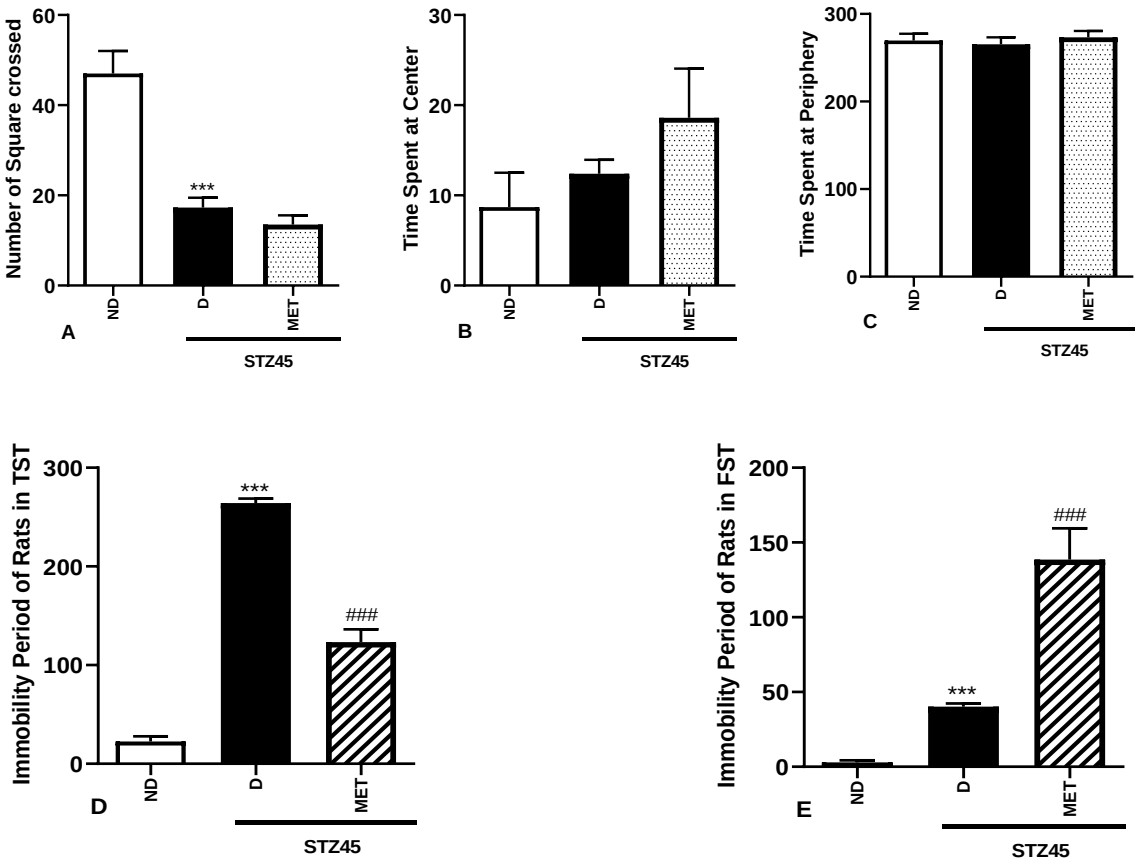

Figure: 3. Effect of various treatments on the locomotor activity and depression-related behavioral alterations of rats.

Values are expressed as mean±S.E.M. n=10 in each group. ***P<0.001 significant difference from the non-diabetic group. ###P<0.001 significant difference from the diabetic group.

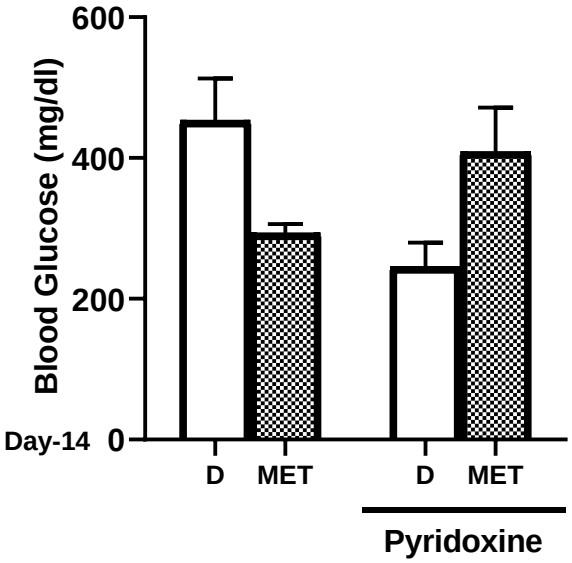

Figure: 4. Effect of combined administration of metformin and pyridoxine on the body weight and blood glucose level of diabetic rats.

Values are expressed as mean±S.E.M. n=10 in each group.

