

Evaluation of the Effect of Dapagliflozin on Atherosclerosis Progression by Interfering with Inflammatory and Oxidative Stress Pathways in Rabbits

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Abstract

Background: Atherosclerosis is a very common disease in which fat deposition in the inner layers of arteries leading to plaques formation. Dapagliflozin is one of a new class of drugs known as the sodium-glucose cotransporter-2 inhibitors, responsible for lowering of the blood glucose level, and enhancing urinary glucose excretion. Dapagliflozin may lower blood glucose levels and at the same time prevent cardiovascular diseases. **Objective:** The objective of this study was to assess the effect of dapagliflozin on atherosclerosis through interfering with inflammatory and oxidative pathways. **Materials and Methods:** Eighteen local domestic male rabbits were used in this study. The rabbits were randomly divided into three groups: Group I rabbits fed normal chow diet for 12 weeks; Group II rabbits fed with 0.5% cholesterol-enriched diet; and Group III rabbits fed with 0.5% cholesterol-enriched diet together with dapagliflozin (1 mg/kg once daily). Blood samples were collected before the study (zero time) and every 4 weeks for the measurement of serum total cholesterol, triglycerides (TGs), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), very LDL-C (VLDL-C), tumor necrosis factor alpha (TNF- α), and endothelin-1 (ET-1). **Results:** Dapagliflozin treatment showed insignificant elevation in total cholesterol and LDL-C, significant decrease VLDL-C and TG, and significant elevation of HDL-C level ($P < 0.05$) compared with the induced untreated group. It was insignificantly decreased inflammatory markers (TNF- α and ET-1), increased aortic total antioxidant capacity, and significantly reduced aortic intima thickness compared with induced untreated group. Dapagliflozin, by slightly interfering with inflammatory and oxidative pathways, may show beneficial effects on atherosclerosis and can attenuate the atherosclerotic lesion formation. **Conclusion:** Dapagliflozin may have a beneficial effect on atherosclerosis by slightly interfering with inflammatory and oxidative pathways and can reduce the atherosclerotic lesion formation; however, our study needs further clinical studies to be carried out on large population.

Keywords: Atherosclerosis, dapagliflozin, inflammatory markers, oxidative stress

INTRODUCTION

Atherosclerosis is a very common disease in which fat deposition in the inner layers of arteries leading to plaques formation.^[1] The exact risk factors of atherosclerosis are unknown, of which main risk factors include hypertension, diabetes mellitus (DM), obesity which can be modified, and atherosclerosis can be delayed or prevented,^[2] while age, genetics, and male gender are nonmodifiable.^[3] Inflammation has been considered to play an important role in the initiation and development of atherosclerosis. For this reason, numerous inflammatory markers have been investigated as candidates for the enhancement of cardiovascular risk assessment.^[4] Tumor necrosis factor alpha (TNF- α) considered the most important

inflammatory marker that maintains low-level systemic inflammation. Several mechanisms were mentioned to interpret the proatherogenic effects of TNF- α on the endothelium, including its action in reactive oxygen species (ROS) formation, reduction of the bioavailability of nitric oxide, and increasing the permeability of endothelium to circulating blood components and cells.^[5]

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How to cite this article: Abd-Ulhussein HK, Rizij FA. Evaluation of the effect of dapagliflozin on atherosclerosis progression by interfering with inflammatory and oxidative stress pathways in rabbits. Med J Babylon 2019;16:20-4.

Access this article online

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10.4103/MJBL.MJBL_104_18

Endothelin-1 (ET-1) is a peptide responsible for pathophysiological changes associated with microvascular and macrovascular disease.^[6] ET-1 may lead to atherosclerosis by its action as a mitogen on smooth muscle cells of vessels and activation of vascular inflammation and cytokine release in atherosclerotic lesions. ET-1 levels of coronary artery and plasma are high in early and late atherosclerosis in humans.^[7]

