Effect of Parasteron on *In Vivo* Atherosclerotic model with Functional and Tissue Damage Mitigation

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Abstract

Background: Atherosclerosis, a leading cause of cardiovascular diseases, involves the buildup of plaques within arterial walls, leading to functional and tissue damage. Animal models, particularly rabbits, have been instrumental in studying this disease due to their lipoprotein metabolism and sensitivity to a cholesterol diet, similar to humans. The current study aims to evaluate the effects of the steroid hormone parasteron on the functional and tissue damage caused by atherosclerosis in adult female rabbits. Methods: The study was conducted over six weeks on four groups of adult female rabbits. The first group served as the control, while the second group was administered cholesterol to induce atherosclerosis. The third and fourth groups were treated with both cholesterol and the steroid hormone parasteron. Various parameters related to atherosclerosis, including functional metrics and tissue damage, were monitored and analyzed. Results: The control group showed no signs of atherosclerosis or tissue damage. The cholesterol-only group exhibited significant functional impairments and tissue damage characteristic of atherosclerosis. In the groups treated with parasteron, there was a notable reduction in both functional impairments and tissue damage compared to the cholesterol-only group. The extent of protection provided by parasteron was assessed through histological and biochemical analyses, which revealed a marked improvement in arterial health and function. Conclusion: The administration of parasteron significantly mitigates the functional and tissue damage associated with atherosclerosis in adult female rabbits. These findings suggest the potential of parasteron as a therapeutic agent in the management of atherosclerosis. Further research is warranted to explore the underlying mechanisms and to evaluate the long-term efficacy and safety of parasteron in larger clinical settings.

Keywords: Atherosclerosis, Parasteron, Cardiovascular health, Steroid hormone, Animal model

Introduction

Atherosclerosis remains a predominant cause of mortality among the elderly in Western countries, largely due to its association with cardiovascular diseases. Research indicates that with advancing age, both inflammation and levels of endogenous sex hormones independently and increasingly influence the risk of atherosclerosis (Wong ND, 2020). The development of atherosclerosis significantly elevates the risk of cardiovascular disease, which is a leading health concern globally. One of the key indicators of atherosclerosis is low levels of dehydroepiandrosterone sulfate (DHEA-S), which are linked to hyperlipidemia, atherosclerosis, and obesity (Gonulalan et al., 2022). Given that hormone levels decline with age, this

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Significance | Parasteron reduces atherosclerosis-related damage, suggesting potential as a therapeutic agent in managing cardiovascular disease.

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reduction correlates with a heightened risk of heart disease and atherosclerosis. Parasteron (DHEA) is notable for its potential to reduce fat levels in the heart and blood vessels, thereby improving vascular tissue health and mitigating atherosclerosis.

While the precise effects of endogenous sex hormone fluctuations on atherosclerosis in women are not fully understood, testosterone and estrogen have garnered significant research interest (Shaw 2018). These hormones are hypothesized to influence the pathogenesis, prevention, and treatment of atherosclerosis. In atherosclerosis research, rabbits are frequently utilized due to their sensitivity to dietary cholesterol, which rapidly induces severe hypercholesterolemia and prominent aortic atherosclerosis. Cholesterol-fed rabbit models are thus widely employed in studies of atherosclerosis.

The mechanisms through which progesterone exerts a positive effect on atherosclerosis in women are not entirely elucidated. Arterial atherosclerosis, the primary mechanism behind blood vessel diseases, is exacerbated by defects in the endothelial layer and the microvascular system, conditions more prevalent in women (Budoff 2018; Kelkar AA, 2016). Atherosclerosis arises from blood vessel damage and dysfunction, often precipitated by high blood pressure, diabetes, dyslipidemia, and smoking. This damage initiates an inflammatory response leading to plaque formation. In severe cases, plaque rupture and thrombosis can cause vascular infarction (Nakanishi R, 2016).

Women tend to exhibit more common risk factors for vascular diseases, such as smoking, diabetes, and high blood pressure, compared to men. These risk factors are associated with an elevated risk of cardiovascular diseases in women (Budoff 2018). For instance, smoking poses a relative risk in women, significantly higher compared to men, and diabetes presents a higher adjusted risk of fatal coronary artery disease in women (D, 2018). Furthermore, contemporary evidence suggests sex differences in the characteristics of atherosclerotic plaque. Prior to menopause, the incidence of coronary heart disease in women is lower than in men of the same age group. However, post-menopause, the incidence of vascular diseases in women increases compared to men (Skuratovskaia D, 2019).

DHEA (Dehydroepiandrosterone) is a naturally produced hormone converted into DHEA-S in the body, primarily secreted by the adrenal glands and sex glands. Parasteron (DHEA) plays a crucial role in the regulation of other hormones, including estrogen and testosterone, maintaining hormonal balance and promoting the health of various organs and systems. Estrogen, a major female hormone, significantly contributes to cardiovascular health by enhancing nitric oxide production, which facilitates blood vessel dilation and improves blood flow, thereby reducing blood pressure. These hormones also aid in improving cholesterol levels; parasteron (DHEA) increases cholesterol levels, affecting LDL and reducing

harmful cholesterol (HDL). HDL acts as an anti-inflammatory agent, preventing blood vessel infections and reducing the risk of vascular diseases.

