British Journal of Pharmaceutical and Medical Research Vol.04, Issue 05, Pg.2048 - 2059, September-October 2019

Available Online at http://www.bjpmr.org

BRITISH JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

Cross Ref DOI : https://doi.org/10.24942/bjpmr.2019.563 Volume 04, Issue 05 Sept-October 2019

ISSN:2456-9836
ICV: 60.37

Research Article

# Ameliorative Effect Of Fluvastatin And Dapagliflozin On Vascular Endothelial Dysfunction In Diabetic Rats

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ARTICLE INFO

Article History:
Received on 07th Sept, 2019
Peer Reviewed on 26th Sept, 2019
Revised on 15th October, 2019
Published on 29th October, 2019

Keywords:
Catecholamines, Endothelial Dysfunction, Fluvastatin and Vascular Reactivity

ABSTRACT

**Objective:** The aim of the present study was to evaluate the ameliorative effects of fluvastatin, dapagliflozin and their combinations on endothelial dysfunction in fructose- induced diabetic rats.

**Methods:** Fluvastatin (4 mg/kg, p.o.), dapagliflozin (2 mg/kg, p.o.) and their combinations were administered for 6 weeks after induction of diabetes by fructose (66%, w/v solution, p.o. for 8 weeks) in wistar rats. The effects were examined on body weight, serum glucose, triglyceride, cholesterol, blood pressure, heart rate, nitric oxide and antioxidant defensive enzymes. After completion of treatment schedule, the blood pressure (BP) was determined by invasive method and vascular reactivity was tested with adrenaline, noradrenaline and phenylephrine. Endothelial dysfunction was determined by acetylcholine and sodium nitroprusside-induced vasorelaxation on isolated rat aortas.

**Results:** Long term treatments significantly decreased body weight gain, serum glucose, triglyceride and cholesterol levels; normalize the heart rate, antioxidant enzymes and vasodilator levels in fructose induced rats. The treatments significantly improved vascular reactivity to catecholamine and endothelial functions with reduction in elevated BP in diabetic rats.

**Conclusion:** Fluvastatin and dapagliflozin were able to reverse endothelial dysfunction, but better ameliorating potential was found when used in combinations.



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## INTRODUCTION:

Metabolic syndrome can be defined as a cluster of interrelated risk factors that are associated with an increased risk of diabetes and cardiovascular disease. [1]It is estimated that around 20-25 percent of the world's adult population have the metabolic syndrome and they are three times as likely to have a heart attack or stroke compared with people without the syndrome. In addition, people with metabolic syndrome have a fivefold greater risk of developing diabetes. [2]Diabetes has become one of the major causes of premature illness and death, mainly through the increased risk of cardiovascular disease (CVD) which is responsible for up to 80% of deaths. [3]

Diabetes is associated with significant morbidity due to specific diabetes related microvascular complications e.g. ischemic heart disease, stroke and peripheral vascular disease, and diminished quality of life.[4]Insulin resistance is associated with many risk factors that commonly precede the development of hyperglycaemia and these typically include obesity, dyslipidaemia, elevated blood pressure, oxidative stress, endothelial dysfunction. [5]It has been suggested that hyperglycaemia and hypertriglyceridemia induce endothelial dysfunction and inflammation through the production of an oxidative stress. Endothelial dysfunction refers to a condition in which the endothelium loses its physiological properties (the tendency to promote vasodilation, fibrinolysis and antiaggregation).[6]Oxidative stress is an increase in the steady-state levels of reactive oxygen species and may occur as a result of increased free radical generation and/or decreased anti-oxidant defence mechanism. [7] It has been shown that, when the activities of superoxide dismutase (SOD, which capture $O_2$•) and catalase (CAT) were maintained, the endothelial function was not altered even in cases of hyperglycaemia.[8]Insulin resistance leads to abnormalities in regulatory mechanism of blood pressure resulting into cardiovascular complications in type-2 diabetes mellitus (DM).The dyslipidaemia is a central player in the development of atherosclerosis and other cardiovascular complications in the setting of insulin resistance in type-2 DM. [9]

The increases in BP and triglyceride level in diabetes are secondary to the hyperinsulinemia, and then a drug intervention that reverses these effects should also attenuate the hypertension and cardiovascular complications. [10] A drug that modulates endothelial functions can significantly alter morbidity and mortality associated with endothelial dysfunction. [11]Fluvastatin, is a HMG-CoA reductase inhibitor. HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonic acid, the rate-limiting step in cholesterol biosynthesis. Fluvastatin belongs to a class statin and is used to reduce plasma cholesterol levels. It has also been shown anti-inflammatory effect on microvascular endothelial independent by its lipid lowering action as well as due to induction of nitric oxide from the vasculature. [12]

Dapagliflozin (Sodium Glucose transporter inhibitor) improves hyperglycaemia by inhibiting renal glucose reabsorption through SGLT2. SGLT2 is a sodium-solute cotransport protein located in the kidney proximal tubule that reabsorbs the majority of glomerular-filtered glucose. It is reasonable to hypothesize that the efficacy of dapagliflozin is independent of the loss of pancreatic β-cell function or the level of insulin resistance and improve endothelial dysfunction. [13]

Therefore, long term treatment with Fluvastatin, dapagliflozin and their combination in fructose-induced rats was evaluated. In this study, an attempt has been made to investigate the effect of Fluvastatin, dapagliflozin and their combination on endothelial dysfunction in diabetic rats.

