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Effects of Atorvastatin as Antioxidants in Diabetic associated Cardiovascular Complications.

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Abstract:

Oxidative stress is pathogenetic hypotheses of vascular complication in diabetes by impaired endothelial dysfunction (ED) and antioxidant status. The endothelium is complex organ essential for controlling vascular functions. Vascular ED leads to pathogenesis of diabetic associated cardiovascular complications. The aim of present study was to evaluate the role of Atorvastatin in diabetic cardiovascular complications. Atorvastatin, *HMG-CoA reductase* inhibitors used as lipid lowering began to emerge; such pleiotropic effects include improvement of ED, increased Nitric oxide (NO) bioavailability, antioxidant, anti-inflammatory activities. Hence to evaluate the effect of Atorvastatin in diabetic vascular complications, we studied the effect of Atorvastatin on acetylcholine responses in thoracic aorta isolated from streptozotocin (60 mg/ kg i.p.) induced 8 weeks diabetic rats. Acetylcholine induced relaxation response was significantly decreased in aortic strips from diabetic with compared to control rats. Lipidperoxidation was significantly increased while *Superoxide dismutase* (SOD) and *Catalase* activity were significantly decreased in aorta of diabetic rats with compared to control rats. The systolic, diastolic and mean arterial pressure (MAP) was significantly increased in diabetic rats with compared to control rats. Diabetic rats treated with Atorvastain (20 & 40 mg/kg/day) for 8 weeks selectively restored ED relaxation response of acetylcholine to near the reactivity observed in vessels from control rats. The enhanced lipidperoxidation, systolic, diastolic and MAP and reduced SOD and Catalase activity were significantly restored to control values following Atorvastain treatment. From results we infer that Atorvastain improves diabetes-induced ED by reducing oxidative stress and blood pressure, increasing relaxation responses of acetylcholine. So it could be an ideal intervention in therapy of diabetic associated cardiovascular complications.

Key words: Atorvastatin, Cardiovascular complication, Vascular Endothelial Dysfunction.

Introduction:

Diabetes mellitus is a major risk factor for the development of cardiovascular disease. Endothelial dysfunction (ED) is encountered early during the development of vascular damage [1]. Animal and human studies have demonstrated that increased oxidative stress largely accounts for the ED in patients with diabetes mellitus type 1 and 2 [2, 3]. As predominant sources of superoxide, the vascular *NADPH oxidase* [4, 5, 6]**,** an uncoupled *endothelial nitric oxide synthatase* (eNOS) [5, 7, 8]**,** *xanthine oxidase* [9] and mitochondria [10] have been identified.

Diabetes mellitus (DM) substantially impairs the vasodilating properties of the endothelium and leads to ED, which can thus be considered the first step in the progression of cardiovascular disease. Endothelial dysfunction in vasculature with macro-vascular damage in diabetes mellitus affects the coronary, carotid and peripheral arteries, hence increased the risk of cardiovascular complication like hypertension, myocardial infraction. The vascular endothelium is target of the diabetic mellitus and ED is thought to play an important role in diabetic vascular diseases [11, 12, 13, 14].

Vascular endothelial cells play a major role in maintaining cardiovascular homeostasis in health. In addition to providing a physical barrier between the vessel wall and lumen, the endothelium secretes a number of mediators that regulate platelet aggregation, coagulation, fibrinolysis and vascular tone. The term endothelial dysfunction refers to a condition in which the endothelium loses its physiological properties, the tendency to promote vasodilation, fibrinolysis and antiaggregation. Endothelial cells secrete several mediators that can alternatively mediate either vasoconstriction, such as endothelin-1 and thromboxane A_2 (TXA_2), or vasodilation such nitric oxide (NO),

prostacyclin and endothelium-derived hyperpolarizing factor (EDHF) [15]. NO is the major contributor to endothelium-dependent relaxation in conduit arteries whereas the contribution of EDHF predominates in smaller resistance vessels. Diabetes mellitus (DM) substantially impairs the vasodilating properties of the endothelium and leads to ED, which can thus be considered the first step in the progression of cardiovascular disease (CVD) [16]**.** Cardiovascular complications, characterized by ED and accelerated atherosclerosis, are the leading cause of morbidity and mortality associated with diabetes. There is growing evidence that excess generation of highly reactive free radicals, largely due to hyperglycemia, causes oxidative stress, which further exacerbates the development and progression of diabetes and its complications [17]**.**