Oxidative stress contributes to atherosclerosis.^[8] Antioxidant is any substance that prevents or removes oxidative damage and inhibits the process of oxidation to a target molecule.^[9] The total antioxidant capacity (TAC) is a mirror of the activity of the antioxidant system.^[10] Dapagliflozin is one of a new class of drugs known as the sodium-glucose cotransporter-2 inhibitors, responsible for lowering of the blood glucose level by inhibiting renal glucose and sodium reabsorption and enhancing urinary glucose excretion.^[11] Epidemiological studies have indicated that cardiovascular diseases (CVDs) become the most common complications and the major leading cause of morbidity and mortality for DM patients.^[12] Dapagliflozin may lower blood glucose levels, and at the same time, prevent CVDs.^[13]

MATERIALS AND METHODS

Eighteen local domestic male rabbits were used in this study. The rabbits were randomly distributed into three groups each group consisted of six rabbits include the following: Group I rabbits fed normal chow diet (at libitum) for 12 weeks; Group II rabbits fed with atherogenic diet containing 0.5% cholesterol for 12 weeks; and Group III rabbits fed atherogenic diet (0.5% cholesterol) with dapagliflozin (1 mg/kg once daily) for 12 weeks. The blood samples were collected at zero time and every 4 weeks on atherogenic diets for the measurement of serum level of total cholesterol triglycerides (TGs), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), very LDL-C (VLDL-C), TNF- α , and ET-1. At the end of the study (after 12 weeks), the aorta was removed for the measurement of TAC level and aortic intima thickness.

RESULTS

There was a statistically insignificant difference ($P > 0.05$) in body weight levels between the control group and the other groups on atherogenic diet suggesting that cholesterol and dapagliflozin had no effect on body weight. Compared with the control group, levels of total cholesterol, TGs, HDL-C, LDL-C, VLDL-C, TNF- α , ET-1, and aortic intima thickness were significantly increased whereas aortic TAC insignificantly decreased ($P > 0.05$). Dapagliflozin treatment showed an insignificant increase in total cholesterol and LDL-C while significantly decreased TG and VLDL-C and significantly increased HDL-C level compared with the induced group ($P < 0.05$). Dapagliflozin insignificantly decreased the elevation in TNF- α , ET-1, significantly decreased aortic intima thickness ($P < 0.05$), and insignificantly increased the

aortic TAC level compared with the induced group as shown in Tables 1-3.

DISCUSSION

The results of the present study indicated that 12 weeks' consumption of atherogenic diet with 0.5% cholesterol increased the serum lipid profile and activated atherosclerosis lesions formation involving the intimal thickening. In addition, the results of the present study revealed that the level of serum TNF- α and ET-1 was significantly elevated in the atherosclerotic rabbits (induced untreated group) and dapagliflozin treatment could insignificantly lower them.

Effects of dapagliflozin on the study parameters

Effect of dapagliflozin on lipid profile

The present study revealed that treatment with dapagliflozin showed different effects on the serum lipid profile involve insignificant increase in total cholesterol and LDL-C level, significant decrease in TG and VLDL-C, and significant increase in HDL-C level compared with the induced untreated group as shown in Figure 1. Leng *et al.* also found that dapagliflozin treatment for 12 weeks significantly lowered TG level in ApoE^{-/-} mice.^[12] Dapagliflozin decreases TG concentration and increases HDL-C was also showed by Min *et al.*^[14] Matthaie *et al.* reported elevation in the total cholesterol level from baseline in patients receiving dapagliflozin treatment.^[15]

Effect of dapagliflozin on inflammatory markers (tumor necrosis factor alpha and endothelin-1)

The result of the present study found that dapagliflozin insignificantly decreased inflammatory markers (TNF- α and ET-1) compared to the induced untreated hypercholesterolemic rabbit as shown in Figure 2. Sato *et al.* found that dapagliflozin significantly decreased serum TNF- α level in diabetic patients with coronary artery disease when treated with dapagliflozin for 6 months^[16] but no data showed the effect of dapagliflozin on serum TNF- α level in rabbits.