## MATERIALS AND METHODS

### Drugs and chemicals

Fluvastatin (Sun pharma laboratories Ltd. Mumbai, India) and dapagliflozin (Sun pharma laboratories Ltd., Mumbai, India) were obtained as gift samples. The drug solution of Fluvastatin was freshly prepared in distilled water and suspension of dapagliflozin was made with 0.5% CMC in distilled water. Biochemical kits for glucose, triglyceride and cholesterol (Auto Span, Surat, India) were used. All the chemicals used were of analytical grade and purchased from standard manufacturers.

**Experimental animals**

Male wistar rats (200 - 220 g) were obtained from Animal Centre of Scientific and Medical Innovation (LACSMI) Biopharm Pvt. Ltd. Pune, India. Animals were housed in polypropylene cages and maintained under the standard laboratory environmental conditions; temperature 25 ± $2^0$ C, 12:12 h L: D cycle and 50 ± 5% RH with free access to food and water *ad libitum*. Animals were acclimatized to laboratory conditions before the test. All the experiments were carried out during the light period (08:00 – 16:00 h). The studies were carried out in accordance with the guidelines given by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi (India). The Institutional Animal Ethical Committee (IAEC) of M.V.P.S College of Pharmacy, Nashik approved the protocol of the study (IAEC/2018/04).

**Induction of Diabetes Mellitus in rats**

Rats were administered with fructose (66% w/v solution, 10 ml/kg/day, p.o., for 6 weeks) to induce DM.[14] The animals showing fasting blood glucose level more than 280 mg/dl and BP over 145 mmHg were selected for the study.

**Experimental design**

The diabetic animals were randomly assigned to six groups (n = 6).Group I (Control) - vehicle (Distilled water; 5 ml/kg, p.o.), Group II (Diabetic control)- Fructose 66% w/v,10ml/kg, p.o.(at interval of 24 hr for 6 week ), Group III –Fructose + Fluvastatin(4mg/kg, p.o), Group IV- Fructose+ dapagliflozin (2mg/kg, p.o), Group V –Fructose + Fluvastatin + dapagliflozin (4mg/kg,p.o.+5 mg/kg, p.o), Group VI - Fructose + atorvastatin( 8 mg/kg, p.o.).The drugs were administered daily for a period of 6 weeks after induction of diabetes.

**In-vivo experimental method**

After induction of diabetes, the treatments were initiated. Body weight of each animal was measured before start of treatment and thereafter weekly of drug treatments. Using tail-cuff method, systolic blood pressure and pulse rate were recorded weekly during the treatments on Power-lab data acquisition system (AD Instruments). [15] Blood samples were collected through retro-orbital plexus under ether-anaesthesia weekly for determination of serum glucose, triglyceride and cholesterol. [16] After completion of treatments, SOD, CAT, LPO and NO levels were measured; BP was determined by invasive method and vascular reactivity was tested with Adrenaline (Adr), NA (Noradrenaline) and PE (Phenylephrine). Acetylcholine (ACh) and nitric oxide (NO)-induced vasorelaxation was studied on isolated rat aortas at end of the treatments. [17]

**Estimation of serum glucose, triglyceride and cholesterol**

The rats were anaesthetized under light ether; blood was removed from the retro-orbital plexus using a capillary in micro sample tubes, serum was separated and used for biochemical investigations. Serum glucose, triglyceride and cholesterol levels were determined by using standard biochemical kits.

**Vascular reactivity to catecholamines**

After the completion of treatment schedule, rats from each group were anesthetized by ketamine and xylazine (75 mg/kg and 15 mg/kg ip. respectively). Right jugular vein was cannulated with fine polyethylene catheter for the administration of drugs. [18] BP was recorded from left common carotid artery using pressure transducer (MLT-125) by direct method on Power-lab data acquisition system (AD Instruments). Heparinised saline (100 IU/ml) was filled in the transducer and in the fine catheter cannulated to the carotid artery to prevent clotting. After 30 min of stabilization, mean change in BP to Adr (1 µg/kg), NA (1 µg/kg) and PE (1 µg/kg) were recorded. [19]

**In-vitro experimental method**

**Endothelial dependent and independent vasorelaxation on isolated rat aortas**

After completion of vascular reactivity studies, rats were sacrificed by cervical dislocation method. Thoracic aorta from the arch down to diaphragm was isolated and a part of it was placed in Krebs solution of composition (mM), NaCl: 118.4; KCl: 4.7; $CaCl_2$: 2.5; $KH_2PO_4$: 1.2; $MgSO_4$: 1.2; $NaHCO_3$: 25.0, Glucose: 11.0, at $37^0$ C and aerated with 95 % $O_2$ and 5 % $CO_2$,and the other part were used for vascular reactivity studies. The aortas were cut into rings of 3 mm length and mounted in organ bath containing 15 ml of Krebs solution. The contractions were recorded by using force transducer

(Power-Lab, AD Instruments). The resting tension of 1 g was applied to the preparation and equilibrated for 3 hr before the experiment with exchange of bathing solution every 15 min. After the equilibration, the rings were exposed to 3 × $10^{-6}$ M PE for precontraction. When the contractile response of PE was plateaued, SNP or Ach were added in a cumulative fashion for NO and endothelial dependent vasorelaxation studies respectively. To verify the integrity of smooth muscle in thoracic aortas, SNP-induced vasorelaxation (a NO donor) was also investigated which causes aorta to relax independent of endothelial nitric oxide. [20]

**Estimation of antioxidants activity**

10 % w/v tissue homogenate of aorta was prepared in ice-cold 0.1 M phosphate buffer (pH 7.4). The post nuclear fraction for catalase assay was obtained by centrifugation (Remi – C - 30, Remi Industries Ltd. Mumbai, India) of the homogenate at 1000 g for 20 min at $4^0$ C; for other enzyme assays, centrifugation was at 12000 g for 60 min at $4^0$ C. Bio-spectrophotometer (BL-200, Elico, India) was used for assay.

