Impact of Dapagliflozin on Atherosclerosis through Inflammation and Oxidative Pathways

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Abstract

Background: Atherosclerosis is a prevalent pathological disorder considered a leading cause of death globally. The pathological process is characterized by fat deposition in the blood vessels, ultimately developing plaques. The atherosclerotic plaque was the final result of the inflammatory reaction and oxidative pathway. Dabagliflozine is a member of hypoglycemic drugs that are classified based on their action as Na-glucose co-transporter 2 inhibitors. Dabagliflozine, besides its blood glucose-lowering effects, may also exhibit cardiovascular protection by reducing oxidative damage. Our research aimed to evaluate how the drug (dabagliflozine) interacted with inflammation and oxidative processes to impact atherosclerosis. Materials and Methods: In this study, eighteen male mice were split into three groups: one fed a regular diet, one fed a cholesterol-rich diet, and one fed a cholesterol-rich diet with dapagliflozin. Blood samples were taken regularly over twelve weeks to analyze serum markers such as TNF-α, endothelin-1, and lipid levels. Results: Initially, there were no significant differences in serum markers among the groups. However, after twelve weeks, the mice treated with dapagliflozin showed notable reductions in TNF-α and endothelin-1 levels (though statistically insignificant). Moreover, there were significant improvements in HDL (the "good" cholesterol) levels, and significant decreases in VLDL and TG (triglycerides) levels ($P<0.05$). Total cholesterol (TC) and LDL (the "bad" cholesterol) levels also decreased, although not significantly. Conclusion: In conclusion, dapagliflozin demonstrated promising protective effects against atherosclerosis in mice by regulating inflammatory cytokine production and improving lipid profiles. These findings suggest that dapagliflozin may have potential therapeutic benefits in managing cardiovascular disease by targeting both inflammation and lipid metabolism. However, further research is needed to validate these results and explore the drug's effectiveness in humans.

Keywords: *Musa Acuminate*, HepG-2 cells, MTT, DAPI/PI/EtBr staining and comet assay.

Significance | Atherosclerotic patients need therapies targeting inflammation and oxidation. This study supports Dapagliflozin's use for clinical benefits in atherosclerosis.

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Introduction

Atherosclerosis is a complex process that can be described as a leading cause of death in many countries. The complication of atherosclerosis is considered the major cause for increasing the rate of mortality in atherosclerotic patients by having a negative effect on the normal function of the cardiovascular system. Since atherosclerotic plaque is considered the final result of two processes, including inflammatory reactions and the oxidative pathway, so it is important to investigate the role of drugs that have attenuation effects on these two pathways in order to develop therapeutic agents with pleotropic effects (Resl & Clodi, 2010).