As HMG-CoA reductase inhibitors (statins), were widely used for treatment of hypercholesterolemia. Statins emerges pleiotropic effects include improvement of ED, increased nitric oxide (NO) bioavailability, antioxidant properties, stabilization of atherosclerotic plaques etc. Additional effects of growing interest include the ability to recruit endothelial progenitor cells (EPCs), a putative immunosuppressive activity, and inhibition of cardiac hypertrophy [18]**.** Understanding the pleiotropic effects of statins, it's important to optimize their use in cardiovascular disease. In addition, the important role of the endothelium in damage repair following a cardiovascular event is emerging. Hence the objective of the study was scientifically evaluating effect of statins on diabetes associated cardiovascular complications.

Materials & Method:

Experimental animals

Adult male wistar rats weighing 200-250 g were used. They were housed in polypropylene cages lined with husk renewed every 24 h under a 12/12 h light/dark cycle at around 22 °C and had free access to tap water and food. The rats were fed on a standard pellets diet. Experimental protocols were approved by Institutional Animal Ethics committee (IAEC).

Induction of experimental diabetes

Healthy Sprague Dawley (SD) rats showing normal blood glucose level in the range of 80-120 mg/dl were used. The rats were injected with Streptozotocin (STZ) (Procured from Sigma Aldrich, U.S.A) in sodium citrate buffer (10 mM) at a single dose of 60-mg/kg body weights intraperitoneally. Blood glucose level was measured after 48 hrs of STZ administration by using blood glucose monitoring instrument, Glucometer (ONE TOUCH, Horizon, TPC 0088AZ, Johnson & Johnson Company; USA). The rats with high blood glucose (more than or equal to 300mg/dl) were selected for further studies as a diabetic group.

Experimental design

In our study, a total of 32 rats (8 normal and 24 streptozotocin diabetic surviving) were used. The rats were divided in to four groups of eight rats each.

Group 1: Normal untreated rats.

Group 2: Streptozotocin (60 mg/kg i.p.) induced diabetic rats.

Group 3: Diabetic rats treated orally with Atorvastatin (20 mg/kg p.o.) for 8 weeks.

Group 4: Diabetic rats treated orally with Atorvastatin (40 mg/kg p.o.) for 8 weeks.

Preparation of tissue homogenate

Rats were scarified by Euthanasia; thoracic aorta was removed after decapitation. Thoracic aorta was homogenized (20 mg/ml of PBS, pH 7.1) and centrifuged at 4 °C (15000 rpm for 10 minutes). The supernant was used for the estimation of various biochemical parameters.

Biochemical analysis

The activity of antioxidant enzymes SOD, Catalase and/or Lipidperoxidation were assayed according to the method of Saggu et al., 1989., Oferely et al., 1979, & Beltowski et al., 2000 respectively [19, 20, 21]**.**

Measurement of blood pressure by non-invasive tail cuff method

Blood pressure was measured in the conscious state at the end of 8^{th} week by non-invasive tail cuff blood pressure recorder (MLT125/R Rat tail cuff/Pulse transducer; ADInstruments Ltd, Australia) attached to the PowerLab (a multiple data acquisition system; ADInstruments Ltd., Australia) To ensure reproducibility, two measurements were made for each animal and the mean value was used. Systolic, diastolic and MAP were calculated.