**Estimation of SOD and CAT activity**

SOD activity was assayed by the method of Kono, wherein the reduction of nitroblue tetrazolium chloride (NBT) was inhibited by the superoxide dismutase, which was measured at 560 nm spectrophotometrically. Briefly the reaction was initiated by the addition of hydroxylamine hydrochloride to the reaction mixture containing NBT and post nuclear fraction of homogenate. The results were expressed as units per milligrams of protein, with one unit of enzyme defined as the amount of SOD required to inhibit the rate of reaction by 50%

Catalase activity was assessed by the method of Luck, where the breakdown of $H_2O_2$ was measured. The assay mixture consisted of 3 ml of $H_2O_2$ phosphate buffer (0.0125 M $H_2O_2$) and 0.05 ml of supernatant of aortic homogenate (10%) and the change in the absorbance were measured at 240 nm. The enzyme activity was calculated using the millimolar extension coefficient of $H_2O_2$ (0.07). The results were expressed as micromoles of $H_2O_2$ decomposed per minute per milligram of protein. [21]

**Estimation of LPO level**

The quantitative measurement of lipid peroxidation was done by the method of Wills. The amount of malondialdehyde (MDA) formed was measured by reaction with thiobarbituric acid at 532 nm. The results were expressed as nanomloes of MDA per milligram of protein, using the molar extension coefficient of chromophore (1.56 x $10^5$ $M^{-1}$ $cm^{-1}$) [22]

**Estimation of NO level**

The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide (NO), was determined with a colorimetric assay using Greiss reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulfanilamide and 2.5% phosphoric acid). Equal volumes of supernatant and Greiss reagent were mixed, the mixture was incubated for 10 min at room temperature in the dark and the absorbance at 543 nm was determined spectrophotometrically. The concentration of nitrite in the supernatant was determined from a sodium nitrite standard curve and expressed as µmol of nitrite per ml of homogenate.[23]

**Statistical analysis**

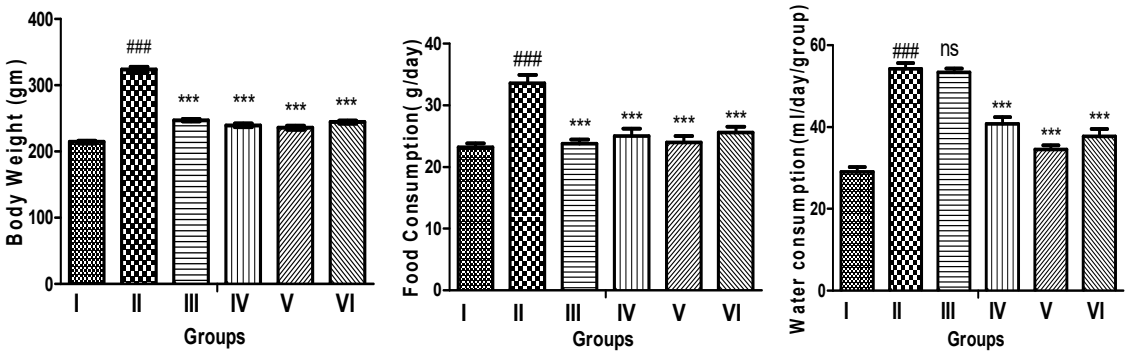
Results are expressed as mean ± SEM, and the statistical analysis of data was done using one-way analysis of variance (ANOVA) followed by Dunnett's test (GraphPad Prism Version 5.00). Probability level less than 0.05 was considered statistically significant.

## RESULTS

**Effect of on body weight, food consumption and fluid intake**

After induction of diabetes the rats showed increase in body weight, food consumption and fluid intake. Treatment with Fluvastatin, Dapagliflozin their combination was able to significantly ($p<0.001$) reduce the diabetes induced body weight, food consumption and fluid intake.″fig.1″

*Figure 1. Effect of Fluvastatin and Dapagliflozin on Body Weight, Food Consumption and Water Consumption. Group I: Vehicle (distilled water 5 ml/kg, p.o.); Group II: Diabetic (Fructose 66 %w/v ,10 ml/kg, p.o.); Group III: Fluvastatin (4 mg/kg, p.o.); Group IV: Dapagliflozin (2 mg/kg, p.o.); Group V: Fluvastatin(4mg/kg, p.o.) + Dapagliflozin (2mg/kg, p.o.); Group VI: Atorvastatin (8mg/kg, p.o.)*