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Atherosclerosis characterized by alteration in the structure of arterial walls that results from accumulation of fatty materials in this blood vessels which united with other elements to form atherosclerotic plaque (Sun et al., 2020). Atherosclerotic plaque considers the final result for collection of processes including inflammatory pathways, endothelial dysfunction with formation of fatty threads infiltrate by immune cells (macrophages, activated neutrophils and dendritic cells) and blood elements associated with changes in lipid profile (more common elevation in cholesterol level) (Frostegård, 2013). .The usual structure of the plaque contains condensing cholesterol center and fibrous coat which adhering to inner vascular walls. As the size of plaque increase the diameter of the vessels decrease, with the time the plaque progression causing narrowing the vascular lumen and blood flow limitation (Abd-Ulhussein & Rizij, 2019). Stable atherosclerotic plaque is not fatal but can cause symptoms of stable angina on exercises or motion state (Narula et al., 2013). Dangerous events can occur when the plaque is ruptured mostly due to damage in the fibrous cap leading to release the content and subsequent processes involving the hemorrhage, lesion and thrombosis which is the starting point for many disorders depending on where these thrombi will fall, including vascular framework brain, lung and heart mainly the coronary arteries causing what is known as acute coronary syndrome. ACS is considered the major cause for increasing the rate of mortality in atherosclerotic patients by a negative effect on normal function of heart (Becker et al., 2012). The exact initiator for this clinical problem is not identified but as we mentioned is combination of many factors associated with risk factors such as comorbid diseases, sedentary life style, obesity and unhealthy diet. The involvement of inflammatory response in the endothelial dysfunction can be reflected by the action of inflammatory mediators such as IL-1, TNF-α, and other cytokines(Pober & Sessa, 2007; Hansson, 2001). Inflammatory pathway well related to atherosclerosis, this relation can observed through in many research which indicated the pro-inflammatory cytokines(IL-1β, IL-6, TNF-α) act as mediators in atherosclerosis process to activate vascular cell, monocyte attraction and adhesion (Probert, 2015).TNF-α well defined as inflammatory cytokine with many roles on different cells, Normally TNF-α found at physiological level as important member in immune responses to regulate the secretion of other immune protein mediators which lead to cell activation and proliferation (Choy & Panayi, 2001). Over stimulation for this cytokine lead to autoimmune disorders (Gao, 2016) and the inflammatory diseases such as inflammatory bowel disease, sepsis, atherosclerosis (Freeman et al., 2014). Endothelin-1define as vasoactive peptide its synthesis highly regulated by the endothelial growth factors and other stimuli (TNF-α, angiotensinII and interlukines) to maintain its divers role in cardiovascular, pulmonary, renal system. It is thought any modification in the synthesis process will be involved in many clinical conditions like cardiomyopathy, asthma, MI, diabetic complications, atherosclerosis and various inflammatory disorders (McKenna et al., 2015). Endothelin-1 directly related to atherosclerotic lesion through the mitogenic action, proliferation of vascular smooth cells, myocardial hypertrophy, release other inflammatory mediators with macrophage infiltration and major complication (Brauner et al., 2000). Dabagliflozine newly developed anti-diabetic agents acted as selective sodium glucose co-transporter 2 inhibitors with pleiotropic action in patient with type 2 diabetic mellitus (Heerspink et al., 2020), antihypergycaemic activity appear as elevation in urinary excretion of glucose and subsequent decrease in blood level of glucose and glucuresis throughout selective inhibition for urinary glucose reabsorption in proximal convoluted tubules (Cannon et al., 2020). Other action which is consider as advantages in such patients involved: In early stage of chronic kidney disease decrease the risk of and blood pressure, moderate reduction in SBP and DBP through diuretic action and reduction in total body weight via reduction in total calorie (Hsia et al., 2017). Recent clinical studies showing neuroprotective effect in diabetic patient receiving sodium glucose co-transporter 2 inhibitors via reduce the risk of Alzheimer's disease and brain damage underlying by atherosclerotic lesion (Weber et al., 2016).

Materials and Methods

Experimental animals

The study utilized 18 domestic male mice with 30- 35gm in weight obtained form animal house facility of Al-Muthanna University and the study conducted in the college of medicine, Al-Muthanna University. Throughout the study, the animal placed in well ventilated cages as groups system in room with temperature degree monitoring system as well as on a 14\10 hours light-dark cycle with free access to water and chow feeding. The protocols for handling, care, and methods was affirmed by local ethical committe.

At the beginning the animal were randomly assigned in to three study group, six male mice involved in each group. In first group the mice received normal chow diet for twelve weeks; In second group the mice received diet containing 0.5% cholesterol to induce the atherosclerosis for same time course in first group (George et al., 1998; Marzolla et al., 2018) ; In third group which is treatment group because the six mice treated with dabagliflozine (1mg\Kg\day)in addition to diet containing 0.5% cholesterol for twelve weeks (Leng et al., 2016; Chai et al., 2015). During study course (12 weeks) the monitoring performed continuously, and samples of blood was collected from all groups to measure the following mentioned parameters; lipid profile as atherosclerotic indicators (Triglyseride, total cholesterol TC, VLDL, LDL, HDL and the inflammatory markers associated with atherosclerosis (Tumer necrosis factor alpha TNF-α, endothelin-1 ET-1) to

investigate the antiinflammmatory role of dabagliflozine rely on the level of inflammatory markers at base line and end line od study. The serum measurement obtained at zero time, the fourth, the eighth, the twelfth week of study (Lindstrom et al., 2015).

