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Pantoprazole Rescue The Vascular Endothelial Dysfunction In Diabetic Rats Through DDAH/ADMA/Enos/NO Pathway

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Running Title: Pantoprazole ameliorates endothelial dysfunction

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Abstract: Proton pump inhibitors (PPIs) are the commonly recommended treatment for gastric abnormalities. The structural scaffold of PPIs (Pantoprazole; PPZ) provides an incalculable chance of association with diverse biological receptors which indicate a huge possibility of pleiotropic therapeutic impact which needs to be explored. Recently, several studies report the cardioprotective events of PPIs, but the underlying mechanism is not clear. Four groups having six animals in each were considered for this study. STZ (50 mg/kg/i.p) was given to induced chronic diabetes mellitus (DM) and vascular endothelial dysfunction (VED). PPZ (4 mg/kg/p.o/daily for 8 weeks) was evaluated against DM induced VED by measuring endothelial relaxation, aortic/serum nitrite/nitrate concentration, asymmetric dimethylarginine (ADMA), aortic superoxide anion generation, serum thiobarbituric acid reactive substances (TBARS) and dimethylarginine dimethylaminohydrolase (DDAH) in the cell lysate of each animals group. PPZ significantly overcome the perturbed level of hyperglycemia measured by blood glucose level, increase the availability of NO measured by aortic/serum nitrite/nitrate concentration. Treatment with PPZ showed the determinate lessening of tissue injuries as it averted increase expression of VED measured by ACh-induced endothelium-dependent relaxation, and diminution in oxidative stress, plasma ADMA level, and DDAH concentration in the cell lysate. The vascular protective potential of PPZ has a strong correlation with the DDAH/ADMA/eNOS/NO signaling pathway. Furthermore, the study also explored the antioxidant activity of PPZ which may also facilitate this protective pathway by increasing the bioavailability of NO in the endothelium.

Keywords: Diabetes mellitus; Endothelial dysfunction; Proton pump inhibitors; Asymmetric dimethylarginine; Pantoprazole.

1. INTRODUCTION

Emerging evidence reports the towering prevalence of diabetes mellitus across the globe. About 425 million are suffering with this insidious metabolic disorder with an additional count of about 352 million people who are at risk. This mounting pervasiveness has been estimated up to 629 million by 2045 [1,2]. This perpetual accumulative incidence of diabetes mellitus (DM)significantly exaggeratesassociated vascular endothelium damage byaltering various vascular derived factor including nitric oxide (Endothelium-derived relaxing factor, NO)[3].NO is synthesized throughout the transition of L-arginine to L-citrulline by endothelial NO synthase (eNOS) [4]. NO signalling is known to produce the consequences of anti-inflammatory, anti-oxidative, anti-proliferative and anti-thrombotic activities [5]. Asymmetric dimethylarginine (ADMA), a foremost endogenous NO synthase inhibitor whichplay a fundamentalrole to regulate endothelial dysfunction. Dimethylarginine dimethylaminohydrolase (DDAH)is a cytosolic enzyme that hydrolase of ADMA and may be an independent predictor of vascular diseases[6]. Diminish performance of DDAH instigates the accumulation of ADMA in variouspathological conditionincludingdiabetes mellitus, atherosclerosis, cardiovascular, and renal diseases [7].

Proton pump inhibitors (PPIs) are basically involved a typical framework of benzimidazolide form (benzimidazole) which is known for the centre of various pharmacological activity including opioid, antihistaminic, antihelmintic, and anticancer medications [8]. PPIs has been uncovered as an ameliorative specialist against various cardiovascular abnormalities as they may prompt vascular relaxation, decrease atrial fibrillation, and have positive inotropic and negative chronotropic impacts [9][10][11]. Ongoing research investigation explored that Omeprazole showed the potential anticancer activity in human colon malignant growth cell lines [12]. Omeprazole and lansoprazole have also been revealed for lower occurrences of tuberculosis [13]. Ghebremariam et al. 2015 uncovered the anti-inflammatory and anti-fibrotic capability of esomeprazole by suppressing the proinflammatory cytokines and interleukins articulation *in vitro* and *in vivo* model of lung injury [14]. Pantoprazole (PPZ) is an expectedly utilized PPIs for the anticipation of gastroesophageal reflux illness and hypersecretory gastric issues including Zollinger-Ellison condition [15]. Rather than its anti-ulcer impacts, PPZ has examined for its pleiotropic activities. Ongoing reports uncovered a huge therapeutic capability of PPZ including anti-inflammatory, anti-apoptotic, and cell reinforcement properties [16][17][18]. However, the direct evidence of the therapeutic efficacy of PPZ with a supportive underlying mechanism against vascular endothelial dysfunction (VED) is not yet known. The present study has been designed to investigate the effect of PPZ against diabetic induced VED in rats.

