

**Research Article****Evaluation of Dapagliflozin in diabetes induced endothelial dysfunction in rats**

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**Abstract**

**Background:** Endothelial cells concerned in modulating vascular tone and structure. Endothelial plays an important role in homeostasis of the body and its dysfunction is correlated with numerous pathophysiology circumstances like diabetes, atherosclerosis. Patients with diabetes at all times show an impairment of endothelium-dependent vasodilation. Hyperglycaemia is the key aspect which develops the endothelial dysfunction in diabetes mellitus. **Objective:** The present study was aimed to investigate the activity of Dapagliflozin to reduce the risk of endothelial dysfunction associated with diabetes. **Materials and Methods:** Type 2 diabetes was induced by a single intraperitoneal injection of STZ (65mg/kg) + NAD (235mg/kg). After the administration of Streptozotocin (STZ) the animal showed marked hyperglycemia. Metformin and Captopril used as the standard drugs and the test drug Dapagliflozin administered for 28 days after the induction of endothelial dysfunction. **Results:** After 28 days of treatment with Dapagliflozin (10,20mg/kg) showed significant reduction in glucose level, glutathione level and improvement in lipid profiles. The treatment showed significant increase in body weight as compared to diabetic control and diabetes + endothelial dysfunction group. **Conclusion:** In the present study investigation, the activity of Dapagliflozin not only reduces the diabetes but also found to reduce the risk of endothelial dysfunction associated with diabetes.

**Keywords:** Diabetes, endothelial dysfunction, atherosclerosis, reactive oxygen species, nitric oxide, Streptozotocin

**Introduction**

The phrase diabetes mellitus illustrate a metabolic disease of a couple of etiology characterize throughout routine hyperglycaemia by means of variance of carbohydrate, fat and protein metabolism significant from defects in insulin secretion, insulin action, or both. Numbers compile by WHO reveal that approximately a hundred and fifty million people incorporate diabetes mellitus & this progression may be twofold via the year of 2050. (Loghmani and Stang, 2005) (Kitabchi et al., 2009)

The international frequency of diabetes mellitus remarkably type 2 diabetes, has bigger than previous than a lot in present day years. Type 2 diabetes alter the vascular openness to greater than a few vasoconstrictor and vasodilators & is a motive vital development of cardiovascular diseases. Lots of the complication in diabetes are associated to total serum glucose

and accelerated era of reactive oxygen species, which go earlier to endothelial dysfunction. (Ghodsi, 2017)

The endothelium is the deepest layer of blood vessels, that's why it's the major organ in the body. It has a range of most vital biological services additional to its location as a mechanical lining. These hold the legislation of leucocyte extravasation, adhesion and subendothelial accretion; the prevention of platelet adhesion that might result in thrombotic tactics; and the legislation of blood vessel patency for defense of right blood flow (Hartge et al., 2007).

Nitric oxide plays an essential part in vascular homeostasis. In endothelial cells, L-arginine convert in the survival of endothelial nitric oxide synthase (eNOS) to L-citrulline and in addition synthesized nitric oxide (Stevena et al., 2017). The chief source for the reduce level of NO production is a decreased action of eNOS. When the phrase and action of eNOS decreases or eNOS becomes uncoupled, reactive oxygen species (ROS) will generate instead of NO. eNOS uncoupled through – decrease of enzyme L-arginine, accretion of endogenous methylarginine and oxidation of BH<sub>4</sub> (Huijie et al., 2016).

When endothelium loses its physiological properties such as:

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the affinity to endorse vasodilation, fibrinolysis, and anti-aggregation, it is known as “endothelial dysfunction” (Ragavan and Monisha, 2016).

The trademark of endothelial dysfunction is the impaired NO bioavailability. In addition, endothelial dysfunction is describe by way of one or more of the following points: decrease endothelium-mediated vasorelaxtion, hemodynamic deregulation, overproduction of growth factors, impair fibrinolytic ability, improved expression of adhesion molecules and extreme new discharge of ROS, inflammatory genes, multiplied oxidative stress.