Preparation of Chemicals

Dapagliflozine powder obtained from sigma-aldrich, the doses of dapagliflozine were used in research is 1mg\Kg\day depened on previous study (Leng et al., 2016). The powder of cholesterol also obtained from sigma-aldrich and the procedure to induce the atherosclerosis disorder rely upon previous research (Zhu et al., 2019).

Statistical Analysis

Statistical analysis of the data was performed using SPSS software, and the results were expressed as mean± SEM (standard error of the mean). In all test results, P≤ 0.05 was considered to be statistically significant.

Results

In the current study, the available results of data expressed as mean± SEM; however, the results rely on data collected at start of the research and at the end week 12; to make the results more valuable, the 95% confidence interval (CI) of the mean was applied, so in all results, the probability value (P≤ 0.05) was considered to be statistically significant.

At the base line (zero week) the data for all groups reveled insignificant difference in the level of serum lipid profile and the inflammatory mediators. However, at the end of the study (12 week) the results could be explained as the following:

Control group versus atherosclerotic induced group

Depending on the data recorded during the course of the study which was used to compare the control group (normal mice without any intervention) and the atherosclerotic-induced group, the results throughout the four monitoring weeks from serum sample collection every four weeks we notice the change in the lipid profile in the second measurement (at the fourth week), except the measures for level of HDL were somewhat steadily fluctuating.The changes in the lipid profile measures for week 4 revealed in Table 1. As shown in Figure (1) and table (2) that display significant elevation (P< 0.05) in cholesterol types, including TC= 801.40± 42.2 , VLDL= 25.40±3.8, LDL= 720.08±44.5, and TG = 124.36± 14.5, that reflect the induction of atherosclerosis and the formation of atherosclerotic plaque. Insignificant elevation (P> 0.05) in good cholesterol (HDL =15.56±0.7 can also distinguished. In the same manner, Figure (3) and table (3) showed the level of inflammatory markers, including TNF-α and ET-1, that demonstrated strong significant elevation (P>0.023) and (P>0.028) against the control group, respectively.

Atherosclerotic induced group versus dabagliflozine treated group

In the same manner, as you can see in the tables (2) and (3), when comparing dabagliflozine- treated mice with an atherosclerotic-induced group at the end of experiment course, the data showed promising results that reflect the cardioprotective action of the drug via demonstrating a significant elevation (P< 0.05) in the level of good cholesterol (HDL = 38.63±2.1) with significantly lower level of VLDL=17.35±0.7 and TG = 86.96±3.6. While the results for both TC and LDL level showed an insignificant reduction. The cardioprotective action represented by elevated level of good cholesterol HDL = 38.63±2.1 can be noticed after the eight week of the study course.

The major point presented in the table (3) is the significant reduction (P> 0.05) in the level of inflammatory markers, indicating the downregulating effect of our selective sodium glucose co-transporter 2 inhibitors.This is evident through the reduction in the serum level of TNF-α (P>0.046) and ET-1 (P>0.048) versus the induced group as depicted in Figure(4). Where the results in the induced group were TNF-α= 190.60± 6.8 and ET-1= 30.51±1.5 versus the compared value in the dabagliflozine treated group were TNF-α= 157.50±2.9 and ET-1= 25.90±0.7.