2. MATERIAL AND METHODS

Study on animals (Wistar albino rats of either sex) were affirmed by CPCSEA, New Delhi, India (Approval No: RITS/IAEC/2016/07/07). All animals were first acclimatized for a week with standard rodent chow and water *ad libitum*.

2.1 Induction of DM

Streptozotocin (STZ; 50 mg/kg, *i.p*., once) disintegrated in freshly prepared citrate buffer (pH 4.5) was given to rodents to instigate experimental DM. The glucose level was checked once

after 72 h of STZ administration. Rodents indicating blood glucose level of more than 300 mg/dL were chosen and named as diabetic rats.

2.2Induction of Experimental diabetic VED

Rats administered STZ (50 mg/kg/ once, *i.p.*) were allowed for 8 weeks to develop experimental VED[19]. The development of VED was evaluated by utilizing an isolated aortic ring structure and assessing aortic and serum nitrite/nitrate concentration. Further, hematoxylin-eosin staining of the thoracic aorta was performed to evaluate the vascular endothelial integrity.

2.3 Isolated rat aortic ring preparation

Sacrificed the rat and incised the thoracic aorta, which was cut into a ring of around 4-5 mm in length and mounted in an organ bath containing Krebs-Henseleit solution of pH 7.4, bubbled with oxygen (95% O2 and 5% CO2) and maintained at 37 ^{0}C. The preparation could equilibrate for 90-min under 1.5 g tension. The isometric contraction was recorded using a force-transducer (Ft-2518) connected to Physiograph (INCO, Ambala, Haryana, India). The aortic ring preparation was primed with 80 mM KCl to assess its functional integrity and to improve its contractility. The cumulative dose-response curve of acetylcholine (Ach; 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M) or sodium nitroprusside (SNP; 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M) were recorded in phenylephrine (3×10^{-6} M)-precontracted ring preparation of the aorta with regular and injured endothelium, respectively[3].

2.4 Estimation of aortic and serum nitrite/nitrate concentration

The aortic tissue was homogenized in 5 mL of phosphate buffer saline of pH 7.4 and centrifuged at 10,000g for 20-min. The supernatant was utilized for assessing aortic nitrite/nitrate and protein evaluation. The carbonate buffer (400 µL, pH 9.0) (arranged by including equivalent volume of 500 mM sodium bicarbonate and 50 mM sodium carbonate to acquire 50 mM carbonate buffer) was added to 100 µL of supernatant got from homogenized aortic example or 100 µL of serum test in an isolated tube. The mixture was mixed with a limited quantity (~0.15 g) of copper-cadmium amalgam (copper and cadmium in a proportion of 1:10). The tubes were brooded at room temperature for 1 h with an intensive shaking to decrease nitrate to nitrite. The response was then halted by including 100 µL of 0.35 M NaOH. Therefore 400 µL of 120 mM zinc sulfate arrangement was added to deproteinize the serum tests. The examples were permitted to represent 10-min and afterward centrifuged (REMI Cooling Centrifuge, India) at 4,000 g for 10-min. Greiss reagent (a combination of 250 µL of 1.0% sulphanilamide arranged in 3N HCl and 250 µL of 0.1% N-naphthylethylenediamine arranged in water) was added to aliquots (500 µL) of clear supernatant, and aortic and serum nitrite/nitrate fixation was assessed spectrophotometrically (LABINDIA 3000, India) at 545 nm. The standard graph of sodium nitrite (0.1–3 nM) was plotted to compute the grouping of aortic nitrite/nitrate (µM/mg of protein) and serum nitrite/nitrate (µM/L)[3]. The protein focus in the homogenized aortic sample was assessed by commercially accessible kits (AGAPPE Diagnostics Ltd., Kerela, India).

2.5 Histological assessment of the integrity of vascular endothelial layer

The histological evaluation of the vascular endothelial layer was performed to assess the structure alteration. The extracted aorta was submerged in 10% neutral buffered formalin, dehydrated in graded concentrations of ethanol, drenched in xylene and implanted in paraffin. A cross over area 5 μm was stained with hematoxylin-eosin. The aortic segment was analysed utilizing Motic Microscope BA310 (Motic, USA) at 40 X to evaluate the integrity of endothelium.

2.6 Assessment of oxidative stress

A. Estimation of aortic superoxide anion

Aorta was chiselled into the cross over rings of 5-6 mm long and set in 5 mL of Krebs-Henseleit arrangement containing nitroblutetrazolium (NBT, 100 μM/L) and incubated at 37 °C for 90-min. The NBT reduction was then ended by including 5 mL of 0.5 N HCl. The aortic ring was minced and homogenized in a combination of 0.1 N NaOH and 0.1% sodium dodecyl sulfate in water containing 40 mg/L diethylenetriamine pentaacetic acid (DTPA). The combination was then centrifuged (REMI Cooling Centrifuge, India) at 20,000 g for 20-min, and the resultant pellets were re-suspended in 1.5 mL of pyridine and kept at 80 °C for 90-min to extricate formazan. The combination was then centrifuged at 10,000 g for 10-min, and the absorbance of formazan was resolved spectrophotometrically at 540 nm[3]. The measure of decreased NBT (picoM/min/mg) was determined utilizing the accompanying equation = $A \cdot V/(T \cdot Wt \cdot \varepsilon \cdot l)$, where 'A' is absorbance, 'V' is the volume of solution (1.5 mL), 'T' is the time duration for aortic rings incubation with NBT (90-min), 'Wt' is the smeared wet load of aortic rings, 'ε' is an annihilation coefficient (0.72 L/mmol/mm) and 'l' is the length of light way (10 mm).

