Protective Effect of Sodium-Glucose Co-Transporter 2 Inhibitor (Dapagliflozin) on Diabetic Retinopathy in Streptozotocin Induced Diabetes in Rats

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Abstract

Background: Diabetic retinopathy is a serious diabetic complication as it is considered the most common cause of blindness in diabetes. There is a link between chronic hyperglycemia and the occurrence and progression of diabetic retinopathy.

Aim of Study: To investigate for the effect dapagliflozin, Sodium-Glucoseco-Transporter2 (SGLT2) inhibitor in reduction of diabetic retinopathy.

Material and Methods: Thirty (30) male albino rats (120-150) were randomly divided into control (n=6); DM (diabetic non treated group, n=10), DM + DAPA (diabetic treated with dapagliflozin for 8 weeks, n=10). Blood samples and retinal tissues were collected to assess glycated hemoglobin (HbA$_{1c}$), serum Interleukin 6 (IL-6), lipid profile and retinal expression levels of hydrogen peroxide (H_2O_2) and Brain Derived Nerve Growth (BDNG) moreover histological assessment was performed.

Results: Diabetes increased all measured parameters while decreased the expression of BDNG. Supplementation of dapagliflozin improved all measured parameters.

Conclusion: This study showed the harmful effect of diabetes on retina which could be mediated by oxidative stress and inflammation. On the other hand, dapagliflozin improved diabetic retinopathy via antioxidant and anti-inflammatory actions also it improved retinal regeneration via increasing the expression of brain derived nerve growth factor.

Key Words: Diabetes – Retinopathy – Dapagliflozin – Interleukin 6 – Brain derived nerve growth factor.

Introduction

DIABETES is a highly established chronic disease in the world. Diabetic Retinopathy (DR) is considered a common micro vascular hurdle of diabetes. Early detection and anticipation of retinopathy in diabetic patients is crucial for preventing vision loss. In order to reach the goal for the efficient medication in diabetic retinopathy, we should seek for understanding the pathogenesis underlying this disease. Although the pathogenesis of DR is unclear, studies have indicated that hyperglycemia, oxidative stress and inflammatory cytokines play a role in the development and the progression of diabetic retinopathy [1]. Hyperglycemia is believed as the first initiating factor in the development of DR, which suggests that strict glycemic control is effective in delaying DR [2]. It is known that hyperglycemia activates protein kinase C, polyol and hexosamine pathways, advanced glycation end products production and these pathways induced mitochondrial dysfunction and endoplasmic reticulum stress and endorse the production of Reactive Oxygen Species (ROS) that promote cellular damage and contribute in the pathogenesis of most diabetic complications [3]. Accumulating evidence has documented that ROS play an important role in the development of diabetic retinopathy. Excess cellular levels of ROS damage of retinal tissue and increased inflammatory process which is believed to play an important role in the development of diabetic retinopathy [4]. Brain Derived Nerve Factor (BDNF) is formed by retinal ganglion cells and in turn plays an essential role in survival of retinal ganglion cells [5]. Diabetes induces imbalance between pro-neurotrophins production and early retinal inflammation. Studies on the levels of neurotrophins might provide a therapeutically effective goal to combat disease progression in diabetic patients [6]. Dapagliflozin sodium-glucose cotransporter 2 inhibitor, it has been shown to improve glycemic index as it works by decreasing re-absorption of glucose in the renal proximal tubule [7]. They have been shown to reduce glyco-

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sylated haemoglobin (HbA$_{1c}$) and fasting plasma glucose levels [8]. The aim of the present study is find out a link between hyperglycemia, increased oxidative stress, inflammation and their effect on brain derived nerve growth factor and the development of diabetic retinopathy and to investigate for the role of dapagliflozin in attenuation of the progression of diabetic retinopathy. This information, which the study sought to provide important for diabetic patients as the prevalence of diabetes may reach 642 million people living worldwide by 2040.

Material and Methods

Thirty male albino rats (120-150gm) were obtained and inbred, with veterinary care, in the animal house of MSA University. They were housed in wire mesh cages at room temperature with normal light-dark cycles and maintained on standard rat chow diet and tap water during the four-week experimental period. They were allowed to accommodate for to the environmental conditions 1 week before starting of the experiment that was conducted in accordance with the ethical guidelines of the committee of the Faculty of Medicine, Cairo University during 2017.