Discussion

In a study conducted by Hetherington and Totary-Jain, (2022), they were found that the atherosclerosis disorder is the mian contributer in cardiovascular events that affected the overwieht individual. Other previous studies reported dabagliflozine as a new oral hypoglycemic agent which also has pleotropic effects that give the drug the capability to fight or prevent the incidence of the cardiovascular complication associated with high readings of lipid profile or high level of blood glucose (Liu et al., 2021; Sugiyama et al., 2018). The present study aimed to show the effect of selective sodium glucose co-transporter 2 inhibitors on the inflammatory and oxidative pathways through their action on the measurments of differents lipoproteins and on the level of inflammatory cytokines .Study results revealed that the atherosclerotic induced group which given 0.5% cholesterol-enriched diet for study period which represented by twelve week showed two important outcomes; firstly is the elevated in serum lipid profile which is the responsible factor for the building of the atherosclerotic plaque and cardiac function deterioration, secondly is activation of the pro-inflammatory cytokines that indicated via the elevation of TNF-α and ET-1 serum level, these two process together is thought to be the responsible for pathological aspect of cardiac complication; however, comparative finding are obtained by (Stiekema et al., 2021; Feijóo-Bandín et al., 2022). Data collected from serum of mice treated with dabagliflozine for 84 days showed significant benefits reveled by the elevation of the HDL readings at week 8 and 12 from

Table 1. The levels of serum lipid profile in mg/dl for animal model at the week 4 the data express as mean± SEM (n=6 mice per each group).

Groups		LDL.4weeks	TC.4weeks	HDL.4weeks	TG.4weeks	VLDL.4weeks
normal control	Mean ± SEM	46.99±7.75	92.50±5.61	9.73±.81	78.70±11.98	15.10±2.75
induced untreated	Mean± SEM	294.69±114.18	348.33±115.7	15.60±3.20	83.70±8.37	16.74±1.67
dapagliflozin group	Mean± SEM	482.20±32.20	531.50±32.41	17.50±2.03	69.95±3.08	13.99±.61

Table 2. The levels of serum lipid profile in mg/dl for animal model at base line and after 12 weeks the data express as mean± SEM (n=6 mice per each group)using independent-Sample T test.

Lipid profile	Control	Atherosclerotic induced	Dabagliflozine treated
Total cholesterol			
Zero week	98.3±3.68	95.33± 2.7	93.91±1.8
12weeks	95.6±2.7	801.40± 42.2*	854.65±18.7
TG			
Zero week	82.08±5.7	68.30±1.7	63.08±2.5
12weeks	73.01±2.3	124.36± 14.5*	86.96±3.6 #
HDL			
Zero week	9.55±0.2	9.93± 0.2	9.0±0.5
12weeks	10.38±0.4	15.56±0.7	38.63± 2.1#
LDL			
Zero week	53.98±4.4	55.61±4.0	59.32±2.4
12weeks	52.06±2.9	720.08±44.5*	769.94±19.6
VLDL			
Zero week	16.17±0.9	13.7±0.3	13.08±0.5
12weeks	14.74±0.4	25.40±3.8*	17.35±0.7#

**P value < 0.05 Atherosclerotic induced group versus control group*

 # P value < 0.05 dabagliflozine treated versus Atherosclerotic induced group

TG: Triglyceride, HDL: high density lipoprotein, LDL: low density lipoprotein, VLDL: very low density lipoprotein.

Table 3. The serum levels of inflammatory markers including TNF-α and ET-1 for animal model at base line and after 12 weeks the data express as mean± SEM (n=6 mice per each group)using independent-Sample T test.

Inflammatory markers	Control	Atherosclerotic induced	Dabagliflozine treated
TNF-α			
Zero week	141.26±15.0	147.43± 6.7	145.66±15.1
12weeks	140.27±20.3	190.60± 6.8*	
157.50±2.9#			
ET-1			
Zero week	23.02±0.8	23.15±1.1	23.54±0.9
12weeks	20.62±0.6	30.51±1.5 *	25.90±0.7#