Patients suffering from diabetes, can leads to an impairment of NO production and action. Endothelium impair due to diabetes mellitus, can cause endothelial dysfunction & which can be considered as the initial tread of the cardiovascular disease (CVD)( Anderson, 2003).Due to diabetes-induced endothelial dysfunction, it may cause a variety of complications such as coronary artery disease (CAD), vascular ischemia, peripheral vascular disease (PVD) etc (Ding and Triggle,2005). Up to 75% patient among diabetes dies due to dysfunction of endothelium.

Sodium-glucose cotransporters 2 (SGLT2), is the chief glucose transporter of the kidney, situated in the S1 and S2 segments of the proximal tubule and is accountable for the reabsorption of .90% of the glucose from primary urine [Rieg et al.,2014]. A new category of antidiabetic drug, SGLT2 inhibition (SGLT2i) reduce the reabsorption of glucose and as a result enhances urinary glucose excretion, as a result lessening both fasting and postprandial hyperglycemia.

As per the prior information of the animal studies recommend that Dapagliflozin produces antidiabetic action. Studies concerned in the treatment of diabetes with Dapagliflozin in diabetic patients also showed effects such as insulin resistance, decrease in dyslipidemia which might helps as the defensive measure for cardiac complications of diabetes (Hippisley and Carol, 2016) (Hink et al., 2001).

With the current study we sought to test whether treatment of diabetic animals with the SGLT2i Empagliflozin improve endothelial dysfunction, oxidative stress, AGE/RAGE signaling and inflammation in a well-characterized rat model of type 2 diabetes mellitus (Hippisley and Carol, 2016) (Sachdeva and Dhingra, 2014).

## Material and methods

### Animals

Albino Wistar rats of either sex weighing 280-300g were procured from the animal house facility of Shri Guru Ram Rai Institute of Technology and Sciences, Patel Nagar, Dehradun for the present protocol. Animals was acclimatized in the animal house facility of the department and housed in the polypropylene

cages with husk bedding (renewed every 48 hours) under 12:12 light dark cycle at 25° C ± 5° C and were fed with standard commercial pellet and water *ad libitum*.

The experimental protocol was approved by *Institution Animal Ethics Committee* (registration no. 264/PO/ReBi/S/2002/CPCSEA) and care of animals was as per guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

### Animal grouping

Animals were randomly allocated into seven groups (n=5 rats per groups)

**Group I-** Normal Control group- Normal saline (1ml/kg) was administered.

**Group II-** Streptozotocin (65mg/kg, I.P) was administered after NAD (235mg/kg i.p) administration.

**Group III-** Metformin (100mg/kg) was administered in STZ treated rats.

**Group IV-** Captopril (50 mg/kg) was administered as a standard.

**Group V-** Captopril (50 mg /kg) + Metformin (100mg/kg) was administered in STZ treated rats.

**Group VI-** Dapagliflozin (10mg/kg) was administered as a test dose.

**Group VII-** Dapagliflozin (20mg/kg) was administered to endothelial dysfunction induced group.

### Induction of diabetes with Streptozotocin (STZ)

A single dose of streptozotocin (65mg/kg) prepared in citrate buffer (pH 4.5) was intraperitoneally administered into overnight fasted rats to induce diabetes in all group except control group. All the rats were allowed free access to water, pallet diet and maintained at room temperature in polypropylene cages. After the administration of STZ, rats were also fed glucose solution (10%) for 12h to avoid hypoglycemia. Rats having serum glucose more than 250mg/dL after 1 week of STZ were considered as diabetic and were selected for further study (Balakumar and Jindal, 2007).