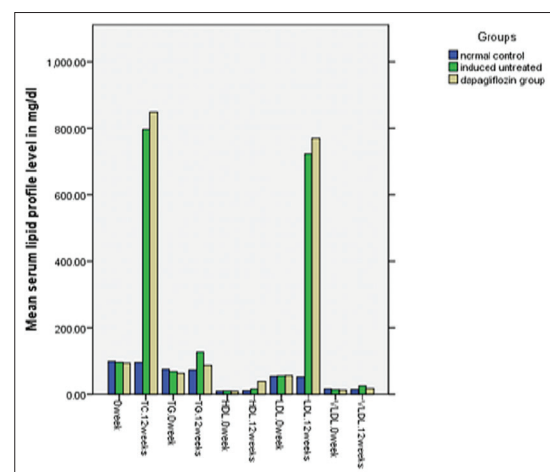


Figure 1: Effect of dapagliflozin treatment on serum lipid profile level in mg/dl during the experimental treatment

Table 1: Changes of serum lipid profile of the three experimental groups

	Total cholesterol	TG	HDL-C	LDL-C	VLDL-C
Normal control group					
Zero time	99.2±3.9	75.5±6.2	9.4±0.29	54±4.41	15.74±0.9
12 weeks	95.5±2.59	73.02±2.32	10.38±0.44	51.9±2.8	14.6±0.46
Induced untreated group					
Zero time	96±4.13	68.13±1.77	9.6±0.3	55±4.1	13.6±1.66
12 weeks	796.6±43.7*	127±18.9*	15.6±0.93*	723.2±45.6*	25.4±3.8*
Dapagliflozin group					
Zero time	93.8±203	62.75±2.8	8.75±0.5	56.5±3.23	12.55±0.56
12 weeks	848.5±20.1*	86.9±3.64*	38.8±2.26*	770.2±19.8*	17.4±0.73*

The result was expressed as mean±SEM ($n=6$ in each group) using paired sample t -test. * $P<0.05$. SEM: Standard error of the mean, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, VLDL-C: Very low-density lipoprotein cholesterol, TG: Triglyceride

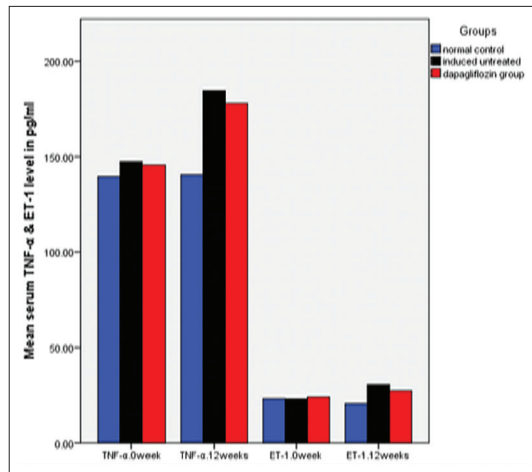


Figure 2: Effect of dapagliflozin treatment on the serum level of tumor necrosis factor alpha and endothelin-1 compared to the normal and induced untreated group

The differences in results may be due to the fact that the drug was used for humans and not for rabbits and this study was longer than our study, and no data had revealed the effect of dapagliflozin on serum ET-1 level. Gaspari *et al.* also found that chronic dapagliflozin treatment for 4 weeks in ApoE^{-/-} adult mice lowered molecular changes and endothelial cell dysfunction associated with early atherogenesis. Endothelial cell dysfunction is a main contributor to early atherogenesis in type 2 diabetic patient. Adhesion molecule upregulation leads to endothelial cell dysfunction and considered to be a precursor of atherosclerosis occurrence. Reduction of endothelial dysfunction and macrophage infiltration and upregulation of intercellular cell adhesion molecule-1 expression, in ApoE^{-/-} mice could give a mechanism of dapagliflozin in modulating the beginning and progression of atherogenesis.^[17]

Effect of dapagliflozin on oxidative stress

This study revealed that dapagliflozin treatment insignificantly increased TAC level in aortic tissue of atherosclerotic hypercholesterolemic rabbit compared with the induced untreated group as shown in Figure 3. Abdel-Wahab *et al.* showed that the serum TAC level was significantly elevated in diabetic Wistar rats treated with dapagliflozin for 12 weeks;^[18]