Vascular reactive study

After 8 week from STZ injection, rats were sacrificed by cervical dislocation and thoracic aorta was isolated from the heart to the diaphragm. It was free from fats and connective tissues. Care was taken not to stretch the vessel. Helical strips of aorta of 3 mm in width and 20 mm in length was cut with sharp iris scissors and placed in 10 ml organ bath containing modified Krebs Henseleit solutions (KHS) of pH 7.4. The solution was continuously aerated with carbogen (95% O_2 + 5 % CO_2) at 37 °C. a resting tension of 2 gm was applied and allowed to equilibrate for 2 hours. Changes in the isotonic contraction were recorded on student's physiograph using isotonic fine movement transducer. During the equilibration and through out the experiments the KHS in the organ bath was changed at every fifteen minutes. ED and manifestations of ED were observed by seeing relaxation response of Acetylcholine chloride in the aortic spiral preparation, which was pre-contracted by phenylephrine (10^{-6} M).

Experimental protocol for vascular reactive study

After 2 hours of equilibration, two wakes up responses of KCl (80 mM) wee taken to check the stability of the tissues. After 15 minutes of gap, for evaluation of manifestation of endothelial dysfunction, tissue was precontracted by phenylephrine (10^{-6} M). After precontracted with phenylephrine, concentration responses curve of Ach. (10^{-9} M- 10^{-2}M in log concentration manner) induced relaxation was constructed.

Statistically analysis

Results were expressed as Mean ± SEM. Statistically differences was determined by Analysis of variance methods (ANOVA) by using statistical computer software Graph Pad Prisom. Only those value showing statistical differences $p<0.05$ considered as statistical significant. The % relaxation response of Ach was expressed in mean ± SEM. Statistically a difference was determined by t-test by using statistical computer software sigma state. Only those value showing statistical differences $p<0.05$ considered as statistical significant.

Results:

Effects of atorvastatin on body weight:

Table 1 showed the effect of Atorvastatin on changes occurred on average body weight. The average body weight was found to be decreased in STZ treated diabetic rats at the end of the 8^{th} week. Only STZ treated rats showed significant differences ($P<0.01$) in prominent loss of body weight at the end of the 8^{th} week as compare to normal untreated rats. STZ induced diabetic rats treated with Atorvastatin (20 & 40 mg/kg) for 8 weeks

Table 1: Effect of *Atorvastatin* on average body weight in STZ treated diabetic rats

Treatment	Body weight (gm)	
	Initial	Final
Normal untreated	246.25 ± 5.54	237.50 ± 5.20 **
STZ (60 mg/kg) treated	238.75 ± 5.54	173.75 ± 5.20 ##
STZ (60 mg/kg) + Atorvastatin (20 mg/kg)	227.50 ± 4.78	227.50 ± 4.78 **
STZ (60 mg/kg) + Atorvastatin (40 mg/kg)	237.50 ± 5.20	231.25 ± 7.46 **

Each value represents the Mean ± SEM for each group of six rats. Final body weight was measured at the end of 8th Week. ##P<0.01 Vs. normal untreated rats. **P<0.01 Vs. only STZ treated rats. (ANOVA, Dunnett's test).

Table 2: Effect of *Atorvastatin* on blood glucose level in STZ treated diabetic rats

Treatment	**Blood glucose level (mg/dl)**	
	Initial	**Final**
Normal untreated	92.71 ± 3.46 **	91.14 ± 2.85 **
STZ (60 mg/kg)	443.66 ± 24.78 ##	506.50 ±15.31 ##
STZ (60 mg/kg) + Atorvastatin (20 mg/kg)	403.33 ± 22.91##	437.38 ± 23.78 ##
STZ (60 mg/kg) + Atorvastatin (40 mg/kg)	431.16 ± 21.87 ##	405.83 ± 8.96 ##

Each value represents the Mean ± SEM for each group of six rats. ##P<0.01 Vs. normal untreated rats. **P<0.01 Vs. only STZ treated rats. (Dunnett's test).

significantly (P<0.01) improved loss of body weight as compared to diabetic rats.