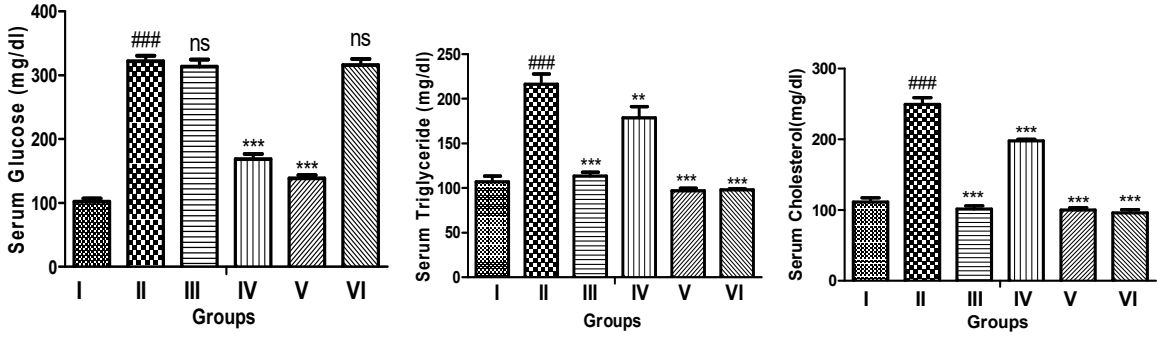



Each value represents mean ± S.E.M. (n=6). # Diabetic rats compared to Normal rats (Student's t-test). * Treatment rats compared to Diabetic rats (One-way ANOVA followed by Dunnett's test). ##, ** p < 0.01, ### p < 0.001.

**Effect on biochemical parameters**

Fructose treated group showed significant (p<0.001) rise in serum glucose, cholesterol and triglycerides levels compared to control group. Long term treatment with HMG CoA reductase (Fluvastatin) and Sodium Glucose transport protein inhibitor (Dapagliflozin) and Fluvastatin-Dapagliflozin combination significantly (p<0.001) reduced elevated serum glucose, cholesterol and triglycerides levels compared to fructose treated group. There was no significant difference in serum glucose, but cholesterol and triglyceride were significant (p<0.01) reduced in group treated with Fluvastatin alone.″fig.2″

*Figure 2.Effect of Fluvastatin and Dapagliflozin on serum glucose, triglyceride and cholesterol levels. Each value represents mean ± S.E.M. (n=6). # Diabetic rats compared to Normal rats (Student's t-test). * Treatment rats compared to Diabetic rats (One-way ANOVA followed by Dunnett's test). ##, ** p < 0.01, ### p < 0.001.*



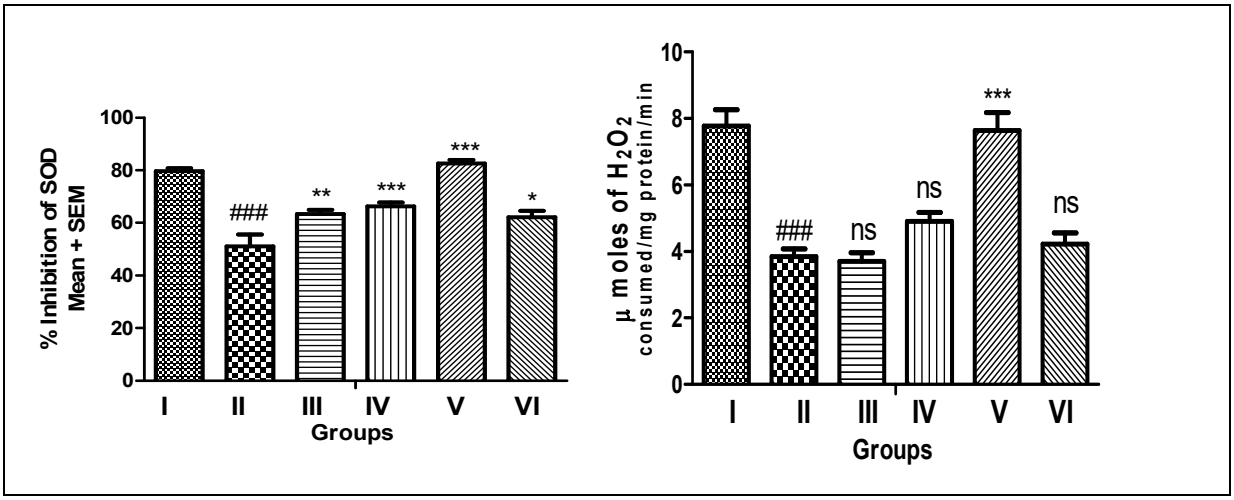

**Effect on heart rate (HR) and blood pressure (BP)( non-invasive and invasive method)**

The control group animals showed the normal BP and HR, whereas fructose treated group showed significant (p<0.001) rise in BP and decrease (p<0.05) in HR as compared to control group. Administration of Fluvastatin, Dapagliflozin and Atorvastatin alone for a period of 6 weeks significantly (p<0.05 and p<0.01 respectively) reduced BP compared to fructose treated group. But the reduction was most significant with the combination of Fluvastatin-Dapagliflozin. All the treatment groups significantly normalized heart rate.″fig.3″

**Effect on antioxidant defense**

The SOD and CAT levels were significantly ($p<0.001$) decreased while the LPO levels were significantly ($p<0.001$) increased in fructose diabetic rats compared to control group. Treatment with Fluvastatin, Dapagliflozin, and their combinations significantly ($p<0.001$) reduced the LPO levels with significant ($p<0.01$) increase in CAT and SOD levels compared to fructose treated group.″fig.6″