**P value < 0.05 Atherosclerotic induced group versus control group*

 # P value < 0.05 dabagliflozine treated versus Atherosclerotic induced group

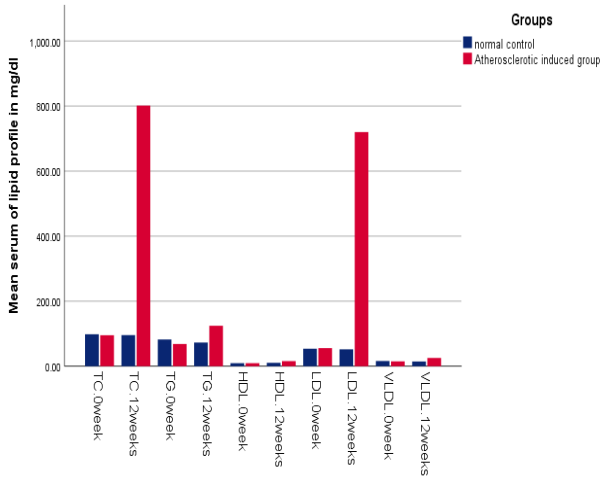

Figure 1. serum level of lipid profile for control group and atherosclerotic induced group in mg\dl

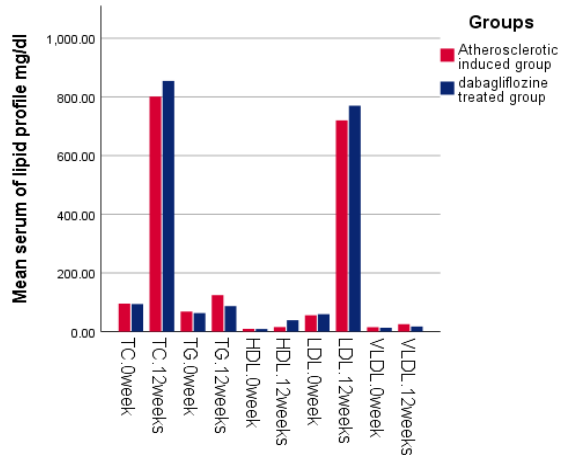

Figure 2. serum level of lipid profile for dabagliflozine treated group and atherosclerotic induced group in mg\dl

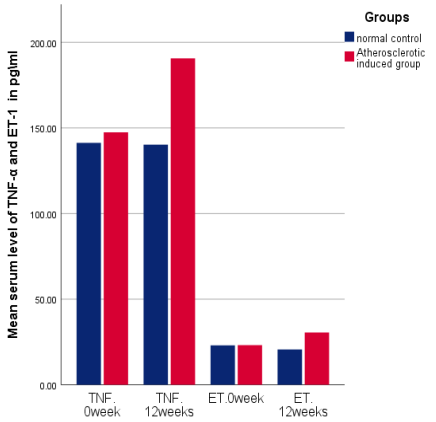

Figure 3. serum level of TNF-α and ET-1 for control group and atherosclerotic induced group in pg\ml

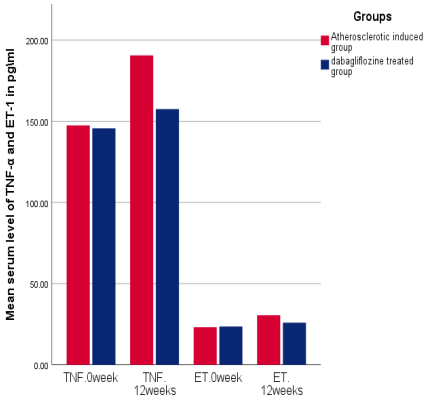

Figure 4. serum level of TNF-α and ET-1 for dabagliflozine treated group and atherosclerotic induced group in pg\ml

the study time, this clinical benefits which explained via the antiatherogenic action which was accomplished by transport the excess of LDL-cholesterol from all parts of body to liver for processing and excretion. So, when noted the data in Figure (3) that revealed the intended drug (dabagliflozine) potentiated the activity of good cholesterol HDL by increasing its level, in addition to the lowering role of anti-diabetic drug on the level of other lipoprotein including very low density lipoprotein and TG. VLDL consist of about two third of TG and less than one third of cholesterol ester and consider the main proatherogenic factor plus its action in the rupture of atherosclerotic plaque. due to this observation of dabagliflozine action that give the protective effect in atherosclerotic induced mice by dual action involving the elevation of antiatherogenic factor and reduction of atherogenic factor. Leng et al., (2016) reported the protective action of dabagliflozine by lowering the serum level of TG in diabetic Apo-/- mice treated for same period of our study.