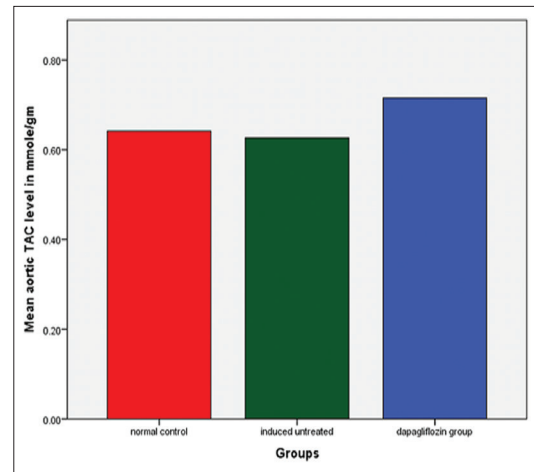


Figure 3: Effect of dapagliflozin treatment on the aortic level of total antioxidant capacity compared to the normal and induced untreated group

however, the differences in results may be due to the fact that rats were used as animal models instead of rabbits and the serum was used instead of aorta for the measurement of TAC level, also Leng *et al.* study on atherosclerotic ApoE^{-/-} mice found that dapagliflozin reduced the aortic production of ROS. Diabetes-accelerated atherosclerosis is usually associated with an increased level of interleukin (IL)-18 and IL-1β by the activation of NLRP3 inflammation involving the formation of ROS. The treatment with dapagliflozin may inhibit ROS-NLRP3 pathway by lowering the blood sugar and lipid and inhibit IL-1β and IL-18 production and reduced the accelerated progression of atherosclerosis in the aortic tissue.^[12]

Effect of dapagliflozin on aortic intima thickness

In the present study, dapagliflozin showed a significant decrease in aortic intima thickness compared with the induced untreated group as shown in Figures 4-6. There was no data available about the effect of dapagliflozin on the aortic intima thickness in atherosclerotic rabbits; however, Leng *et al.* found that dapagliflozin retarded the onset and the development of aortic lesions in ApoE^{-/-} mice with diabetes, indicated that dapagliflozin might be interfere with the formation of diabetic atherosclerosis. The main mechanisms that are responsible for the dapagliflozin benefits in atherosclerosis may be the

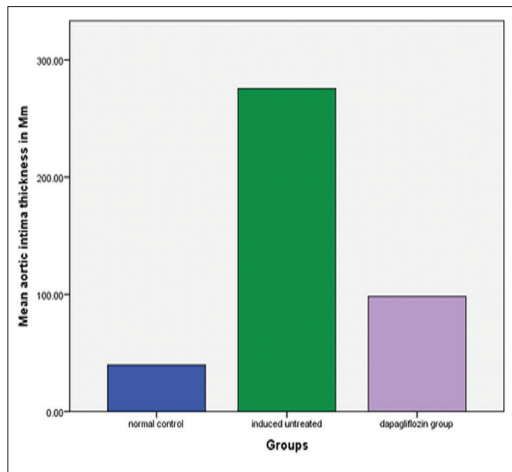


Figure 4: Effect of dapagliflozin treatment on aortic intima thickness compared to the normal and induced untreated group

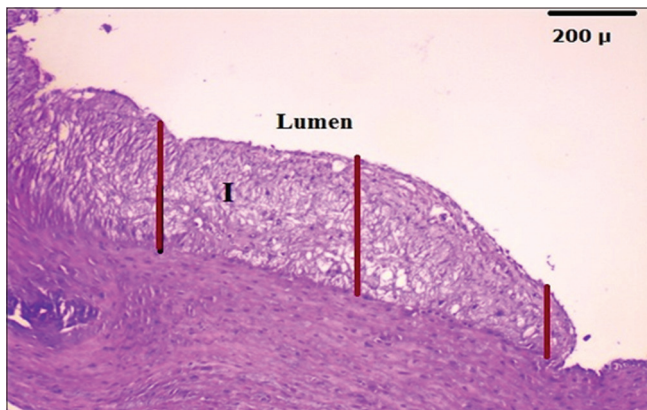


Figure 5: Photomicrograph of the transverse section of aorta of rabbit fed atherogenic diet for 12 weeks (untreated group) shows diffuse intimal thickening and lipids deposition in intima (I). The section stained with hematoxylin and eosin ($\times 10$)