Effect of atorvastatin on blood glucose level in diabetic rats:

Tables 2 illustrated the effect of Atorvastatin on blood glucose level. Initially there was significant (P<0.05) increase in blood glucose level in all groups treated with single dose of STZ as compared to normal untreated rats. At the end of 8th week, Atorvastatin administered at a dose of 20 & 40 mg/kg in STZ treated diabetic rats did not show any significant difference in the decrease in blood glucose level as compared to normal untreated rats.

Antioxidant activity of atorvastatin in diabetic rats

Table 3 illustrated the effect of Atorvastatin on antioxidants profile of *Superoxide dismutase* (SOD), *Catalase* and lipidperoxidation. Rats treated with only single dose of STZ showed significantly (P<0.01) decreases in the activity of antioxidant enzyme, SOD and *Catalase* in thoracic aorta as compared to normal untreated rats. At the end of the 8th week, STZ induced diabetic rats treated with Atorvastatin (20 & 40 mg/kg) significantly (P<0.01) increased the activity of antioxidant enzyme, SOD and *Catalase* when compared to only STZ treated diabetic rats. There was no significantly (P<0.05) difference observed as compared to normal untreated rats means improved the antioxidant enzyme activity extended to the normal level. The concentration of MDA content increase significantly (P<0.05) in single dose of STZ treated rats as compared to normal untreated rats. STZ induced diabetic rats treated with Atorvastatin at doses of 20& 40 mg/kg for a period of 8 weeks exerted a significantly (P<0.01) improved effect on increased concentration of MDA content in only STZ treated diabetic rats as compared to only STZ treated diabetic rats.

Table 3: Effects of Atorvastatin on *Superoxide dismutase* (SOD), *Catalase* & Lipidperoxidation in STZ treated diabetic rats.

Treatment	**SOD (Units/mg protein)**	**Catalase (Units/mg of protein)**	**MDA content (n mol/gm of tissue)**
Normal untreated rats	12.14 ± 0.40 **	7.68 ± 0.52 **	45.58 ± 1.25 **
STZ (60 mg/kg) induced diabetic rats.	5.62 ± 0.44 $^{\#\#}$	3.84 ± 0.26 $^{\#\#}$	68.01 ± 0.68 $^{\#\#}$
STZ (60 mg/kg) + Atorvastatin (20 mg/kg)	11.33 ± 0.72 **	6.81 ± 0.36 **	42.93 ± 1.08 **
STZ (60 mg/kg) + Atorvastatin (40 mg/kg)	12.27 ± 0.56 **	7.80 ± 0.31 **	38.96 ± 1.79 $^{\#\,**}$

Each value represents the Mean ± SEM for each group of six rats. $^{\#}$P<0.05 & $^{\#\#}$P<0.01 Vs. normal untreated rats. *P<0.05 **P<0.01 Vs. only STZ treated rats. (Dunnett's test).

Table 4: Effects Atorvastatin on Blood pressure in STZ treated diabetic rats

Treatment	**Blood pressure in mmHg**		
	Systolic	**Diastolic**	**MAP**
Normal untreated rats	112.11 ± 3.06**	79.49 ± 3.99 *	91.47 ± 2.53**
STZ (60 mg/kg) induced diabetic rats.	151.98 ± 2.83 $^{\#\#}$	90.74 ± 1.81$^{\#}$	111.16 ± 1.38$^{\#\#}$
STZ (60 mg/kg) + Atorvastatin (20 mg/kg)	118.06 ± 1.73**	83.69 ± 3.05	95.48 ± 1.18**
STZ (60 mg/kg) + Atorvastatin (40 mg/kg)	112.74 ± 5.15**	79.41 ± 3.21	90.52 ± 3.45**

Each value represents the Mean ± SEM for each group of six rats. $^{\#}$P<0.05, $^{\#\#}$P<0.01 Vs. normal untreated rats. *P<0.05, **P<0.01 Vs. only STZ treated rats. (Dunnett's test).