The association between atherosclerosis and inflammatory pathways it presented via Hedin and Matic, (2019) also, they wree considered the atherosclerosis is chronic inflammatory disorder and mentioned the incorporation of the inflammatory cells and cytokines in the formation of atherosclerotic lesion. So, groups of drugs with multiple effects on the lipid profile and inflammatory cytokines have attenuation effect on atherosclerosis. As the data documented in the Figure (4) which explained the role of studied drug on atherosclerotic mice that showed reduction in serum level of TNF-α, this protein in addition to other inflammatory mediators activate cascade events involving in the endothelial change and damaged for vessels however the suppression in the level of inflammatory mediators also reported by Leng et al., (2016) in diabetic mice . Endothelin-1 play critical role in the stimulation of several inflammatory cells which drive the underlying pathological events of atherosclerosis so decrease its secretion or antagonize its action can categorize as therapeutic target for the treatment. Another study, stated there is marked decline in function of heart and kidney associated with elevation of ET-1 level as a part of the systemic responses and the patient treated with dabagliflozine 10mg once daily for 12 months showed significant reduction (P= 0.029) in the level of endothelin-1in heart failure patients (Yeoh et al., 2023). This results consistent with the slight reduction in ET-1 that seen in the mice treated with dabagliflozine after induction of atherosclerosis which indicated the anti-inflammatory action of study drug. The difference in results may related to differences in dose since our study used 1mg\Kg\day which consider higher than dose used for heart failure patients (10mg once daily) however present study used animal model while Yeoh et al., (2023) used dabagliflozine for human with heart disorder. As mentioned previously by many scientists that the progression of atherosclerosis had strong correlation with cardiac disorders such as cardiomyopathy, myocardial infraction, heart failure and other cardiovascular events (Zhu et al.,2018). So any drug can ameliorated this progression of atherosclerosis also can lowering the incidence of cardiac complication in patient with atherosclerosis and might be consider as cardio protection agent. In the present study we can thought the proposed mechanism of action of our drug is through the all previous pathways; moreover, dabagliflozine offer cardio protection role via two dimension, first; modification in lipid metabolism, second; modification in inflammatory mediator levels specifically TNF-α and ET-1. The benefit effect of dabagliflozine on heart function also reported by other research via the improvement in left ventricular function and infract size reduction in ischemic reperfusion rat model (Tanajak et al., 2018)

Conclusion

The study results presents useful data which indicated that dabagliflozine has attenuated action on atherosclerosis process via two pathways including the modification of lipid profile and reduction the inflammatory cytokines to increase the stability of atherosclerotic plaque and prevent its rupture. So, the need for more investigation about the dabagliflozine it important to rule out the full action for clinical use is important. It is recommended to study the signaling pathway which is responsible for antixodative action of dabagliflozine with the assessment for the oxidative marker.

Author contribution

Z.A.A. conducted all portions of the study, she wrote the article, S,A.R. and Z.I.J.S. performed the tests, reviewed the design, and supervised the work.

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Competing financial interests

The authors have no conflict of interest.

References

Abd-Ulhussein, H. K., & Rizij, F. A. J. (2019). Evaluation of the effect of dapagliflozin on atherosclerosis progression by interfering with inflammatory and oxidative stress pathways in rabbits. Medical Journal of Babylon, 16(1), 21.

Becker, L., Prado, K., Foppa, M., Martinelli, N., Aguiar, C., Furian, T., ... & Rohde, L. E. (2012). Endothelial dysfunction assessed by brachial artery ultrasound in severe sepsis and septic shock. Journal of critical care, 27(3), 316-e9.

Brauner, J. S., Rohde, L. E., & Clausell, N. (2000). Circulating endothelin-1 and tumor necrosis factor-α: early predictors of mortality in patients with septic shock. Intensive care medicine, 26, 305-313.

Cannon, C. P., Perkovic, V., Agarwal, R., Baldassarre, J., Bakris, G., Charytan, D. M., ... & Mahaffey, K. W. (2020). Evaluating the effects of canagliflozin on cardiovascular and renal events in patients with type 2 diabetes mellitus and chronic kidney disease according to baseline HbA1c, including those with HbA1c< 7% results from the CREDENCE trial. Circulation, 141(5), 407-410.