B. Estimation of serum TBARS

One mL of 20% trichloroacetic acid was added to 100 μL of serum and 1.0 mL of 1% TBARS reagent (a combination of an equivalent volume of 1% thiobarbituric acid aqueous solution in 1 M NaOH (50 mg/mL), and glacial acetic acid), blended and incubated at 100 C for 30-min. The sample was cooled on ice and centrifuged (REMI Cooling Centrifuge, India) at 1000 g for 20-min. The serum concentration of TBARS was estimated spectrophotometrically at 532 nm[20]. The standard graph was plotted by utilizing 1,1,3,3-tetramethoxypropane (0.1 nM to 1 nM) to ascertain the amount of serum TBARS.

2.7 Determination of ADMA concentration

The concentration of ADMA was determined by high-performance liquid chromatography (HPLC). The presence of protein content in the serum was separated by adding 5-sulfosalicylic acid. HPLC was done utilizing a Shimadzu LC-6A fluid chromatograph with Shmadzu SCL-6A framework regulator and Shimadzu SIC-6A autosampler. O-Ph thaldiade-hyde adducts of methylated amino acids and internal standard ADMA formed by precolumn blending and were observed by fluorescence detector (model RF 530) set at λ^{ex}= 338 and λ^{em}=425 nm. Tests were eluted from the segment utilizing a direct slope containing versatile stage A made out of 0.05 M (pH 6.8) sodium acetic acid derivation methanoltetrahydrofuran

(81:18:1 v:v:v) and portable stage B made out of 0.05 mM sodium acetic acid derivation methanol-tetrahydrofuran (22:77:1 v:v:v) at a flow-rate of 1 ml/min[21].

2.8 DDAH activity assay

The potency of endothelial DDAH was calculated by measuring the metabolic concentration of ADMA. The endothelial cell was isolated from rodents as described in previous report. In-brief, animals were anesthetized (10 mg/ml pentobarbital sodium) and the abdominal aorta were exposed. The aorta was dissected out and immersed in Dulbecco's modified eagle medium (DMEM) and 20% fetal bovine serum (FBS) mixture having 1000 U/ml of heparin. The connecting tissue was rapidly removed, and lumen was washed with serum free DMEM. Now the tissue was incubated with collagenase type II solution for 45 minutes at 37 C. The endothelial cells were removed from aorta by flushing with mixture of DMEM and 20% FBS. Endothelial cells were centrifuged at 1200 rpm for 5 minutes and precipitate was re-suspended with DMEM and 20% FBS mixture [22].The cell lysates wereseparated, and ADMA was added (finalconcentration 500 μM). A 30% sulfurosalicylic acid was immediately mixed to normal control groupto inactivate DDAH (0% DDAH activity) whereas other groups wereincubated at 37 °C for 2 h before adding 30% sulfurosalicylic acid. The ADMA level in each group was estimated by HPLC and the differences in ADMA level of these groups endorse the potential of DDAH action. For each investigation, DDAH movement of cells presented to typical adapted medium was characterized as 100%, and DDAH action in different conditions was communicated as rates of the ADMA used as compared to control[23].

2.9 Experimental protocol

Four groups having six rats (weight around 200-220 gm) each, were utilized in the current study where normal control, diseases control, drug *per se* such, and PPZ treated groups were used. PPZ at a portion of 4 mg/kg/*p.o* suspended in 0.25% carboxymethylcellulose was directed every day for about two months. The outline of test convention is given as:

Group I (*Normal Control*), rats were maintained on standard food and water, and no treatment was given. Group II (*Diabetic Control*), rats were administered STZ (50 mg/kg, *i.p.*, once) dissolved in citrate buffer of pH 4.5, and were allowed for 8 weeks to develop experimental VED. Group III (PPZ *per se*), normal rats were administered PPZ (4 mg/kg/*p.o.*) for 8 weeks. Group IV (PPZ treatment), diabetic animals received daily treatment of PPZ (4mg/kg/*p.o*) for 8 weeks against induction of VED as mentioned in group 2.