### Biochemical parameters assessment

**Collection of blood sample:** using heparinized capillary glass tubes and estimated following biochemical parameters-

### Serum glucose estimation

Serum glucose level was predictable by glucose oxidase/peroxidase method by using commercially accessible enzymatic glucose oxidase-peroxidase method. The quantitative determination of the activity of serum

glucose in serum was done using the GOD-POD kit (Transasia bio-medicals Ltd, Baddi, India.) (Saydam et al., 1997)

### Vascular reactivity

The vascular reactivity towards Norepinephrine (NE) as a vasoconstrictor and Acetylcholine (Ach) as an endothelium-dependent vasodilator was assessed using the isolated aortic ring preparation. Briefly, abdominal aortas were rapidly placed in warm Krebs' solution and dissected free of surrounding tissue before being cut into transverse rings of 3–5 mm length. An aortic ring was mounted in 10 ml water jacketed organ bath system containing Krebs' solution of the following composition (g/l): Sodium chloride ( NaCl) 6.9, Potassium chloride ( KCl) 0.35, Potassium dihydrogen Phosphate (KH<sub>2</sub>PO<sub>4</sub>) 0.16, Magnesium sulphate (MgSO<sub>4</sub>·7H<sub>2</sub>O) 0.3, Calcium chloride (CaCl<sub>2</sub>·2H<sub>2</sub>O) 0.37, Sodium bicarbonate (NaHCO<sub>3</sub>) 2.1, and glucose 1.05. The organ bath solution was continuously aerated with carbogen (a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub>) and its temperature was kept at 37°C. The mounted aortic ring was suspended horizontally between 2 hooks passed through its lumen. The preparation was allowed to equilibrate for about 2 h under a resting tension of 2 g during that time any change in the resting tension was readjusted. NE and Ach were freshly prepared by dissolving them in Kerbs' solution. Serialized dilutions of each vasoactive agent were arranged such that cumulative add-ons to the bath gave a concluding bath concentration. For testing the relaxant effect of Ach, Pre-contraction with NE was carried out first with a concentration that produces approximately 60–70% of the maximum contractile response. Contractile responses to NE were expressed as percentage of maximal response while relaxant responses to the vasodilator Ach were expressed as percentage relaxation of the pre-contraction value (Balakumar and Jindal, 2007).

### Histological staining of aorta ring

(1) Rat aortic rings were preset in 4% paraformaldehyde and surrounded in paraffin. Deparaffinized tissue sections were stained with hematoxylin and eosin.

(2) For Von Kossa staining,

Cells / tissues washed with PBS for three times and for 15 minutes tissues/cells were fixed with 4% paraformaldehyde. Paraffinized tissue sections were deparaffinized. Washed the cells/tissues three times with PBS and one time with sterilized water, after that incubated with 5% silver nitrate solution and exposed to UV for 20 minutes until their color turned dark blue. After that remove the silver nitrate solution, and then cells were washed three times with sterilized water and dried.

### Activity of glutathione peroxidase (GSH-Px activity)

The GSH-Px activity of enzymatic extract was determined according to a modification of the method proposed by Paglia

and Valentine (Cristina et al., 2013).

### Estimation of total cholesterol

The quantitative determination of the activity of total cholesterol in serum was done using the total cholesterol estimation kit (Transasia bio-medicals Ltd, Baddi, India.)

### Assessment of body weight changes

Each rat was weighed independently two times, first at the start of the experiment (initial weight) and second 24h after the administration of the last dose of either drug (final weight). The diversity in body weight of each rat was calculated and expressed as percentage change according to the following:

$$\% \text{ change in body weight} = \{(\text{Final weight} - \text{initial weight}) / \text{Initial weight}\} \times 100$$

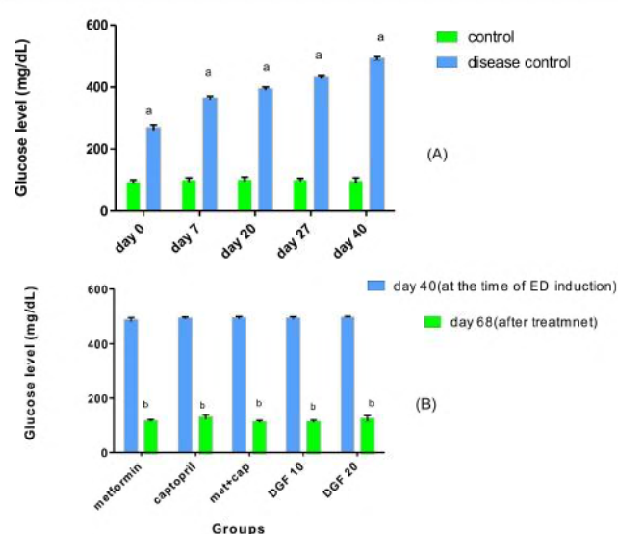
### Statistical analysis

All data were expressed as mean  $\pm$  standard error mean (SEM). All data were statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple range test for multiple comparison among various groups respectively.