Treatment with Atorvastatin at doses of 40 mg/kg to diabetic rats for a period of 8 weeks exerted a significant (P<0.05) decreased the concentration of MDA content beyond the level of MDA concentration observed in normal untreated rats.

Effects of atorvastatin on blood pressure in stz treated diabetic rats

Table 4 showed the effect of Atorvastatin on blood pressure in STZ treated diabetic rats. Initially the blood pressure was measure in all groups and it was normal. Rats treated with only single dose of STZ showed significant (P<0.01) increased systolic, diastolic and Mean Arterial Pressure (MAP) in STZ treated rats as compared to normal untreated rats. Treatment with Atorvastatin (20 & 40 mg/kg) for 8 weeks significantly (P<0.01) decreased systolic, diastolic and MAP as compared to only STZ treated diabetic rats. There was no significant (P<0.05) difference observed in treatment with Atorvastatin (20 & 40 mg/kg) as compared to normal untreated rats means maintain the blood pressure up to normal level.

Vascular reactivity studies on rat thoracic aorta:

Effect of Acetylcholine (Ach) on Rat Thoracic aorta of Normal rats.

Figure 1 illustrated the **c**oncentration response curve of Ach. (10^{-9}M to 10^{-2}M) induced relaxation in thoracic aorta of normal rats (Endothelial intact & Endothelial denude), precontracted with Phenylephrine (PE) (10^{-6}M). Values are

expressed in Means ± SEM. % relaxation of Ach significantly inhibited in endothelial denude thoracic aorta of the control rat as compared to endothelial intact control rats. *P<0.05, **P<0.01 Vs. Control (Endothelial +).

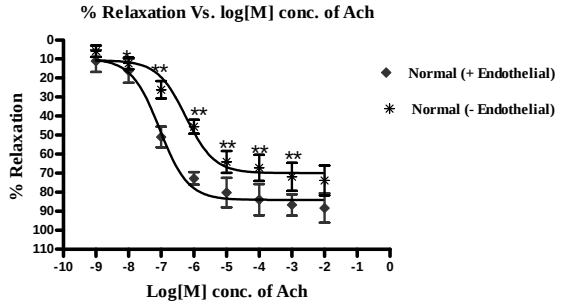

Figure 1: Concentration response curve of Ach. (10^{-9}M to 10^{-2}M) induced relaxation in thoracic aorta of normal rats (Endothelial intact & Endothelial denude) precontracted with Phenylephrine (PE) (10^{-6}M).

Effect of Acetylcholine (Ach) on Rat Thoracic aorta of Normal and STZ treated diabetic rats.

Figure 2 showed the concentration response curve of Ach. (10^{-9}M to 10^{-2}M) induced relaxation in thoracic aorta of normal (endothelial intact) and diabetic rats, precontracted with PE (10^{-6}M). Values are expressed in Means ± SEM. % relaxation of Ach significantly decreased in diabetic rat thoracic aorta as compared to control. *P<0.05, **P<0.01 Vs. Control.

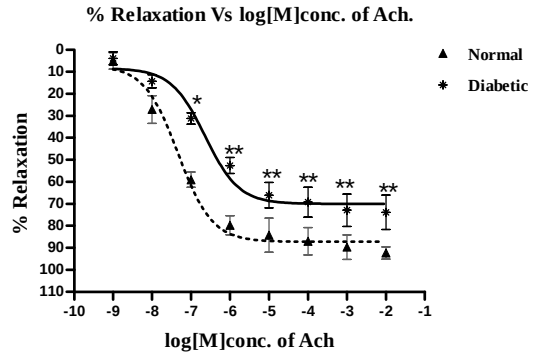

Figure 2: Concentration response curve of Ach. (10^{-9}M to 10^{-2}M) induced relaxation in thoracic aorta of normal (endothelial intact) and diabetic rats, precontracted with PE (10^{-6}M).