2.10 Statistical analysis

All values were expressed as mean ± SD. Data for isolated aortic ring preparation were statistically analysed using repeated measures of analysis of variance (RM-ANOVA), followed by Student-Newman-Keuls Method. The endothelium-dependent relaxation (Ach, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M) and endothelium-independent relaxation (SNP, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M) in between all groups were statistically analysed using one way ANOVA, followed by Tukey's multiple comparison test. Rest of all values were expressed in mean ± SD and were statistically dissected by utilizing one-way ANOVA followed by Tukey's

multiple comparison post-hoc tests utilizing Sigma Plot (12.0, from Systat Software, Inc., San Jose California USA). A '*p*' estimation of under 0.05 was considered as measurably significant.

3. RESULTS

All the analysis was carried at the end of investigational procedure (after 8 weeks). Around an average of 10% body weight were reduced in diabetic rats. Administration of PPZ (4 mg/kg/day, *p.o.,* 8 weeks) to normal rats did not produce any statistically significance difference in *per se* treatment on various parameters as compared to normal rats.

3.1 Effect of PPZ on blood glucose

A significant surge in blood glucose level was noticed in STZ-administered rats (at the end of 8 weeks) as compared to normal rats. The treatment of PPZ (4 mg/kg/day, *p.o.,* 8 weeks)moderately diminished the raised blood glucose concentration in diabetic rats (Table 1).

3.2 Effect of PPZ on Ach-induced endothelium-dependent relaxation

Animal treated with PPZ showed dose-dependent relaxation in phenylephrine (3 X 10^{-6} M) pre-constricted rat aortic ring preparation by Ach (10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M) in endothelium-dependent manner. Conversely, a significant diminution of ACh-induced endothelium-dependent relaxation was observed in the isolated aortic ring of diabetic rats. The treatment of PPZ (4 mg/kg/day, *p.o.,* 8 weeks) moderately reinstated this ACh-mediated endothelium-dependent relaxation indiabetic rat aortic preparation(Figure 1-A).

3.3 Effect of PPZ on SNP-induced endothelium-independent relaxation

Animal treated with PPZ (4 mg/kg/day, *p.o.,* 8 weeks)showed dose-dependent relaxation in phenylephrine (3 X 10^{-6} M) pre-constricted rat aortic ring preparation by SNP (10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M) in endothelium-independent manner. Conversely, there was no change in SNP-induced endothelium-independent relaxation in diabetes and PPZ treated group (Figure 1-B).

3.4 Effect of PPZ on serum and aortic nitrite/nitrate concentration

The ratio of nitrite/nitrate concentration was observed in serum and aortic tissue, respectively. A significant reduction of serum and aortic nitrite/nitrate concentration was noticed in diabetic control rats as compared to normal control rats. However, the treatment of PPZ (4 mg/kg/day, *p.o.,* 8 weeks)in diabetic rats substantially inhibited the declined concentration of nitrite/nitrate ratio of serum and aortic samples (Figures 2-A and 2-B respectively).

3.5 Effect of PPZ on the integrity of vascular endothelium

Structural alteration due to any pathological change was observed by histopathological study (by hematoxylin-eosin staining). Histological photomicrographs showed disruption of endothelial cell lining in the aorta of diabetic rats as compared to the normal control rats, thatpresented an identical endothelial layer. Whereas the treatment of PPZ(4 mg/kg/day, *p.o.,*

8 weeks)partially blocked the loss of endothelial cell layer in isolated aorta of diabetes rats(Figures 3).

3.6 Effect of PPZ on aortic superoxide anion generation and serum TBARS

The measurement of aortic superoxide anion generation was carried by evaluating reduce NBT concentration. In the present study a significant rise in aortic superoxide anion generation was noticed in diabetic control rats as compared to normal control rats (Table 1). Similarly, a distinct upturn in serum TBARS level was observed in diabetic control rats as compared to normal control rats (Table 1). However, the treatment of PPZ (4 mg/kg/day, *p.o.,* 8 weeks)significantly inhibited diabetes-induced expansions of aortic superoxide anion generation and serum TBARS.

3.7 Effect of PPZ on concentrations of ADMA and activity of DDAH

The plasma concentration of ADMA was significantly increase in diabetic control rats as compared to normal control rats. Treatment with PPZ(4 mg/kg/day, *p.o.,* 8 weeks)significantly inhibited the elevated concentration of plasma ADMA level indiabetes mellitus rats (Figure 4-A).

Secondly, the cell lysate from diabetic rats showed a significant diminish of DDAH activity in endothelial cells as compared to the cell lysate of normal control rats. However, the cell lysate from PPZ (4 mg/kg/day, *p.o.,* 8 weeks)treated rats revealed theinhibition of significant attenuation the endothelial DDAH activity as compared to diabetic rats (Figure 4-B).