### Results

#### Effect of Dapagliflozin on serum glucose level (mg/dL)

Administration of streptozotocin produced a significant ( $p \leq 0.01$ ) increase in glucose level in rats as compared to normal control group. Treatment with Dapagliflozin in diabetic rats (10 mg/kg p.o, 20 mg/kg p.o) showed significant ( $p \leq 0.01$ ) decrease in glucose level as compared to glucose level at day 40 (Figure 1A & B).



**Figure 1** (A) Serum glucose level in control group and diabetic rats group from day zero to day 40. (B) Effect of Dapagliflozin on serum glucose level in diabetic rats (mg/dL). Here data was compared between day 40 and day 68. All data are expressed as mean  $\pm$  SEM, n=5, a= ( $p \leq 0.01$ ) vs control and b= ( $p \leq 0.01$ ) vs day 40

### Effect of Dapagliflozin on vascular reactivity

#### Effect of Dapagliflozin on NE-induced contraction

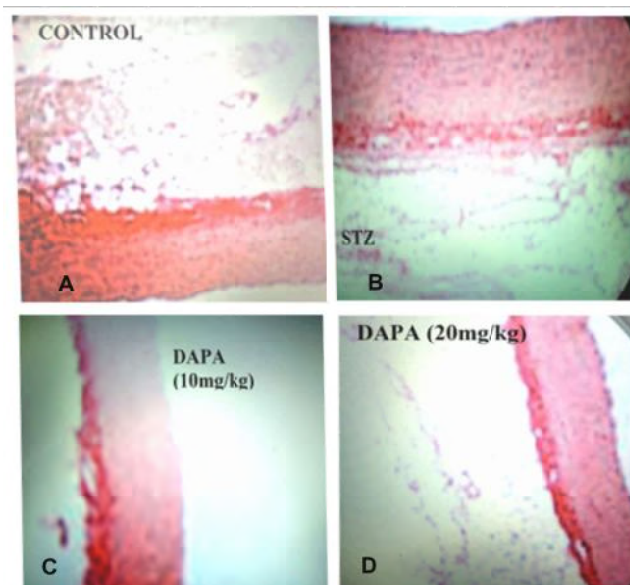
Collective concentrations of NE, initial from log D -7.0 to -4.0, created a concentration-dependent contraction of aortic rings isolated from normal rats. A most contraction was achieved at a concentration of -4.7 NE, reaching about  $92.35\% \pm 2.76$  of the maximal response. Aortic rings isolated from diabetic rats showed reduction, particularly at concentrations from -5.9 to -5.02 ( $p \leq 0.01$ ), as compared to normal group. Oral treatment of hyperglycaemic rats with DGF 10mg/kg and DGF 20mg/kg ( $p \leq 0.05$ ) decrease the vascular response of aortic rings as compared to the disease control group (Figure 2A).