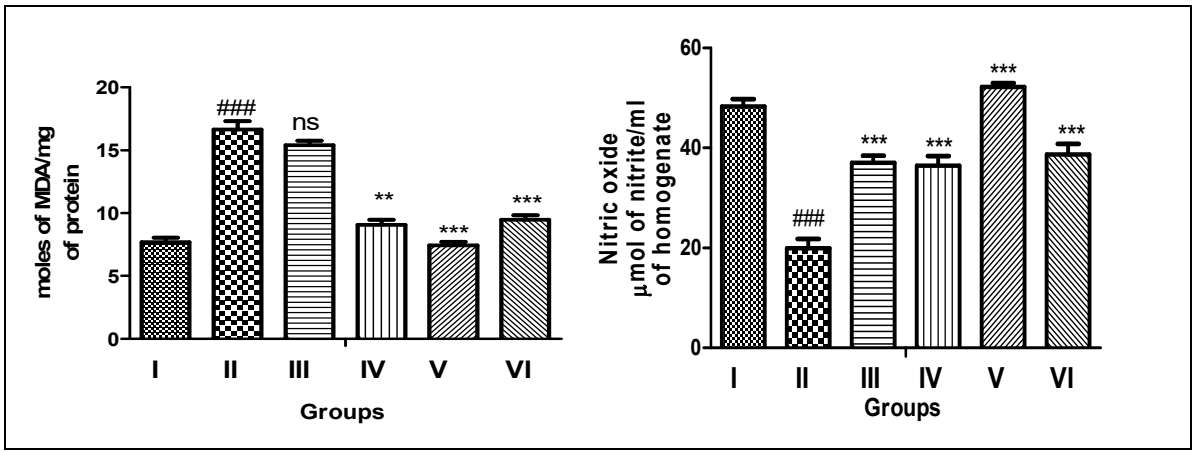
*Figure 6. Effect of Fluvastatin and Dapagliflozin on superoxide dismutase (SOD) and Catalase (CAT) level in rats.*




**Effect on NO Levels**

The NO levels were significantly ($p<0.001$) decreased in fructose diabetic rats compared to control group. Treatment with Fluvastatin, Dapagliflozin and Atorvastatin significantly ($p<0.01$) increase NO levels compared to fructose treated group. But was most significant with Fluvastatin and Dapagliflozin ($p<0.001$) combination.″ fig.7″

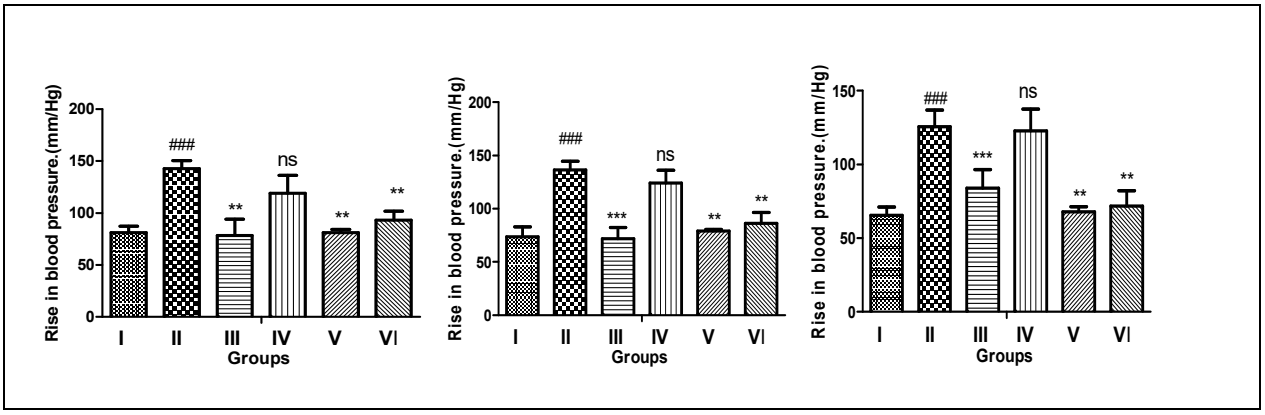
*Figure 7.Effect of Fluvastatin and Dapagliflozin on Lipid Peroxidation(LPO) and Nitric Oxide(NO) level in rats.*




**Effect on vascular reactivity to catecholamines**

The control group showed normal vascular response to Adr, NA and PE (1 µg/kg, iv.), whereas fructose treated group showed a significant ($p<0.001$) elevation in mean change in BP to Adr, NA and PE. Treatment with Fluvastatin, dapagliflozin and atorvastatin significantly ($p<0.05$) reduced the mean change in BP to Adr, NA and PE compared to fructose treated group. But most significant ($p<0.001$) reduction in the mean change in BP to Adr, NA and PE was observed with Fluvastatin and dapagliflozin combination.″ fig.4″

*Figure 4. Effect of Fluvastatin and Dapagliflozin on vascular reactivity to Adrenaline (1µg/kg), noradrenaline (1µg/kg) and Phenylephrine (1µg/kg) respectively.*