4. DISCUSSION

Administration of STZ induce DM in experimental animal and the chronic diabetes condition leads to induction of VED in 8 weeks[24]. Endothelial damage was evaluated in isolated aortic ring preparation by significant diminution in ACh-induced endothelium-dependent relaxation without altering SNP-induced endothelium-independent relaxation that endorse the occurrence of VED in diabetic rats. It is a well-known statement that release of endothelium-derived relaxing factor (NO) is predominantly intervened through Ach-induced endothelium-dependent relaxation [3,25]. Consequently, the decreased accessibility of NO in the tunica intima might have performed a central role in the diminished ACh-induced endothelium-dependent relaxation in the diabetic rat aorta[3,25]. This contention is defended by the consequences obtained in the current experiment asthe aortic and serum concentrations of nitrite/nitrate were extensively condensed in diabetic rats. Moreover, the consequence also revealed the significant reduction in DDAH and increased expression of ADMA in diabetic animals which support the existing report where ADMA has been consider as a chief pathological factor for VED[26,27].

The structural scaffold of PPZ provides an incalculable chance of association with diverse biological receptors which indicate a huge possibility of pleiotropic therapeutic impact. PPZ is an ordinarily utilized PPIs against gastroesophageal reflux disorder and other gastric problems [28]. In addition, some recent reports also revealed the numerous pleiotropic action of PPI (PPZ) including anti-inflammatory [16], anti-proliferative [17], antiapoptotic and antioxidant properties [18]. Moreover, the outcomes of some ongoing clinical investigations demonstrated the lower risk of hyperglycaemia by PPIs treatment [29,30]. The therapeutic

outcomes of PPZ in the present investigation revealed the significant amelioration in blood glucose level on chronic use that instigate its important anti-diabetic potential. Furthermore, the treatment of PPZ endorse the increased ACh-induced endothelium-dependent relaxation which may reflect the upsurge level of NO. The statement has further confirmed by the other outcomes of study where the PPZ treated rats showed substantial improvement in aortic and serum NO level as compared to diabetic control animals. The mentioned consequence of upsurge level of NO may also associate with reduced insulin dependent NO degradation in treatment groups. As a previous report revealed that endothelial NO synthase (eNOS)/NO activity may also be moderated by insulin which is a key hormone in metabolic homeostasis[20,31,32].Moreover, the antioxidative potential was observed in the PPZ treated rats which was estimated by reduced level of superoxide anion generation and serum TBARS. The reduced oxidative stress might also be responsible to induce the protective potential of PPZ against VED as suggested by previous report where oxidative stress has been found as key pathological hallmark for endothelial dysfunction[33,34].

The underlying mechanisms for the development of endothelial dysfunction in diabetes mellitus are multifaceted and comprise of oxidative stress, inflammatory cascades, and alter bioavailability of NO[35]. Indeed, numerous molecular events result in dysregulation of endothelial function including reduced bioavailability of tetrahydrobiopterin, uncoupling of eNOS, increased production of ROS, increased level of asymmetric dimethyl arginine, and increased expression of nuclear factor κB (NFκB) [36,37]. eNOS may be obstructed by endogenous products of arginine metabolism (ADMA) which further leads to reduce formation of NO and endothelial dysfunction [27,38]. Thus, various prospective revealed that ADMA has been an independent predictor of vascular functions[27,38]. DDAH-2 indorses the metabolism of ADMA and produce a potential action in the regulation of acute inflammatory response and ultimately endothelial dysfunction[7,26]. The treatment of PPZ in diabetic induced vascular injured animal showed reduced level of DDAH as compared to diseases group. Similarly, the outcomes further revealed the PPZ produce substantial upsurge of plasma ADMA level and NO concentration. This whole narrative suggest the possible underlying molecular mechanism where increased level of DDAH significantly metabolised ADMA concentration and lowered the accumulation of ADMA which further facilitate the upregulated eNOS dependant NO generation and may be responsible for the endothelial protection against diabetes induced VED. The biochemical consequences were confirmed by histological observation where the integrity of endothelium was restored in PPZ treated animals as compared to diseases group. A similar histological lesion may indorse in the previous report by excessive exposure of reactive oxygen species and low level of NO[3,39]. This restoration of endothelium signifies the protective mechanism of PPZ against VED.Treatment with PPZ presents determinate lessening of tissue injuries. Intriguingly, the PPZ treatment averted the diabetes mellitus-induced VEDas measured in increase expression of ACh-induced endothelium-dependent relaxation, aortic/serum NO level, anddiminution in oxidative stress, and plasma ADMA level.

Convincingly, it may be determined that chronic treatment of PPZ partially, but significantly prevents experimental DM and -induced VED. This vascular protective potential of PPZ have strong corelation with the DDAH/ADMA/eNOS/NO signalling pathway. Moreover, the study also explored the antioxidant activity of PPZ which may also facilitate this protective

pathway by increasing the bioavailability of NO in endothelium. This possible underlying mechanism need to be further explored for better understanding of signalling mechanism.

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Figures/Table Legends

Table 1: Effect of PPZ on blood glucose level, Reduced NBT and Serum TBARS. All data are expressed as Mean ± SD, where $^{\#}p< 0.05$represents significantas compared with normal and $^{*}p< 0.05$ represents significant as compared to diseasescontrol.