Figure 3 showed the concentration response curve of Ach. (10^{-9}M to 10^{-2}M) induced relaxation in thoracic aorta of normal (endothelial denude) and diabetic rats, precontracted with PE (10^{-6}M). Values are expressed in Means ± SEM.

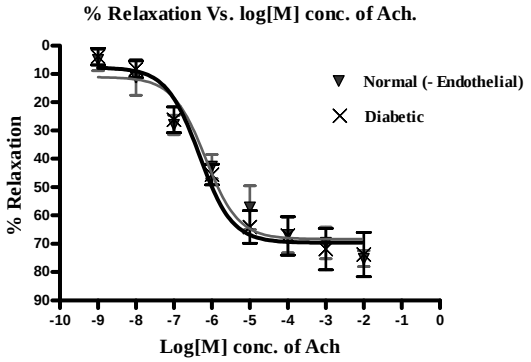

Figure 3: Concentration response curve of Ach. (10^{-9}M to 10^{-2}M) induced relaxation in thoracic aorta of normal (endothelial denude) and diabetic rats, precontracted with PE (10^{-6}M).

Effect of Acetylcholine (Ach) on Rat Thoracic aorta of Diabetic rats treated with Atorvastatin.

Figure 4 illustrated **c**oncentration response curve of Ach. (10^{-9}M to 10^{-2}M) induced relaxation in thoracic aorta of normal rats, diabetic rats & diabetic rats treated with Atorvastatin (20 & 40 mg/kg) precontracted with PE (10^{-6}M). Values are expressed in Means ± SEM. % relaxation of Ach significantly inhibited in diabetic rat as compared to control rats. Diabetic rats treated with Atorvastatin showed a significant difference with compared to STZ treated Diabetic rats. *P<0.05, **P<0.01 Vs. Control. #P<0.01 Vs. diabetic rats,

Discussion:

The rats showed symptoms of type 1 diabetes with prominent loss of body weight. Results suggest that treatment with Atorvastatin showed improvement in their body weight indicating that the Atorvastatin

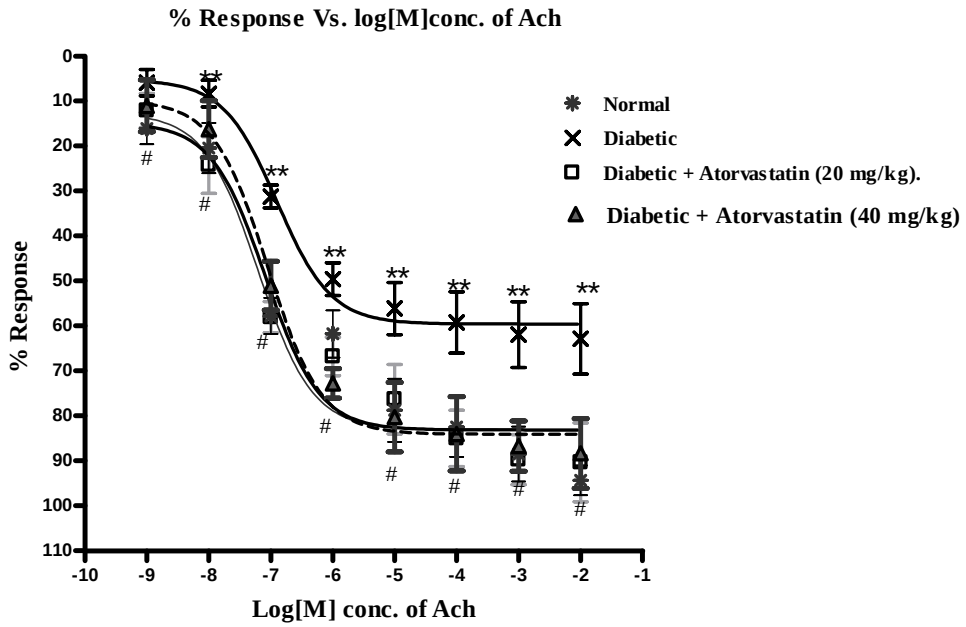

Figure 4: Concentration response curve of Ach. (10^{-9}M to 10^{-2}M) induced relaxation in thoracic aorta of normal rats, diabetic rats & diabetic rats treated with Atorvastatin (20 & 40 mg/kg) precontracted with PE (10^{-6}M).