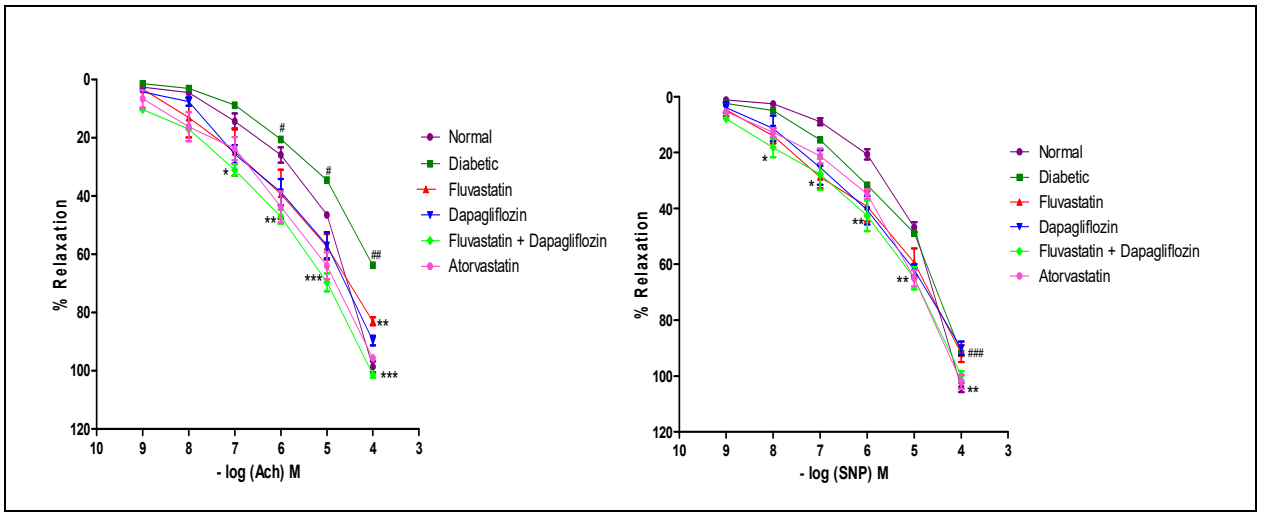



Each value represents mean ± S.E.M. (n=6). # Diabetic rats compared to Normal rats (Student's t-test). * Treatment rats compared to Diabetic rats (One-way ANOVA followed by Dunnett's test). ##, ** p < 0.01, ### p < 0.001.

**Effect on vascular endothelial function**

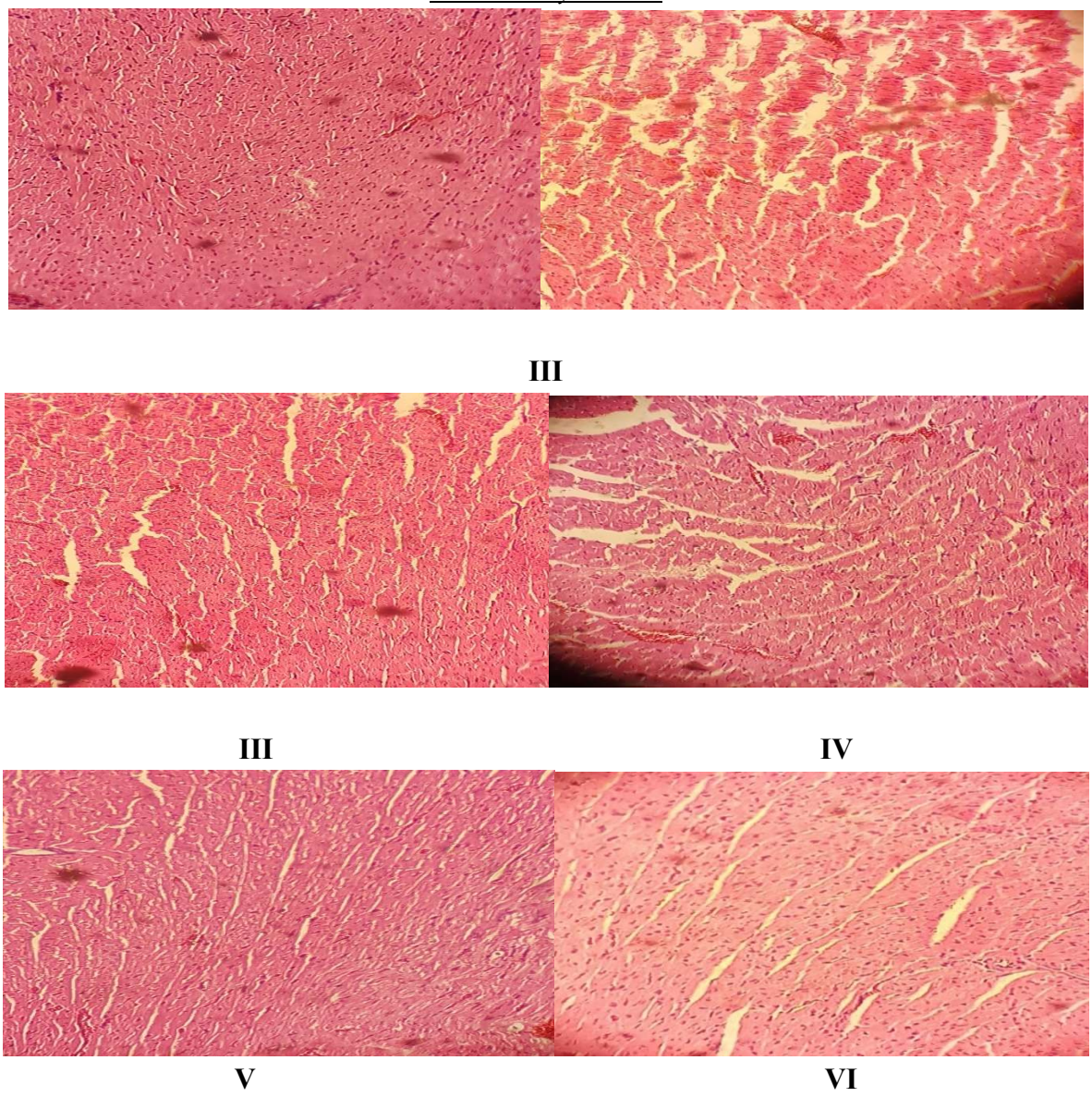
Sodium nitroprusside-induced cumulative relaxation response curves, on aortas isolated from fructose treated group showed significant (p<0.001) reduction in relaxation, indicating diabetes induced vascular damage. The aortas of control group animals showed normal relaxant response to cumulative doses of ACh ($10^{-7}$ M to $10^{-3}$ M). The aortas from fructose treated group showed significant (p<0.01) impairment of relaxation with cumulative doses of ACh. Treatment showed significant (p<0.01) improvement in relaxation, which indicates improvement in endothelial function in diabetic rats. Improvement in relaxation was most significant (p<0.001) with Fluvastatin and dapagliflozin combination compared to fructose treated group."Fig.5"