Assessments	Normal Control	Diabetic Control	PPZ per se	PPZ Treatment
Blood glucose level (mg/dl)	97.93 ± 5.34	324.65 ± 15.37$^{\#}$	98.91 ± 14.54	142.52 ± 12.77*
Reduced NBT (Pico mol/min/mg)	17.09 ±1.54	27.48 ±1.53$^{\#}$	16.83 ±1.24	23.76 ±1.27*
Serum TBARS (µ mol/L)	8.01 ±0.84	15.79 ±1.15$^{\#}$	8.37 ±1.02	11.15 ±0.92*

Table 1: Effect of PPZ on blood glucose level, Reduced NBT and Serum TBARS. All data are expressed as Mean ± SD, where #$p< 0.05$ represents significant as compared with normal and *$p< 0.05$ represents significant as compared to diseases control.

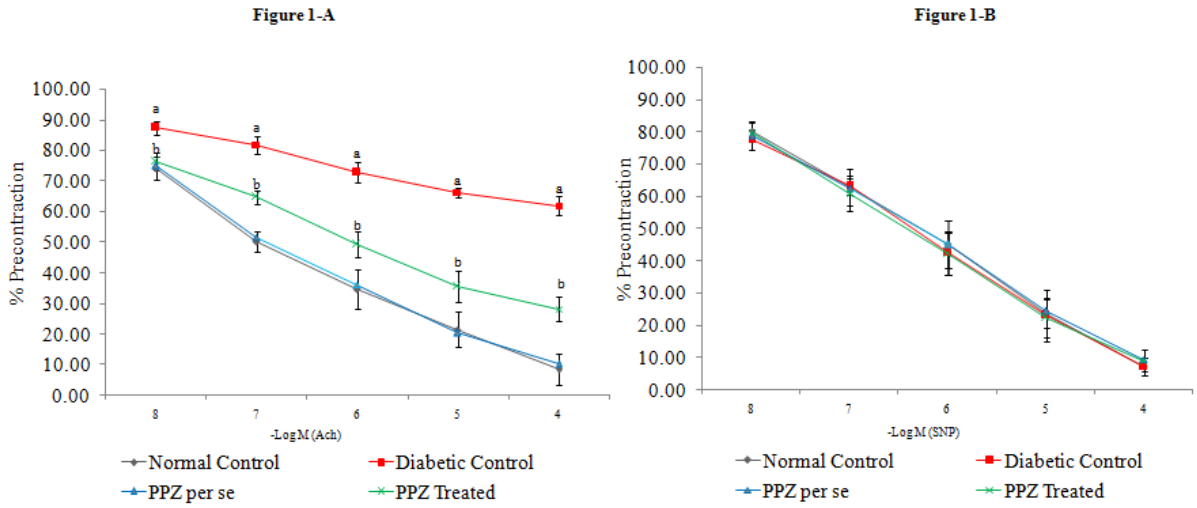

Figure 1: Effect of PPZ on Ach-induced endothelium-dependent relaxation in the thoracic aorta of diabetic rats, responses were represented as percentage of maximum contraction induced by phenylepherine (3 X 10^{-6} M) **(1-A)**, SNP-induced endothelium-independent

relaxation in the thoracic aorta of diabetic rats, responses were represented as percentage of maximum contraction induced by phenylepherine (3 X 10^{-6} M) **(1-B).***All values were expressed as mean ± SD. a = p<0.05 vs Normal Control; b = p<0.05 vs Diabetic Control.*