Chai, M., Ji, Q., Zhang, H., Zhou, Y., Yang, Q., Zhou, Y., ... & Zhao, Y. (2015). The protective effect of interleukin-37 on vascular calcification and atherosclerosis in apolipoprotein E-deficient mice with diabetes. Journal of Interferon & Cytokine Research, 35(7), 530-539.

Choy, E. H., & Panayi, G. S. (2001). Cytokine pathways and joint inflammation in rheumatoid arthritis. New England Journal of Medicine, 344(12), 907-916.

Feijóo-Bandín, S., Aragón-Herrera, A., Otero-Santiago, M., Anido-Varela, L., Moraña-Fernández, S., Tarazón, E., ... & Lago, F. (2022). Role of sodium-glucose co-transporter 2 inhibitors in the regulation of inflammatory processes in animal models. International Journal of Molecular Sciences, 23(10), 5634.

Freeman, B. D., Machado, F. S., Tanowitz, H. B., & Desruisseaux, M. S. (2014). Endothelin-1 and its role in the pathogenesis of infectious diseases. Life sciences, 118(2), 110-119.

Frostegård, J. (2013). Immunity, atherosclerosis and cardiovascular disease. BMC medicine, 11(1), 1-13.

Gao, W., Liu, H., Yuan, J., Wu, C., Huang, D., Ma, Y., ... & Ge, J. (2016). Exosomes derived from mature dendritic cells increase endothelial inflammation and atherosclerosis via membrane TNF-α mediated NF-κB pathway. Journal of cellular and molecular medicine, 20(12), 2318-2327.

George, J., Afek, A., Gilburd, B., Blank, M., Levy, Y., Aron-Maor, A., ... & Shoenfeld, Y. (1998). Induction of early atherosclerosis in LDL-receptor–deficient mice immunized with β2-glycoprotein I. Circulation, 98(11), 1108-1115.

Hansson, G. K. (2001). Immune mechanisms in atherosclerosis. Arteriosclerosis, thrombosis, and vascular biology, 21(12), 1876-1890.

Hedin, U., & Matic, L. P. (2019). Recent advances in therapeutic targeting of inflammation in atherosclerosis. Journal of vascular surgery, 69(3), 944-951.

Heerspink, H. J., Stefánsson, B. V., Correa-Rotter, R., Chertow, G. M., Greene, T., Hou, F. F., ... & Wheeler, D. C. (2020). Dapagliflozin in patients with chronic kidney disease. New England Journal of Medicine, 383(15), 1436-1446.

Hetherington, I., & Totary-Jain, H. (2022). Anti-atherosclerotic therapies: milestones, challenges, and emerging innovations. Molecular Therapy, 30(10), 3106-3117.

Hsia, D. S., Grove, O., & Cefalu, W. T. (2017). An update on SGLT2 inhibitors for the treatment of diabetes mellitus. Current opinion in endocrinology, diabetes, and obesity, 24(1), 73.

Leng, W., Ouyang, X., Lei, X., Wu, M., Chen, L., Wu, Q., ... & Liang, Z. (2016). The SGLT-2 inhibitor dapagliflozin has a therapeutic effect on atherosclerosis in diabetic ApoE–/– mice. Mediators of inflammation, 2016.

Lindstrom, N. M., Moore, D. M., Zimmerman, K., & Smith, S. A. (2015). Hematologic assessment in pet rats, mice, hamsters, and gerbils: blood sample collection and blood cell identification. Clinics in Laboratory Medicine, 35(3), 629-640.

Liu, Z., Ma, X., Ilyas, I., Zheng, X., Luo, S., Little, P. J., ... & Xu, S. (2021). Impact of sodium glucose cotransporter 2 (SGLT2) inhibitors on atherosclerosis: from pharmacology to pre-clinical and clinical therapeutics. Theranostics, 11(9), 4502.