#### Effect of Dapagliflozin on Ach-induced relaxation

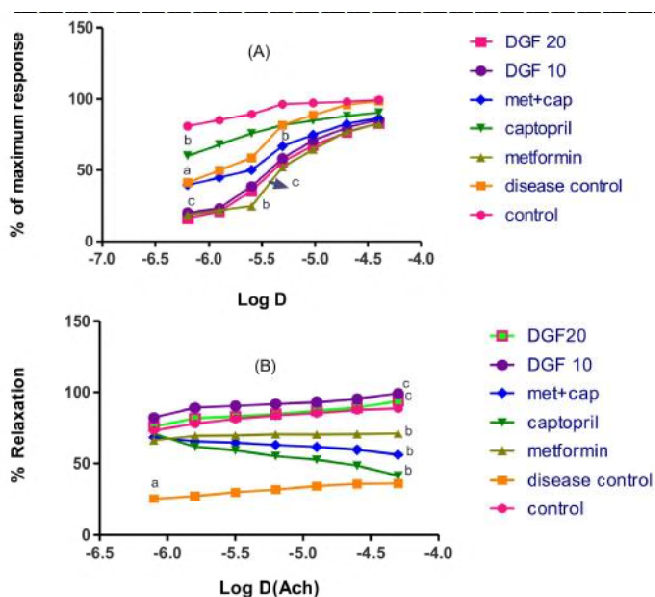
Collective concentrations of Ach, initial from log D -6.5 to -4.0, created a concentration-dependent relaxation of aortic rings isolated from normal rats. A most contraction was achieved at a concentration of -4.9 NE, reaching about  $82.99\% \pm 2.12$  of the maximal response. Aortic rings isolated from diabetic rats showed reduction, particularly at concentrations from -5.86 to -4.9 ( $p \leq 0.01$ ), as compared to normal group. Oral treatment of hyperglycaemic rats with DGF 10mg/kg and DGF 20mg/kg ( $p \leq 0.05$ ) improve the vascular response of aortic rings as compared to the disease control group (Figure 2B).

#### Effect of Dapagliflozin on aorta rings

Histological staining for aortic wall thickness exposed no major increase in thickening in the disease control group but significant decrease in aortic wall thickening was observed by both doses of Dapagliflozin (10mg/kg and 20mg/kg) (Figure 3).



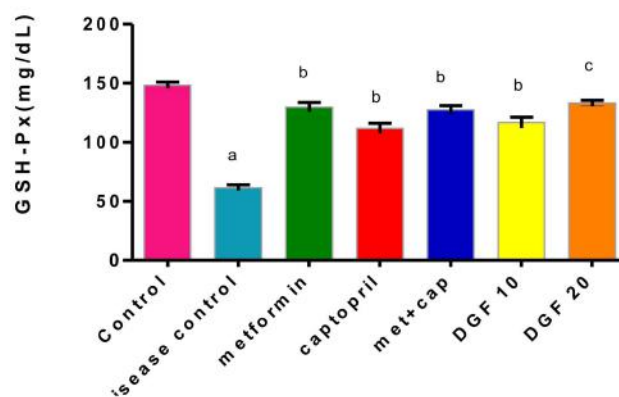
**Figure 3.** Effect of Histological staining of aorta ring in – (A) Control group, (B) Disease control group, (C) Dapagliflozin (DAPA) (10mg/kg) treated group and (D) Dapagliflozin (DAPA) (20mg/kg) treated group.



**Figure 2 (A & B).** Effect of Dapagliflozin on (A) NE-induced contraction and (B) Ach-induced relaxation. Values are expressed as Mean  $\pm$  SEM, n=5 in each group. a=( $p \leq 0.01$ ) vs control, b=( $p \leq 0.05$ ) vs disease control, c=( $p \leq 0.05$ ) vs disease control.

#### Effect of Dapagliflozin on GSH-Px activity (mg/dL)

Administration of streptozotocin followed by endothelial dysfunction in diabetic rats, produced a significant ( $p \leq 0.001$ ) decrease in GSH-Px level in rats as compared to normal control. Treatment with Dapagliflozin in diabetic + endothelial dysfunctional rats (10 mg/kg p.o) showed significant ( $p \leq 0.001$ ) increase in GSH-Px level as compared to diabetic control group and 20 mg/kg p.o showed significant ( $p \leq 0.05$ ) increase in GSH-Px level as compared to diabetic + endothelial dysfunction group (Figure 4).