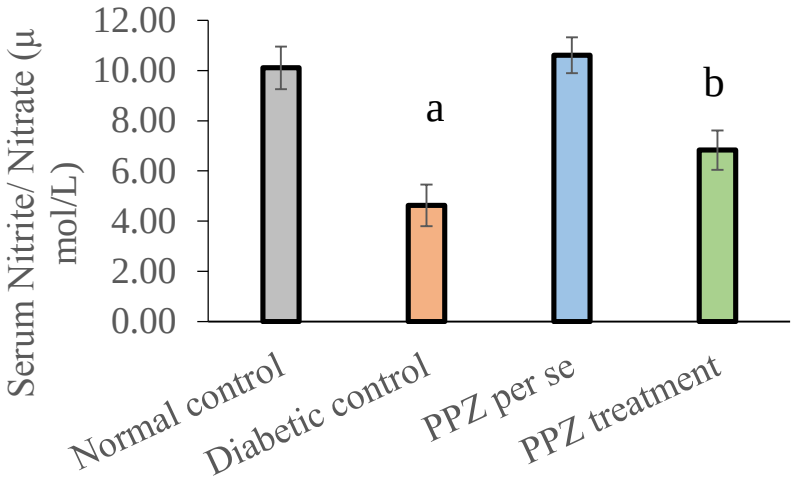

Figure 2-A

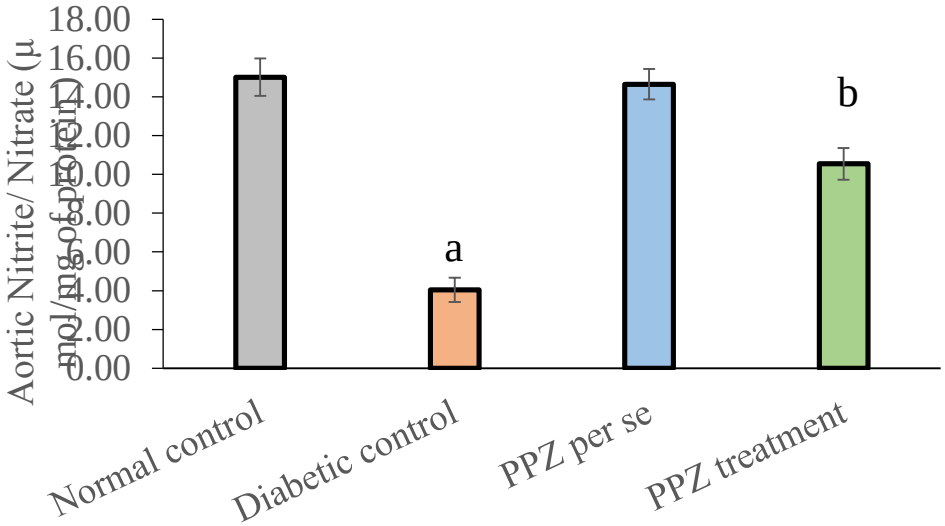

Figure 2-B

Figure 2: Effect of PPZ on serum nitrite/nitrate concentration (µ mol/L) in diabetic rats **(2-A)**, aortic nitrite/nitrate concentration (µ mol/mg of protein) in diabetic rats **(2-B).***All values were expressed as mean ± SD. a = p<0.05 vs Normal Control; b = p<0.05 vs Diabetic Control.*