Marzolla, V., Armani, A., Mammi, C., Feraco, A., & Caprio, M. (2018). Induction of atherosclerotic plaques through activation of mineralocorticoid receptors in apolipoprotein E-deficient mice. JoVE (Journal of Visualized Experiments), (139), e58303.

McKenna, S., Gossling, M., Bugarini, A., Hill, E., Anderson, A. L., Rancourt, R. C., ... & Wright, C. J. (2015). Endotoxemia induces IκBβ/NF-κB—Dependent Endothelin-1 expression in hepatic macrophages. The Journal of Immunology, 195(8), 3866-3879.

Narula, J., Nakano, M., Virmani, R., Kolodgie, F. D., Petersen, R., Newcomb, R., ... & Finn, A. V. (2013). Histopathologic characteristics of atherosclerotic coronary disease and implications of the findings for the invasive and noninvasive detection of vulnerable plaques. Journal of the American College of Cardiology, 61(10), 1041-1051.

Pober, J. S., & Sessa, W. C. (2007). Evolving functions of endothelial cells in inflammation. Nature Reviews Immunology, 7(10), 803-815.

Probert, L. (2015). TNF and its receptors in the CNS: The essential, the desirable and the deleterious effects. Neuroscience, 302, 2-22.

Resl, M., & Clodi, M. (2010). Diabetes and cardiovascular complications: Epidemiologie zur Morbidität und Mortalität. Wiener Medizinische Wochenschrift, 160, 3-7.

Stiekema, L. C., Willemsen, L., Kaiser, Y., Prange, K. H., Wareham, N. J., Boekholdt, S. M., ... & Kroon, J. (2021). Impact of cholesterol on proinflammatory monocyte production by the bone marrow. European Heart Journal, 42(42), 4309-4320.

Sugiyama, T., Yamamoto, E., Bryniarski, K., Xing, L., Fracassi, F., Lee, H., & Jang, I. K. (2018). Coronary plaque characteristics in patients with diabetes mellitus who presented with acute coronary syndromes. Journal of the American Heart Association, 7(14), e009245.

Sun, L., Zhang, W., Zhao, Y., Wang, F., Liu, S., Liu, L., ... & Xu, Y. (2020). Dendritic cells and T cells, partners in atherogenesis and the translating road ahead. Frontiers in immunology, 11, 1456.

Tanajak, P., Sa-Nguanmoo, P., Sivasinprasasn, S., Thummasorn, S., Siri-Angkul, N., Chattipakorn, S. C., & Chattipakorn, N. (2018). Cardioprotection of dapagliflozin and vildagliptin in rats with cardiac ischemia-reperfusion injury. Journal of Endocrinology, 236(2), 69-84.

Weber, M. A., Mansfield, T. A., Cain, V. A., Iqbal, N., Parikh, S., & Ptaszynska, A. (2016). Blood pressure and glycaemic effects of dapagliflozin versus placebo in patients with type 2 diabetes on combination antihypertensive therapy: a randomised, double-blind, placebo-controlled, phase 3 study. The lancet Diabetes & endocrinology, 4(3), 211-220.

Yeoh, S. E., Docherty, K. F., Campbell, R. T., Jhund, P. S., Hammarstedt, A., Heerspink, H. J., ... & McMurray, J. J. (2023). Endothelin-1, Outcomes in Patients With Heart Failure and Reduced Ejection Fraction, and Effects of Dapagliflozin: Findings From DAPA-HF. Circulation, 147(22), 1670-1683.

Zhu, J., Liu, B., Wang, Z., Wang, D., Ni, H., Zhang, L., & Wang, Y. (2019). Exosomes from nicotine-stimulated macrophages accelerate atherosclerosis through miR-21-3p/PTEN-mediated VSMC migration and proliferation. Theranostics, 9(23), 6901.

Zhu, Y., Xian, X., Wang, Z., Bi, Y., Chen, Q., Han, X., ... & Chen, R. (2018). Research progress on the relationship between atherosclerosis and inflammation. Biomolecules, 8(3), 80.