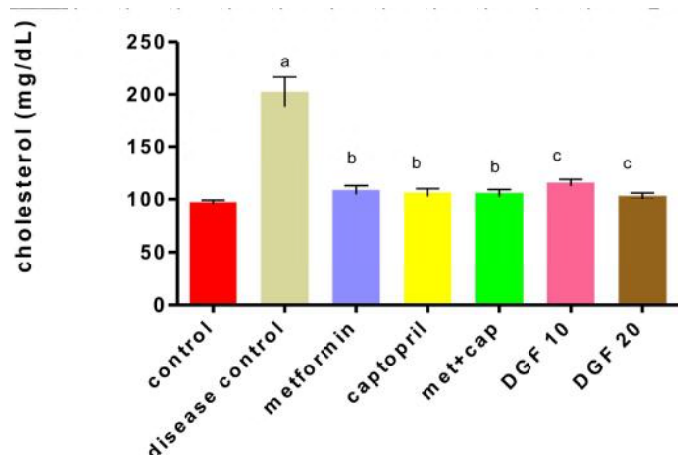
**Figure 4.** Effect of Dapagliflozin on GSH-Px activity (mg/dL). Values are expressed as Mean  $\pm$  SEM, n=5 in each group. a=( $p \leq 0.001$ ) vs control, b=( $p \leq 0.001$ ) vs disease control, c=( $p \leq 0.05$ ) vs disease control

### Effect of Dapagliflozin on total cholesterol (mg/dL)

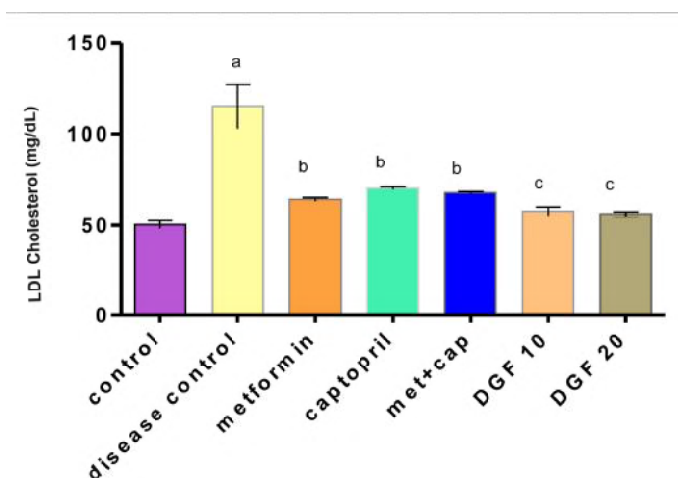
Administration of streptozotocin followed by endothelial dysfunction in diabetic rats, produced a significant ( $p \leq 0.001$ ) increase in total cholesterol level in rats as compared to normal control. Treatment with Dapagliflozin in diabetic + endothelial dysfunctional rats (10 mg/kg p.o) showed significant ( $p \leq 0.001$ ) decrease in total cholesterol level as compared to diabetic control group and 20 mg/kg p.o showed significant ( $p \leq 0.05$ ) decrease in total cholesterol level as compared to diabetic + endothelial dysfunction group (Figure 5).

### Effect of Dapagliflozin on LDL cholesterol (mg/dL)

Administration of streptozotocin followed by endothelial dysfunction in diabetic rats, produced a significant ( $p \leq 0.001$ ) increase in LDL cholesterol level in rats as compared to normal control. Treatment with Dapagliflozin in diabetic + endothelial



**Figure 5.** Effect of Dapagliflozin on total cholesterol(mg/dL). Values are expressed as Mean  $\pm$  SEM, n=5 in each group. a= ( $p \leq 0.001$ ) vs control, b=( $p \leq 0.001$ ) vs disease control, c=( $p \leq 0.05$ ) vs disease control.