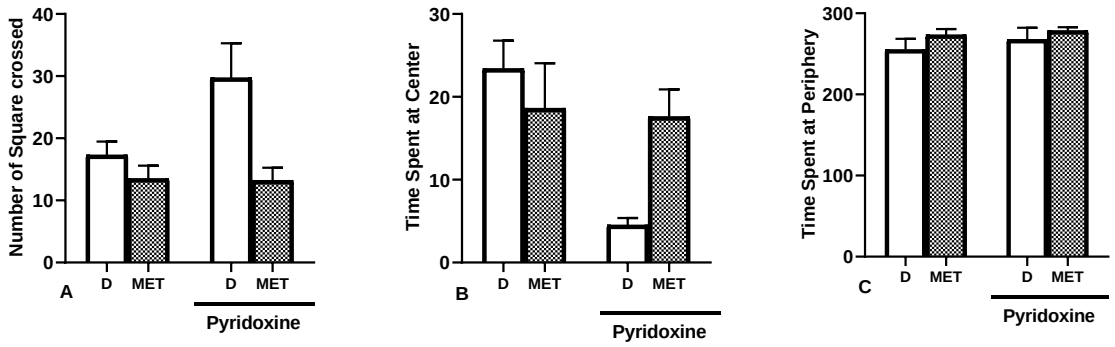

Figure: 5. Effect of combined administration of metformin and pyridoxine on the performance of diabetic rats in OFT.

Values are expressed as mean±S.E.M. n=10 in each group.

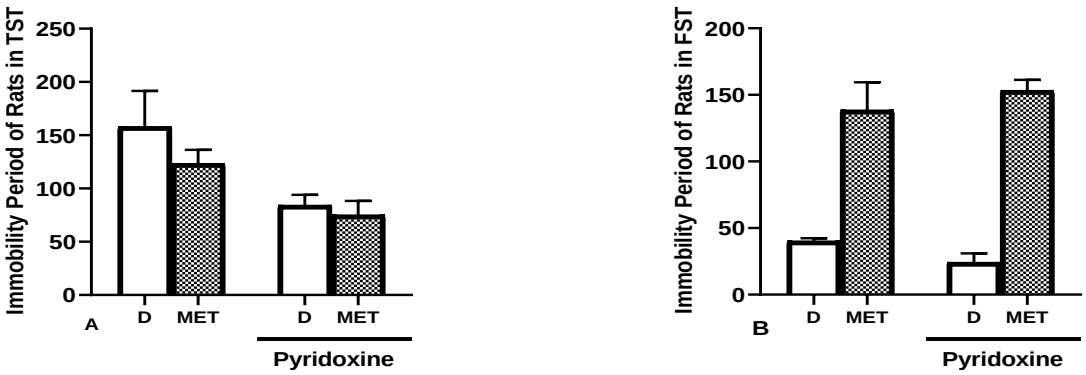

Figure: 6. Effect of combined administration of metformin and pyridoxine on the immobility period of diabetic rats in TST and FST.

Values are expressed as mean±S.E.M. n=10 in each group.

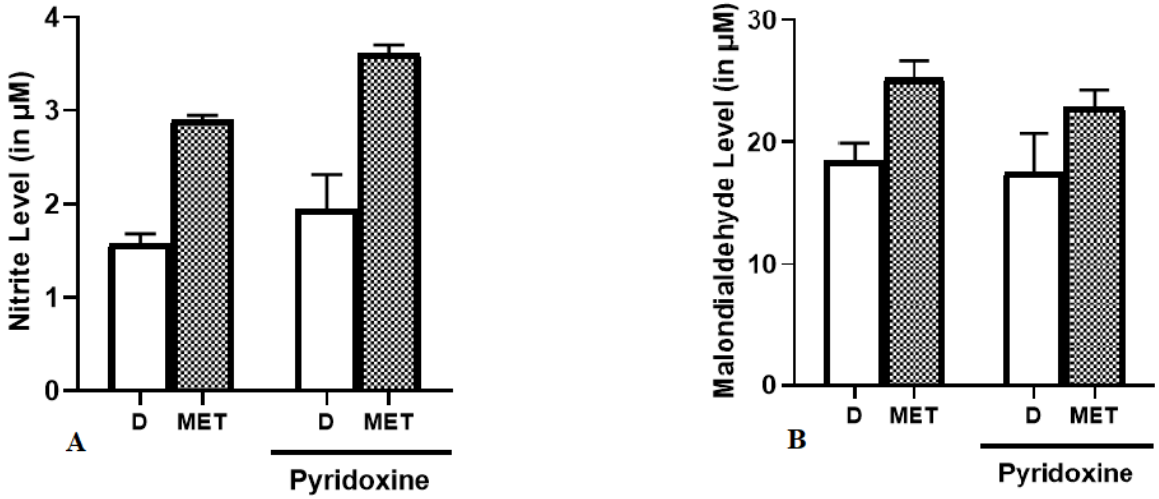

Figure: 7. Effect of combined administration of metformin and pyridoxine on the plasma nitrite level and MDA level.

Values are expressed as mean±S.E.M. n=10 in each group.