Figure 3

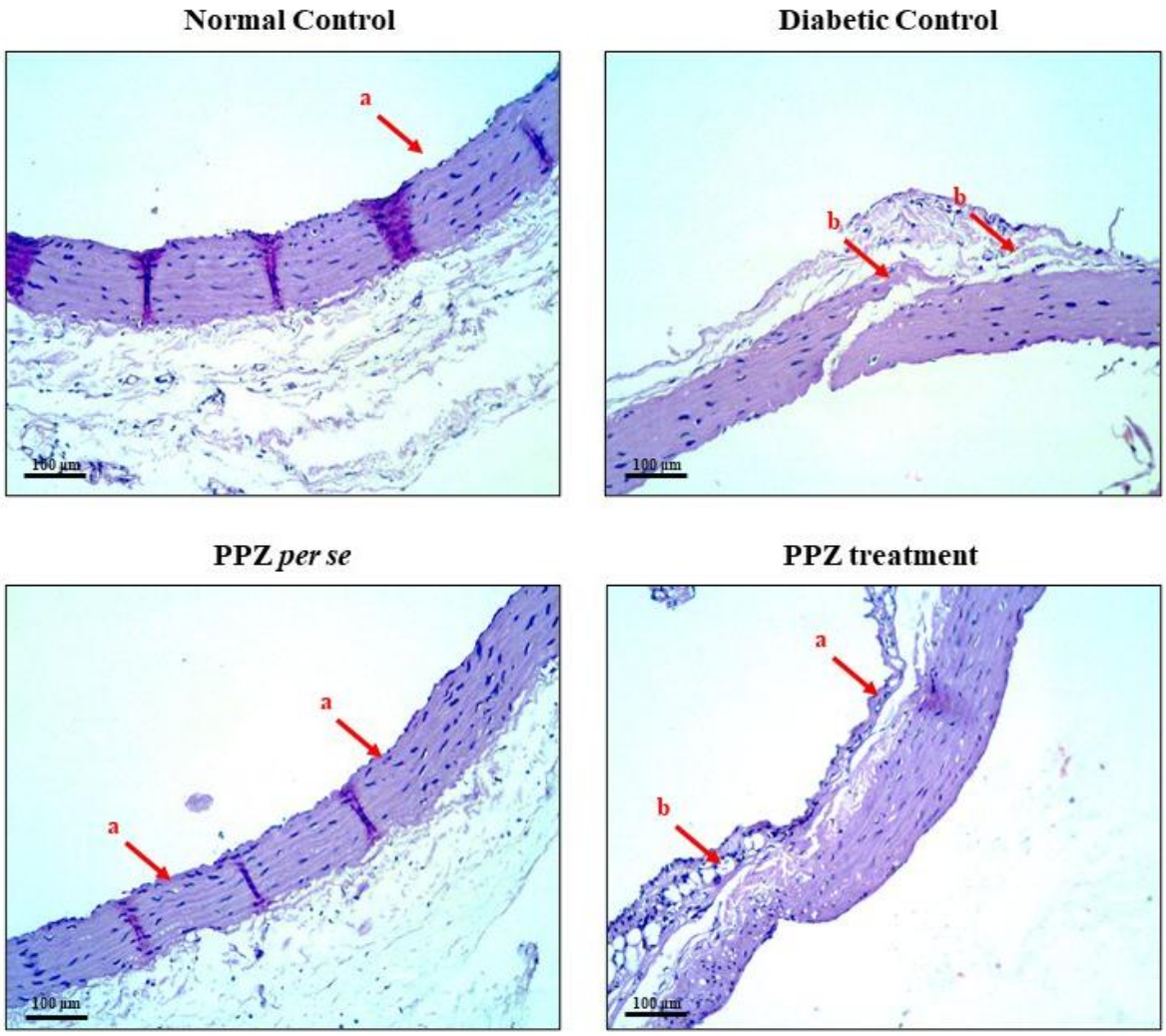

Figure 3: Figure portrays the effect of PPZ on the integrity of the vascular endothelial layer in the aorta isolated from each group using inverted microscope (Cosmo Laboratory Equipment) at 40X (scale bar = 100 µm). 'a' shows the normal histological architecture of healthy vascular endothelium, whereas 'b' shows the damage in endothelium layer.

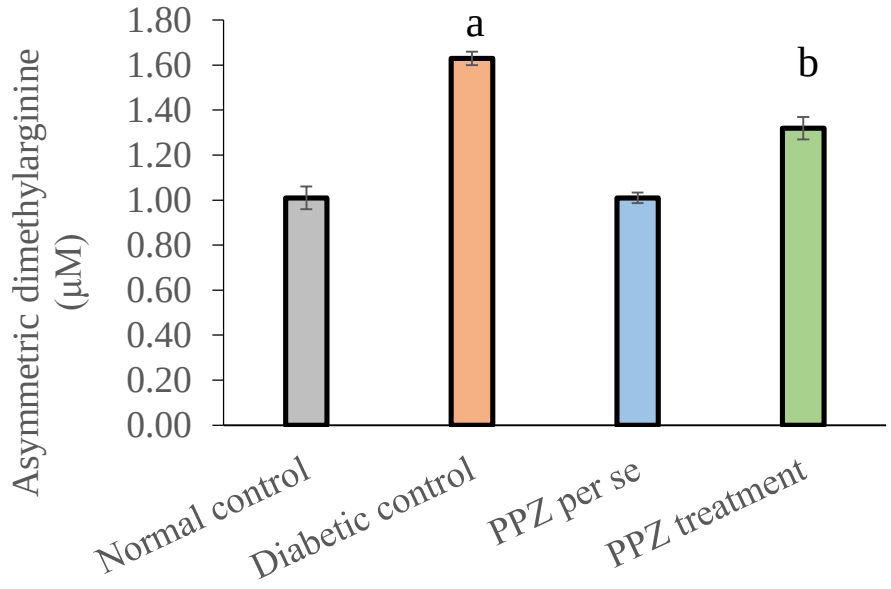

Figure 4-A

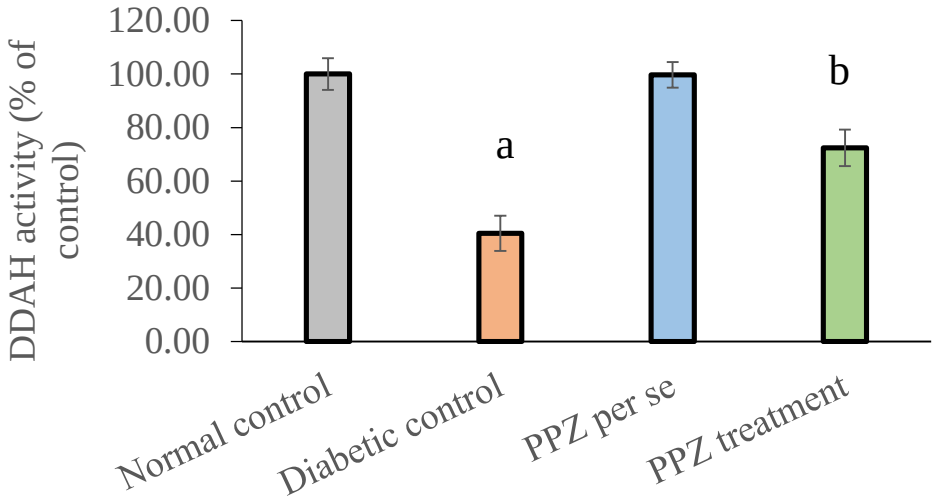

Figure 4-B

Figure 4: Effect of PPZ onblood plasmaconcentration of asymmetric dimethylarginine(µM) in diabetic rats **(4-A)**, DDAH activity in endothelial cells lysate of diabetic rats **(4-B)**. *All values were expressed as mean ± SD. a = p<0.05 vs Normal Control; b = p<0.05 vs Diabetic Control.*