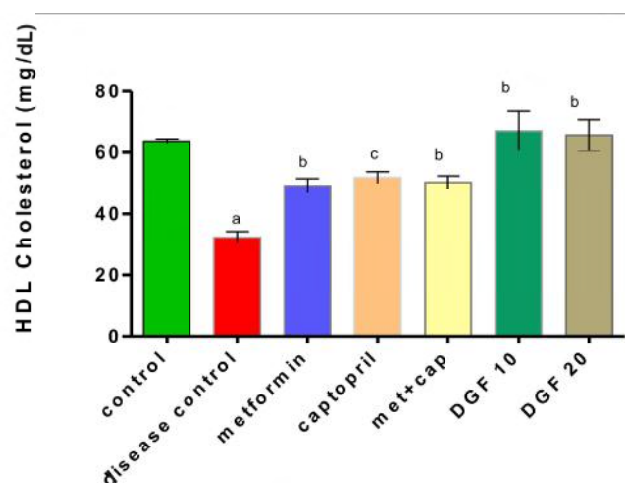


**Figure 6.** Effect of Dapagliflozin on LDL cholesterol (mg/dL). Values are expressed as Mean  $\pm$  SEM, n=5 in each group. a= ( $p \leq 0.001$ ) vs control, b=( $p \leq 0.001$ ) vs disease control, c=( $p \leq 0.05$ ) vs disease control

dysfunctional rats (10 mg/kg p.o) showed significant ( $p \leq 0.001$ ) decrease in LDL cholesterol level as compared to diabetic control group and 20 mg/kg p.o showed significant ( $p \leq 0.05$ ) decrease in LDL cholesterol level as compared to diabetic + endothelial dysfunction group (Figure 6).

### Effect of Dapagliflozin on HDL cholesterol (mg/dL)

Administration of streptozotocin followed by endothelial dysfunction in diabetic rats, produced a significant ( $p \leq 0.001$ ) decrease in HDL cholesterol level in rats as compared to normal control. Treatment with Dapagliflozin in diabetic + endothelial dysfunctional rats (10 mg/kg p.o) showed significant ( $p \leq 0.05$ ) increase in HDL cholesterol level as compared to diabetic control group and 20 mg/kg p.o showed significant ( $p \leq 0.05$ ) increase in HDL cholesterol level as compared to diabetic + endothelial dysfunction group (Figure 7).



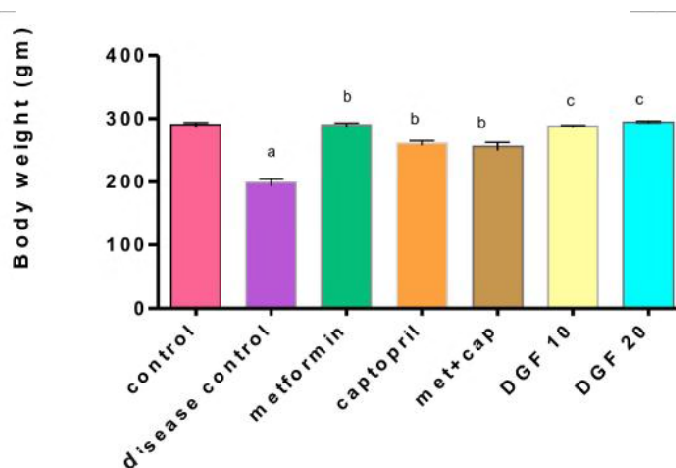
**Figure 7.** Effect of Dapagliflozin on HDL cholesterol (mg/dL). Values are expressed as Mean  $\pm$  SEM, n=5 in each group. a= ( $p \leq 0.001$ ) vs control; b= ( $p \leq 0.05$ ) vs disease control, c=( $p \leq 0.01$ ) vs disease control

### Effect of Dapagliflozin on body weight

Administration of streptozotocin followed by endothelial dysfunction in diabetic rats, produced a significant ( $p \leq 0.001$ ) decrease in body weight in rats as compared to normal control. Treatment with Dapagliflozin in diabetic + endothelial dysfunctional rats (10 mg/kg p.o) showed significant ( $p \leq 0.001$ ) increase in body weight as compared to diabetic control group and 20 mg/kg p.o showed significant ( $p \leq 0.05$ ) increase in body weight as compared to diabetic + endothelial dysfunction group (Figure 8).