Experimental protocol:

The rats were divided randomly as follows: Control group: Normal control (n=10), diabetic non treated group (DM) (n=10), diabetic treated with Dapagliflozin (n=10) (Forxiga; AstraZeneca Egypt): Started 6 weeks after induction of DM at a dose of 0.5mg/Kg/day corresponding to dose taken by human in type II diabetes orally for 4 weeks.

Induction of diabetes: Streptozotocin (STZ) (Sigma Aldrich, USA)was injected single intraperitoneal injection in a dose of 55mg/kg and then diabetic animals with blood glucose level above 300mg/dl were rendered diabetic.

Preparation of dapagliflozin: Tablets were curshed, weighed according to the calculated dose then were dissolved in tab water. Each rat received its prepared dose via oral gavage.

Determination of body weight:

Body weights were measured at the end of the experimental period (10 weeks).

Animals were sacrificed a day after the last treatment under ketamine anesthesia and retina were harvested. From each rat one retina was preserved in foil and kept freezed at –80°C for biochemical analysis, the other retina was kept in

formalin (10%) for histological analysis. Whereas plasma was collected for estimation of blood glucose, HbA$_{1c}$, lipid profile and IL-6.

Biochemical measures:

- *Estimation of HbA$_{1c}$:* It was assessed via rodent hemoglobin A1c (HbA$_{1c}$) assay kit.

- *Estimation of lipid profile:* It was assessed via Commercially available kits (BioMed, Cairo, Egypt).

- *Estimation of serum IL-6 (Interleukin 6):* It was assessed via Elisa kit supplied by (MyBio Source, USA), according to manufacture instruction.

- *Estimation of retinal expression of $_{H2O2}$ (hydrogen peroxide):* It was assessed via Colorimetric method kit supplied by (Biodiagnostic, Egypt).

- *Estimation of retinal expression of BDNG (brain derived nerve growth factor):* It was assessed via Western blot kits supplied by Bio Basic Inc. Markham Ontario L3R 8T4 Canada.

- *Histological analysis:* From each rat, one eye was sectioned into two halves along the horizontal axis. For light microscopy, ocular globes were fixed for 24hr in 10% formalin, embedded in paraffin and processed for sectioning at thickness of 5 µm. Multiple parallel serial sections at different levels were taken from both halves. Sections were stained with hematoxylin and eosin (H & E) then examined microscopically. The sections were analyzed using an Olympus BX5 1 microscope equipped with camera, with magnification 100x in all examined groups. Histological analysis was done in a private histological laboratory.

Statistical analysis:

Data are expressed as mean ± SD. Statistical analysis was performed using one-way ANOVA (Graph pad prism, release 7). A *p*-value of <0.05 was considered statistically significant Tukey method for multiple comparison ($p<0.05$).

Results

Body weight: Results showed significant decrease ($p<0.05$) in the body weight in the diabetic group (DM), diabetic + Dapagliflozin (DM + DAPA) in comparison to normal control group Fig. (1).

Biochemical measurements:

- *Glycated hemoglobin (HbA$_{1C}$):* Results showed significant increase ($p<0.05$) in the HbA$_{1C}$ % in DM group and in DM + DAPA in comparison to normal control group. However there was signif-

icant decrease (*p*<0.05) in its percentage in DM + DAPA in comparison to diabetic group Fig. (2).

- *Cholesterol:* According to (Table 1) cholesterol levels showed significant increase in DM, and in DM + DAPA in comparison to normal control group. However, there was significant decrease (*p*<0.05) in the cholesterol levels with DM + DAPA in comparison to DM.

- *Triglycerides:* Results showed significant increase (*p*<0.05) in the triglycerides levels in DM and DM + DAPA in comparison to normal control group. However there was significant decrease (*p*<0.05) in the triglycerides levels with DM + DAPA in comparison to DM group (Table 1).

- *High Density Lipoproteins (HDL):* Our results showed significant decrease (*p*<0.05) in the HDL levels in DM and DM + DAPA in comparison to normal control group. However there was significant increase (*p*<0.05) in its levels with DM + DAPA in comparison to diabetic group (DM) (Table 1).

B- *Assessment of Interleukin 6:*

Results showed significant increase (*p*<0.05) in Interleukin 6 levels in DM and DM + DAPA in comparison to normal control group. However, there was significant decrease (*p*<0.05) in its level with DM + DAPA in comparison to diabetic group (DM) (Table 1).