*Figure 5.Effect on vascular endothelial dysfunction*




SNP: Sodium nitroprusside; ACh: Acetylcholine. Each value represents mean ± S.E.M. (n=6). # Diabetic rats compared to Normal rats (Student's t-test). * Treatment rats compared to Diabetic rats (One-way ANOVA followed by Dunnett's test). ##, ** p < 0.01, ### p < 0.001.

*Figure 8. Effect of Fluvastatin and Dapagliflozin on histopathology of rat hearts in fructose-induced metabolic syndrome.*




## DISCUSSION

The present study demonstrates that HMG-CoA reductase inhibitor (Fluvastatin) and Sodium glucose transporter inhibitor (Dapagliflozin) significantly improved endothelial dysfunction in experimental diabetic rats. Insulin resistance induced by administration of fructose in rats was associated with an increase in oxidative stress. Insulin resistance was also associated with an early development of moderate hypertension and vascular remodelling of arteries. [23]

In the present investigation, it has been found that the characteristic and cardinal sign produced by oral administration of 66% fructose are similar and consistent with those reported earlier. The fructose administered rats showed increase in weight gain this might be due to increase in the adiposity of the body. Fluvastatin and Dapagliflozin combination significantly reduced fructose induced weight in diabetic rats.

In this study, serum glucose level was significantly increased in fructose fed diabetic rats indicating the presence of hyperglycaemia. The

combination of Fluvastatin with Dapagliflozin was significantly able to reduce the hyperglycaemia. Fructose fed diabetic rats showed significantly elevated serum triglycerides level. Increases in blood triglyceride level has been shown to reduce the number of insulin receptors, thereby reducing insulin sensitivity leading to development of hyperinsulinemia, which further causes complications of metabolic syndrome. [24]It has been found that the combination of Fluvastatin with Dapagliflozin significantly reduced the elevated serum triglyceride level in fructose fed diabetic animals. Dapagliflozin alone was also able to reduced serum triglyceride level but less significantly than Fluvastatin and Dapagliflozin combination. Insulin is known to increase the activity of lipoprotein lipase, an important regulatory enzyme of triglyceride uptake by this mechanism hyperinsulinemia may partially overcome.[25] As in present study Fluvastatin in combination with Dapagliflozin significantly reduced triglyceride level, this might be because of its property to increase insulin sensitivity, which in turn increases the activity of lipoprotein lipase which regulates triglyceride uptake, as stated earlier.

In the metabolic syndrome, adipocytes are resistant to the action of insulin, and lipolysis continues unchecked. Increased release of free (i.e., nonesterified) fatty acids from adipocytes and their delivery to the liver provide additional substrate for hepatic production of cholesterol which contributes to the susceptibility to atherosclerotic disease.[26] In this study, serum cholesterol level of fructose fed diabetic rats increased significantly indicating the presence of atherogenic dyslipidemia. The combinations of Fluvastatin with Dapagliflozin reduced serum cholesterol level in fructose fed diabetic rats.

In the present investigation fructose fed diabetic rats showed significant increase in systolic blood pressure. The combination of Fluvastatin with Dapagliflozin and Atorvastatin treatment significantly reduced the elevated blood pressure in fructose fed hypertensive rats. Dapagliflozin treatment did not show reduction in elevated blood pressure in fructose fed hypertensive rats. Heart rate was found to be lower in fructose fed diabetic rats; suggesting early autonomic dysfunction due to diabetes. [27] Long term treatment with the Fluvastatin and its combinations with Dapagliflozin restored the heart rate to normal. This showed that the autonomic dysfunction was normalized by the treatment with the drugs.