have beneficial effect in preventing loss of body weight of diabetic rats (Table-1).

It is well-established models that STZ increase the blood glucose level and causes diabetes mellitus. In present study we have observed that STZ treated rats showed increased in blood glucose level at 48 hrs after administration of single dose of STZ and at the end of the 8th weeks. The results indicate that treatment with Atorvastatin has no significant effects on blood glucose level (Table-2).

Oxidative stress is an imbalance between reactive oxygen species (ROS) and the antioxidant defense mechanisms of a cells or tissue which leads to the lipidperoxidation and inactivation of many enzymes [22]**.** In the present study STZ induced diabetes produced generation of highly free radicals ROS & reactive nitrogen species (RNS) by autoxidation of glucose [23]**,** unpaired of eNOS [24, 25, 26] and decreased antioxidant defenses enzymes activity [27]. In addition to hyperglycemia induced mitochondrial overproduction anion radicals play a key role in the activation of the stress sensitive pathways [28]. Also these free radical mediated peroxidation of membrane phospholipids and consequences changes in membrane permeability are responsible for diabetes induced macrovascular damage. Clinically complication in diabetes may be due to dysfunction of key antioxidant enzymes. Exogenous administration of Atorvastatin has been demonstrated to provide protection from these changes either by scavenging free radical or by antioxidant activity (Table 3).

Increased generation of superoxide (O_2^-) and other ROS and decreased plasma or tissue concentration of SOD and *catalase* enzymes in both clinical as well as experimental diabetes are reported. [29, 30]**.** In present study there was decrease in the activity of SOD and catalase observed in STZ induced experimental diabetes (Table 3). Decreased activity of SOD and catalase in diabetes can leads to excess availability of O^{2-} and

hydrogen peroxide (H_2O_2) in the biological system, which in terns generated hydroxyl (OH^-) radical resulting in the propagation of lipidperoxidation.

SOD, important endogenous antioxidants enzymes of first line defense, which catalyses the disputation of superoxide radicals. In the present study, the results indicate that Atorvasrtatin administration at doses of 20 & 40 mg/kg restored the activity of SOD and catalase to the normal levels due to potent antioxidant property and their pleotropic effects (Table 3). Taken together these results support the idea that the antioxidant property of Atorvastatin.

Oxygen free radical mediated lipid peroxidation of unsaturated fatty acid was clearly implicated in pathogenesis and progression of various diseases such as atherosclerosis, hypertension and IHD [31]**.** ROS can also alter lipids and proteins accelerated formation of AGEs; NO rapidly react with superoxide to forms peroxynitrate ($ONOO^-$), which may promote low density lipo protein (LDL) oxidation. OH^- is responsible for attack by radicals on phospholipids rich cell membrane leading to lipid peroxidation.

Lipid peroxidation plays an important role in macrovascular cells damage. Enormous amount of ROS, like O_2^-, H_2O_2 and OH^-, are produced during diabetes. Significant elevation in the concentration of thiobarbituric acid reactive substances (TBARS) expressed as a content of malondialdehyde (MDA) observed in diabetic rats (Table 3). In vivo lipidperoxidation is a radical chain reaction consisting of chain reaction and propagation. During chain initiation reaction, an alkyl radical is formed by abstracting one of the two hydrogen's on bisallylic carbon atoms from the polyunsaturated fatty acids moiety of phospholipids bilayers. This ultimately leads to lipid hydro peroxides formation, which further attacks the neighboring polyunsaturated fatty acids. Unstable lipid hydroperoxides could also interact with DNA and forms unstable adducts. Highly reactive radicals such as OH^- have the propensity to attack biological membranes and biomolecules by abstracting hydrogen and initiating free radical chain reaction and consequent lipid peroxidation. As the cellular antioxidant status determines the susceptibility to oxidative damage, which usually alters in response to oxidative stress and there fore the SOD activity can inversely be correlated with MDA content.