C- *Assessment of retinal Hydrogen peroxide level:*

Hydrogen peroxide results showed significant increase (*p*<0.05) in DM and DM + DAPA in comparison to normal control group. However there was significant decrease in the percentage with DM + DAPA in comparison to diabetic group (DM) (Table 1).

D- *Assessment of retinal brain derived nerve growth factor levels:*

In this study, we found that BDNF was decreased significantly (*p*<0.05) in DM and DM + DAPA in comparison to normal control group. However there was significant increase (*p*<0.05) in its levels with DM + DAPA in comparison to diabetic group (Table 1).

Histological results: In the present work, control group showed normal retinal histology, intact ten layers of retinal structure and no edema is present in retinal tissue Fig. (3A). Diabetic group (DM) fatty changes are the most pronounced. Moreover blood effusion and arteriole remodeling was observed in the loose layers Fig. (3B,C). Administration of Dapagliflozin (DM + DAPA) prevent changes to the structure of the retinal arterioles. The drug was capable of reducing effusion of retinal capillaries and minimizing arteriole remodeling, factors that contribute to the progression of diabetic retinopathy Fig. (3D).

Table (1): Mean ± SD of serum cholesterol, triglycerides, high density lipoproteins, interleukin 6 (IL-6) and retina expression levels of hydrogen peroxide (H_2O_2), Brain Derived Nerve Growth factor (BDNG).

Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	High density lipoproteins (mg/dl)	IL-6 (pg/ml)	Hydrogen peroxide (H_2O_2)	Brain derived nerve growth factor (BDNG)
Control	160.6±11.3	62.8±7.63	55.1±2.86	14.6±1.01	110.2±4.9	1.005±0.008
DM	227.1±14.1 *	107.1±4.83*	21.6±1.21*	101.7±6.73 *	584.1±112.4*	0.2±0.03 *
DM + DAPA	181.1±6.18 *@	76.1±2.32 *@	37±2.61 *@	75.01±8.7*@	207.2±7.46 *@	0.63±0.1 *@

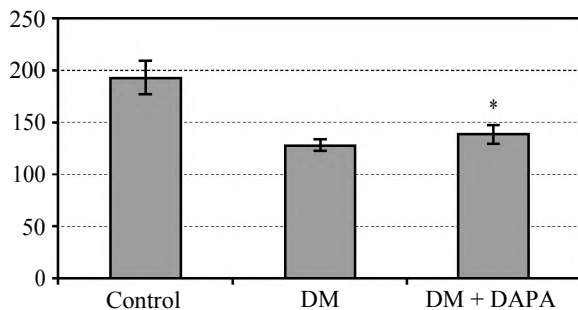*: Significant (*p*<0.05) in comparison to control.   @: Significant (*p*<0.05) in comparison to DM.

Fig. (1): Mean ± SD of body weight at the end of the experimental period.

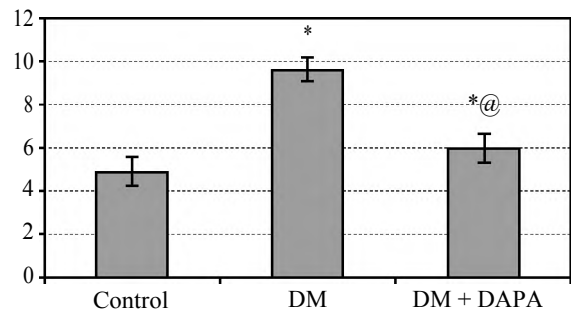   *: Significant (*p*<0.05) in comparison to control.

Fig. (2): Mean ± SD of Glycated hemoglobin% (HbA$_{1c}$) at the end of the experimental period.