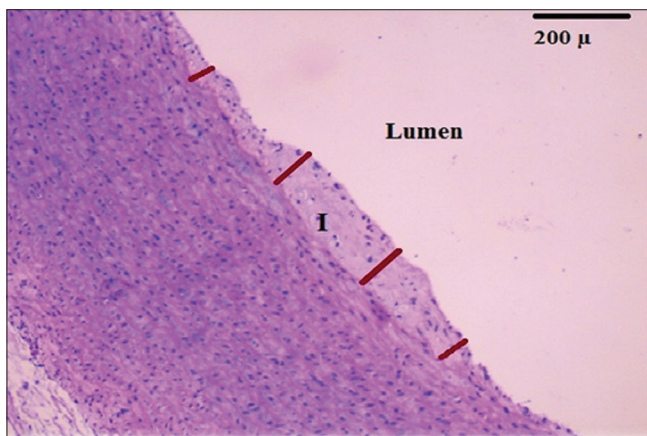


Figure 6: Photomicrograph of the transverse section of rabbit aorta fed on atherogenic diet with dapagliflozin treatment for 12 weeks (dapagliflozin group) shows significant reduction of intima thickness compared to the induced untreated group, (I = intima). The section stained with hematoxylin and eosin ($\times 10$)

inhibition of inflammatory response during the pathogenesis of atherosclerosis which in turn suppress the infiltration of

Table 2: Changes of serum inflammatory markers (tumor necrosis factor alpha and endothelin-1) of the three experimental groups

	TNF- α (pg/ml)	ET-1 (pg/ml)
Normal control group		
Zero time	139.64 $\pm$ 14.5	23.2 $\pm$ 0.8
12 weeks	140.59 $\pm$ 20.47	20.62 $\pm$ 0.67
Induced untreated group		
Zero time	147.43 $\pm$ 6.8	23.02 $\pm$ 1.2
12 weeks	184.49 $\pm$ 8.3*	30.51 $\pm$ 1.53*
Dapagliflozin group		
Zero time	145.6 $\pm$ 15.08	23.97 $\pm$ 0.93
12 weeks	178 $\pm$ 6.4*	27.4 $\pm$ 0.97*

The result was expressed as mean $\pm$ SEM ($n=6$ in each group) using paired sample t -test. * $P<0.05$. SEM: Standard error of the mean, TNF- α : Tumor necrosis factor alpha, ET-1: Endothelin-1

Table 3: The means of rabbits aortic (total antioxidant capacity and intima thickness) of the three experimental groups at the end of 12 weeks

	Aortic TAC (mmole/g)	Aortic intima thickness (μ m)
Normal control group	0.64 $\pm$ 0.05	39.8 $\pm$ 1.2
Induced untreated group	0.62 $\pm$ 0.03	275.46 $\pm$ 33.2*
Dapagliflozin group	0.72 $\pm$ 0.03	98.16 $\pm$ 0.68**

Data were expressed as mean $\pm$ SEM ($n=6$ in each group) using independent sample t -test. * $P<0.05$ compare to normal control, ** $P<0.05$ compared to induced untreated. SEM: Standard error of the mean, TAC: Total antioxidant capacity

macrophage into the lesions suggesting its benefit for lesion stability.^[12]

CONCLUSION

The results of this study indicate that dapagliflozin may have a beneficial effect on atherosclerosis by slightly interfering with inflammatory and oxidative pathways and can reduce the atherosclerotic lesion formation; however, our study needs further clinical studies to be carried out on large population.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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