### Discussion

Diabetes mellitus is global health problem and it is growing frequently worldwide. Diabetes is referred as a metabolic disorder which is characterized by relative and absolute deficiency of insulin secretion or insulin resistance. Diabetes is said to be foremost cause of mortality and morbidity in the



**Figure 8.** Effect of Dapagliflozin on body weight. Values are expressed as Mean  $\pm$  SEM, n=5 in each group. a= ( $p \leq 0.001$ ) vs control; b= ( $p \leq 0.001$ ) vs disease control, c= ( $p \leq 0.05$ ) vs disease control

world. Diabetes is the disorder which is related to other complications such as cardiomyopathy, retinopathy, nephropathy and neuropathy (Loghmani and Stang, 2005; Kitabchi et al., 2009; Ghodsi, 2017).

Hyperglycemia promotes the risk of cardiovascular diseases as it can alter protein kinase C (PKC) by stimulating the formation of diacylglycerol. Several factors which may lead to the development of atherosclerosis include diabetes associated dyslipidaemia (reduction in HDL cholesterol and increase in LDL cholesterol and triglycerides levels) and the oxidation of elevated levels of glucose within the cell that stimulate the production of ROS which increase oxidative stress (Oelze et al., 2014).

In this study Dapagliflozin was used as a test drug for the treatment of diabetes induced endothelial dysfunction. As per the previous reports of the animal studies suggest that Dapagliflozin produces antidiabetic action. Studies involved in the treatment of diabetes with Dapagliflozin in diabetic patients also showed effects such as reduction in dyslipidemia, insulin resistance which may help as the protective measure for cardiac complications of diabetes (Ding and Triggle, 2005; Rieg et al., 2014).

The model used for the inducing diabetes in this study is streptozotocin (STZ) induced diabetes model. Diabetes was induced in rats by administration of STZ in the single dose of 65mg/kg by intraperitoneal injection in rats leads to the selective loss of pancreatic beta cells.

Outcome of the current study discovered that STZ-induced hyperglycaemia in rats was accompanied by some vascular and biochemical changes. The vascular changes were evidenced by alterations in the reactivity of isolated aortic rings towards a vasoconstrictor as well as vasodilators. The biochemical

changes, as in otherwise, were evidenced by a greater than before level of LDL, total cholesterol and serum glucose level with the decreased action of erythrocytic GSH-Px and HDL. STZ-induced hyperglycaemia was also detected with a deep loss in body weight. These results were in accordance with earlier reported figures.

Data obtained from present study demonstrate that the treatment with the SGLT2i Dapagliflozin in STZ treated rats prevents the enlargement of endothelial dysfunction, oxidative stress and inflammation in an animal model of type II diabetes mellitus. These valuable effects are generally due to antioxidant and anti-inflammatory effects of test compound, i.e. inhibition of the activity of NADPH oxidase and decreased serum levels of the AGE precursor methylglyoxal. These antioxidant and anti-inflammatory effects are probable due to glucose lowering effects. Glucose lowering is probably due to deduction of glucose by the kidney via SGLT2 inhibition but may also be secondary to improved glucose consumption by restored insulin production and signaling, all of which prevent AGE formation, metabolic dysfunction, oxidative stress and impairment of vascular function (Cristina et al., 2013).

Dapagliflozin (10mg/kg and 20mg/kg p.o) showed reduction in serum glucose level, total cholesterol level, LDL level and increase the level of HDL cholesterol, GSH-Px activity and also increase the body weight of the rats. Results from the study by assessing the different parameters indicate that Dapagliflozin produces cardioprotective property and could be used as a therapeutic treatment in diabetes induced endothelial dysfunction.

### Conclusion

From the above discussion and result it can be concluded that: Dapagliflozin shows an endothelial protective as well as anti-diabetic effect in streptozotocin induced diabetic rats. Treatment with Dapagliflozin showed decrease in serum glucose level, cholesterol level, increase in HDL cholesterol level and decrease in LDL cholesterol level. Glutathione peroxidase activity and body weight decreases in streptozotocin induced diabetic rats but with treatment of Dapagliflozin glutathione peroxidase activity and body weight increases. The present study results showed, treatment with Dapagliflozin significant difference in the thickness of aorta ring which is the evidence for the therapeutic potential in diabetes induced endothelial dysfunction.

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### Conflicts of interest

We declare that we have no conflict of interest.

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