   * : Significant (*p*<0.05) in comparison to control.
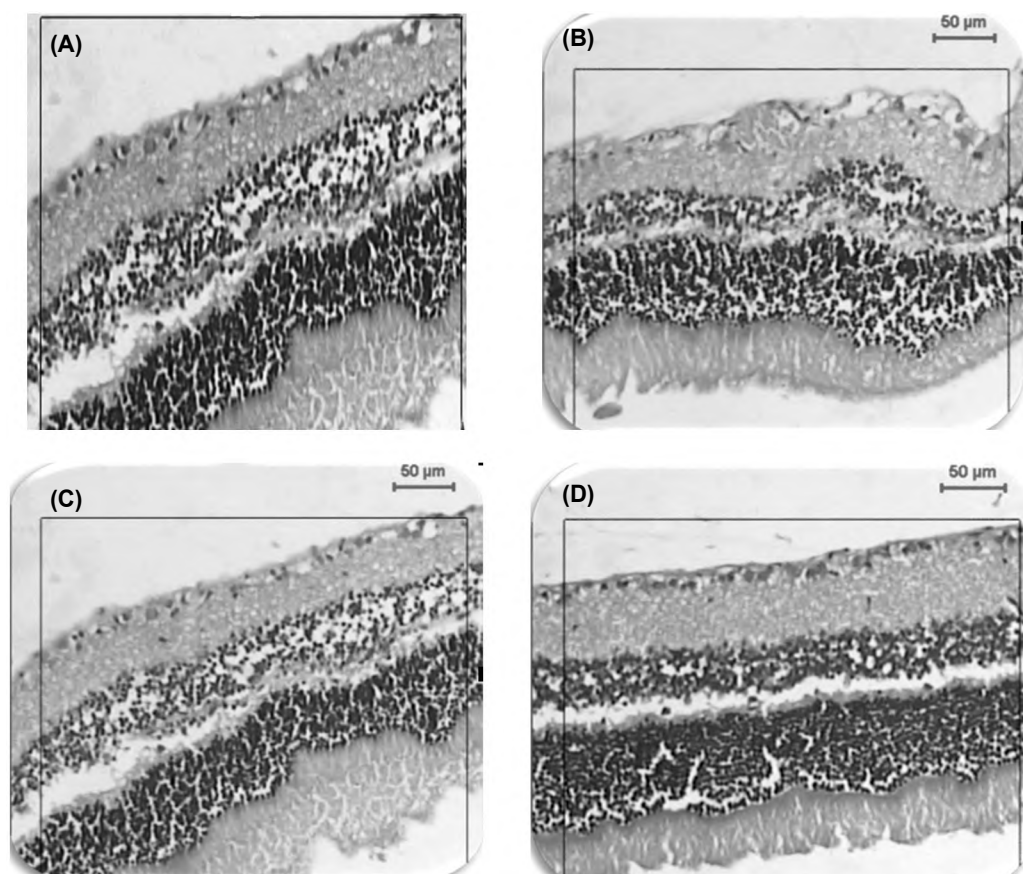   @: Significant (*p*<0.05) in comparison to DM.

Fig. (3): Photomicrograph pictures of retinal sections stained with H & E X100. (A) Control group: The retinal structure was continuous, and its capillary structure was normal. (B,C): DM group: The tissue layers of the retina were edematous, and the retina developed telangiectatic vessels (black arrow). Many ganglion cells developed vacuolar degeneration (white arrow). (D): DM ± DAPA group: Dapagliflozin appeared to prevent changes to the structure of the retinal arterioles.The drug was capable of reducing hyperperfusion of retinal capillaries and minimizing arteriole remodelling, factors that contribute to the progression of diabetic retinopathy.

Discussion

Accumulating evidence has documented that diabetic retinopathy is a common vascular disorder associated with diabetes. Hyperglycemia associated with diabetes is often accompanied with increased oxidative stress production that causes cellular damage and deterioration of organ function [10]. The results of the present study revealed that diabetes decreased body weight and increased retinal oxidative stress and inflammation as detected by increased H_2O_2 and increased IL-6, on the other hand diabetic retina showed decreased the expression of brain derived nerve growth factor. Together with histological damage observed by increased retinal fat deposition and bloody accumulation in different fields. This may be an effect of the high lipid profile detected in our result [11]. Proposed that, hyperlipidemia might contribute in DR as it predisposes in endothelial dysfunction and breakdown of the blood retinal barrier [12]. Moreover in diabetes, the sorbitol pathway increases in activity in tissues like retina, kidney, sorbitol does