In the present study a marked rise in MDA content with a concomitant decrease in SOD activity demonstrates STZ induced diabetes causes oxidative stress in thoracic aorta by decreased efficiency of antioxidant enzymes and increased lipid peroxidation and impaired vascular dysfunction. However treatment with Atorvastatin (20 & 40 mg/kg) decreased the elevated level of TBARS by decreasing concentration of MDA contents as compared to diabetic rats (Table 3).

Mitochondrial are the major endogenous sources of superoxide and superoxide is a casual link between elevated levels of blood glucose and major biochemical pathways postulated to be involved in the development of oxidative stress, ROS & RNS and vascular complication in diabetes like hypertension which correlated well with increasing in systolic blood pressure in diabetic rats. In present study results suggest that treatment with Atorvastatin 20 & 40 mg/kg maintain the systolic blood pressure up to the normal levels (Table 4). The reason behind this might be considered as a good antioxidant activity and hence less amount of production of ROS and RNS, which are mainly responsible for generation of vascular dysfunction.

In both type 1 and type 2 diabetes, diabetic complications in target organs arise from chronic elevations of glucose. The pathogenic effect of high glucose, possibly

in concert with fatty acids, is mediated to a significant extent via increased production of ROS and RNS and subsequent oxidative stress. Amongst the ROS, O_2^-, OH^-, and H_2O_2 are implicated in the impaired relaxation responses to Ach (A marker for endothelial dysfunction) [32, 33]**.** Earlier reported that Type 1 diabetes is associated with impaired responsiveness to NO and with impairment in Ach-stimulated NO release [34]**.** In present study, the Ach induced relaxation was impaired in diabetic rats as compared to normal rats (Figure 2). In addition there is no significance difference was observed in rat thoracic aorta of diabetic rats and endothelial denude thoracic aorta of normal rats (Figure 3). The results suggested that generation of oxidative stress in diabetes may leads to the ED. Thus conclusively that ROS are generated in experimental diabetic rats, which causes the vascular dysfunction. The results suggest that treatment with Atorvastatin at doses of 20 & 40 mg/kg restored the endothelial dysfunction by observing the Ach induced relaxation in Atorvastatin treated rats for 8 weeks (Figure 4).

Conclusion:

Due to prolonged hyperglycemia, rats have developed oxidative stress leading to causes imbalance between free radicals and antioxidant defense mechanism like SOD and *Catalase* enzymes. The decreased level of SOD and *catalase* enzymes and increased the lipidperoxidation leads to the ED and increased the vascular complication like hypertension. So first, antioxidant therapy needs to be improved the vascular complication in diabetes mellitus. At present scenario many types of non-enzymatic and synthetic anti-oxidants are available which can improve some aspects of ED in diabetes. Atorvastatin emerges pleiotropic effects include improvement of ED, increased NO bioavailability, antioxidant properties, stabilization of atherosclerotic plaques etc. Additional effects of growing interest include the ability to recruit endothelial progenitor cells (EPCs), a putative immunosuppressive activity, and inhibition of cardiac hypertrophy. Present investigation focused its ability as improved the antioxidant profile and improved ED and prevents the vascular complication like hypertension in diabetes. By the results of the present study, we can predict that Atorvastatin can be used as a co-therapy in diabetes mellitus for treating the vascular complication in diabetes as suggested in folklore remedies

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