In the present investigation, the effect on the vascular reactivity to the infusion of various catecholamines such as adrenaline, noradrenaline and phenylephrine was studied. There was significant increase in pressor response to adrenaline, noradrenaline and phenylephrine in the fructose fed diabetic rats. This increase in pressor response might be due to presence of hypertension in fructose fed diabetic rats. Treatment with combination of Fluvastatin with Dapagliflozin significantly reduced the increase in pressor response to adrenaline, noradrenaline and phenylephrine in fructose fed rats. Their ability to decrease the pressor response attributes to their ability to restore the elevated blood pressure in fructose fed rats. Dapagliflozin alone did not showed significant reduction in the pressor response to adrenaline, noradrenaline and phenylephrine in fructose fed rats.

It is known that ACh induces endothelium-derived NO release in blood vessels causing vessels to dilate. Endothelial dysfunction is a clinical feature of metabolic syndrome, there is decreased availability of endothelium derived NO for proper vasodilatation to occur. In present investigation, there was normal relaxant response to cumulative doses of ACh of isolated aortas of control group rats. Whereas relaxant response to cumulative doses of ACh was significantly reduced of aortas isolated from fructose fed diabetic rats. This finding is consistent with those reported earlier which indicates presence of endothelial dysfunction. Treatment with Fluvastatin and Dapagliflozin combination significantly improved the relaxant response to ACh of aortas isolated from fructose fed diabetic rats.

SNP (Sodium Nitroprusside) mediates its vasorelaxant effects in contractile cells are *via* NO released in the vascular smooth cells.SNP induces production of NO in the endothelial cells by the activation of NOS. [17] Aortic relaxation responses to SNP were taken in preconstructed aortic rings with phenylephrine. It has been found that there is normal relaxant response observed to cumulative doses of

SNP from isolated aortas in normal rats. Whereas relaxant response to cumulative doses of SNP were significantly reduced on aortas isolated from fructose fed rats. Fluvastatin and its combination with Dapagliflozin showed significant relaxant responses to SNP.

Oxidative stress is one of the major reasons of endothelial dysfunction in metabolic syndrome. [28] So to find out oxidative stress various levels of antioxidant enzymes in rat aorta were measured. SOD is one of the most important enzymes in the antioxidant defence system of the body. The major function of SOD is to catalyse the conversion of superoxide anion radicals (the first product of oxygen radical formation) to $H_2O_2$ and hence reduces the toxic effects due to this radical or other free radicals derived from secondary reactions. CAT, which is present virtually in all mammalian cells, is responsible for the removal of $H_2O_2$. Glutathione peroxidase (GPX) is a cytosolic enzyme that is complementary to CAT to detoxify $H_2O_2$ and organic hydroperoxides. [29] The levels of SOD, CAT, LPO and NO were measured. The levels of antioxidants like SOD, CAT and NO were normal in normal rats. While there was significant reduction in antioxidant like SOD, CAT and NO observed in rats treated with fructose. This reduction of SOD and NO was restored significantly by treatment with Fluvastatin and Dapagliflozin. Thus, drug combination was able to reduce oxidative stress in rats treated with fructose fed diet. LPO value was normal in control normal. While increased in control group that may be due to obesity. LPO level was reduced to normal after treatment combination.

In histopathological study, we observed that fructose treated diabetic rat heart revealed focal myonecrosis and lymphocytic infiltration (myocarditis). Due to microvascular endothelial damage leading to tissue ischemia which triggers and anti-inflammatory cascade leading to local tissue damage and ischemic necrosis. The reperfusion of ischemic tissue associated with endothelial dysfunction is manifested as impaired endothelium dependent dilation in arterioles along with increased oxygen radical, with less nitric oxide. The resulting imbalance between superoxide and nitric oxide in endothelial cell leads to the production and release of anti-inflammatory mediators (TNF and platelet activating factor) along with increased biosynthesis of adhesion molecules. The inflammatory cascade increases intra compartmental ischemia with further worsening of myonecrosis. [30] In present study fructose treated group showed focal myonecrosis and lymphocytic infiltration whereas treatment with Fluvastatin, Dapagliflozin and combinations of Fluvastatin and Dapagliflozin showed better architecture as compared to fructose diabetic rats.

The result of the study indicated that Fluvastatin and dapagliflozin both have significant impact on endothelial dysfunction associated with Fructose induced metabolic syndrome (diabetics). The combination of Fluvastatin and dapagliflozin was found to be most effective in the management of endothelial dysfunction as compared to their individual drugs.

## CONCLUSION

The present work clearly concluded that Fluvastatin and Dapagliflozin reversed endothelial dysfunction by reducing metabolic and cardiovascular complications. But combination of Fluvastatin with Dapagliflozin showed significant effect on endothelial dysfunction. Therefore, combination of these may be preferred to treat endothelial dysfunction in diabetes, provided that the present finding could be extrapolated in human studies.

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**How To Cite This Article:**
Vandana S. Nade, Ananta Titare, Nasar A. Kardus, Laxman A. Kawale, Swapna Lajurkar *Ameliorative effect of Fluvastatin and Dapagliflozin on vascular endothelial dysfunction in diabetic rats* Br J Pharm Med Res , Vol.04, Issue 05, Pg. 2048 - 2059, September - October 2019. ISSN:2456-9836 Cross Ref DOI : https://doi.org/10.24942/bjpmr.2019.563

**Source of Support:** Nil **Conflict of Interest:** None declared