not easily diffuse through cell membranes; as a result, it gathers and grounds osmotic damage [13]. Accumulation of ROS can lead to increased vascular permeability and causes damage of vascular endothelium and retinal odema [14]. It has been reported that oxidative stress-induced cell death primarily due to increased necrosis that convinces the expression of an inflammatory genes [15]. In a similar study reported by [16] elucidated that diabetic group showed significant increase in serum glucose, glycosylated haemoglobin (HbA_{1c}), Total Cholesterol (TC) and retina malondialdehyde (MDA). Our results depicted significant decrease in the level of brain derived nerve growth factor in diabetic group these results are in accordance with [17] as they delectated that level of BDNF was significantly decreased in patients with diabetic retinopathy patients and the retina of diabetic rats as compared to control groups non-diabetic group. The low expression of brain derived nerve growth factor could be attributed to increased oxidative stress which was mentioned by [18] as they observed elevation in the markers of oxidative stress in the

cerebral cortexes of experimental groups decreased the expression level of brain derived nerve growth factor. From the previous knowledge we could conclude that therapies that target to reduce blood glucose, oxidative stress and lipid levels could be an efficient key to reach the goal of reducing the progression of diabetic retinopathy. In the present work using dapagliflozin the sodium-glucose cotransporter 2 inhibitor resulted in obvious improvement in retinal oxidative stress as detected by a significant decrease in the level of H_2O_2 and decreased inflammation by decreased serum level of IL-6. Histopathological examination, showed decreased retinal damage and lowers fatty deposition and blood accumulation in the retina. Dapagliflozin administration for six weeks has been shown to improve diabetic control and reduce blood pressure in patients with type 2 diabetes mellitus, parameters associated with the early stages of vascular remodeling [20]. Moreover results showed that dapagliflozin lowers the plasma lipids and these findings are in accordance to [21]. A study mentioned that treatment with dapagliflozin prevented renal podocyte injury, glomerular pathology, dapagliflozin also decreased renal lipid accumulation. Moreover, renal inflammation and oxidative stress were lower in the dapagliflozin-treated WD-fed mice than in the untreated western-fed mice [19]. Results of this study are opposite to [21] as they mentioned that dapagliflozin has no effect of lipid profile in a study on patients, dapagliflozin reduced HbA_{1c} by 0.9% and body weight by 3.1kg, mainly attributable to reduction of body water and lean mass but no change was detected in HDL or cholesterol. Results in this study showed that dapagliflozin lowers IL-6 level as these results are in accordance to [22] as they found that Dapagliflozin increased glucose uptake and reduced the secretion of pro-inflammatory chemokines in cardiac endothelial cells. The role of dapagliflozin on brain derived nerve growth factor is still unclear, results in this research depicted significant increase in the expression level of brain derived nerve growth factor and this could be attributed to its role in improvement in all measured parameters. In conclusion, this study accomplished that medication of dapagliflozin is effective in limiting the progression of diabetic retinopathy.

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التأثيـر الوقائـى لمثبط ناقل الصوديوم الجلوكوز (2) (dapagliflozin) على إعتلال الشبكية السكرى فى مرض السكرى المستحث بالستريتوزوتوسين فى الجرذان

إعتلال الشبكية السكرى لمرض السكرى يعتبر من الأسباب الأكثر شيوعا للعمى فى مرض السكرى. هناك صلة بين إرتفاع السكر فى الدم المزمن وحدوث وتطور إعتلال الشبكية السكرى.

الهدف من الدراسة: البحث عن تأثير عقار dapagliflozin فى الحد من إعتلال الشبكية السكرى والآلية المستخدمة.

المواد والطرق: ثلاثون (30) ذكور جرذان آلبينو (120-150) تم تقسيمهم عشوائيا مجموعة الضبط (عدد=6)، (المجموعة غير المعالجة بالسكرى=10)، DM + DAPA (مجموعة السكرى المعالجة ب داباجليفلوزين لمدة 8 آسابيع=10). تم جمع عينات الدم وآنسجة الشبكية لتقييم (HbA$_{1c}$)، وinterleukin-6، ومستويات التعبير عن الشبكية من بيروكسيد الهيدروجين (H$_2$O$_2$) ونمو الأعصاب المشتقة من الدماغ (BDNG) وتم آيضا عمل فحص ميكروسكوبى لآنسجة الشبكية.

النتائج: زيادة جميع المعلمات فى مجموعة مرض السكرى مع إنخفاض BDNG. فى حين تحسن جميع القياسات مع dapagliflozin.

الخلاصة: آظهرت هذه الدراسة الآثار الضارة لمرض السكرى على شبكية العين والذى يمكن آن يحدث بوساطة الإجهاد وإلتهاب الأكسدة. من ناحية آخرى، قام dapagliflozin بتحسين إعتلال الشبكية السكرى.