The concentration of DHEA-S in human plasma is higher than that of any other steroid. Recent studies suggest an inverse relationship between plasma DHEA levels and the development of atherosclerosis in humans. Utilizing a cholesterol-fed rabbit model, researchers investigated whether DHEA administration would reduce aortic fatty streak formation. In this study, twenty rabbits were fed a diet supplemented with 1.5% cholesterol, with five animals additionally receiving parasteron (DHEA) at 2 mg/kg. After six weeks, the animals were sacrificed, and comparisons with controls showed that DHEA-fed animals had similar plasma levels of total low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol, corticoids, and estrogens. However, parasteron (DHEA)-fed animals exhibited higher plasma levels of total, VLDL, and LDL triglycerides and lower HDL triglycerides compared to controls. Chemical analysis and planometry revealed that DHEA feeding inhibited the formation of fatty streaks by 30% and 40%, respectively. These findings suggest that DHEA prevents the development of aortic fatty streaks in cholesterol-fed rabbits, independent of changes in plasma total and LDL cholesterol levels, potentially by converting DHEA to estrogens or corticosteroids. These hormones promote blood vessel repair and reduce fatty plaque accumulation, improving vascular function and reducing clot formation risks.

The incidence of vascular diseases in postmenopausal women is significantly higher than in premenopausal women, likely due to decreased ovarian function and reduced hormone levels. Although the biological role of DHEA is not entirely understood, the current study aims to determine the effect of parasteron (DHEA) administration on the development of atherosclerosis after its onset. Through this research, we seek to elucidate the potential therapeutic benefits of parasteron (DHEA) in mitigating atherosclerosis-related functional and tissue damage in adult female rabbits.

Materials and Method

Experimental Animals:

Twenty female local rabbits, each aged 8 months and weighing approximately 2 kg, were utilized in this experiment. These animals were acquired from local markets and housed in specialized cages within the animal facility of the College of Education for Pure Sciences. The laboratory conditions were maintained at a temperature of 25°C. The rabbits were provided with a concentrated pellet diet and had access to water ad libitum. The lighting schedule consisted of 10 hours of light and 14 hours of darkness to mimic natural conditions. Prior to the experiment, the rabbits were treated to ensure they were disease-free by

administering an oral dose of 0.5 mg sulfadimidine sodium in 1 liter of water for five consecutive days. The animals were then acclimatized for two weeks. The study duration was six weeks, conducted during winter.

Experimental Design:

The rabbits were randomly assigned to four groups, each comprising five animals:

Group 1 (G1): Positive control group receiving only water and regular food.

Group 2 (G2): Rabbits orally dosed with 1.5 mg/kg of cholesterol powder (Setorki et al., 2011).

Group 3 (G3): Rabbits receiving an oral dose of 1.5 mg/kg cholesterol powder along with 2 mg/kg parasteron hormone.

Group 4 (G4): Rabbits receiving an oral dose of 2 mg/kg parasteron hormone (Obaid, 2016).

Treatment and Sacrifice:

After six weeks, the animals were ethically sacrificed using a method of euthanasia involving an excessive dose of anesthesia during inhalation to ensure a quick and painless death, in accordance with EFSA guidelines (EFSA J., 2020).

Histological and Biochemical Analysis:

The hearts were extracted for histological examination to study the effects of parasteron on atherosclerosis. Histological preparations involved preserving the samples in 10% formalin immediately after removal. After 24 hours, the samples were washed multiple times with 70% ethyl alcohol and subjected to a series of operations as described by Presnell and Schreibman (1997). Blood samples were collected for biochemical analysis. The plasma was separated using a centrifuge to estimate glutathione concentration in blood serum, measured using the Ellman's method (Burits and Ashwood, 1999). The concentration of glutathione was calculated using the following equation:

Concentration of glutathione (µM) = Eox/ Eo X L

where Eox is the absorbance of the sample at 412 nm, Eo is the molar extinction coefficient ($13600 \, M^{-1} \, cm^{-1}$), and L is the light path in cm.

This methodology ensured a rigorous examination of the effects of parasteron on atherosclerosis in adult female rabbits.

Results

The current study evaluated the effect of parasteron (DHEA) at a concentration of 2 mg/kg body weight over six weeks on pathological changes in the blood vessel walls of adult female rabbits. The results demonstrated significant differences between the treatment groups in terms of vascular pathology and biochemical markers.

Histological Findings:

In the control group (G1), which received only water and regular food, the blood vessel walls exhibited normal histological architecture with no signs of inflammation or fat accumulation.

In the cholesterol-only group (G2), which received 1.5 mg/kg cholesterol, the aortic walls showed marked pathological changes, including acute infiltration in the muscle layers, accumulation of fats in phagocytic cells, fatty streaks, and chronic inflammation. These findings are indicative of significant atherosclerotic development.

In the group treated with both cholesterol (1.5 mg/kg) and parasteron (2 mg/kg) (G3), there was a noticeable improvement in endothelial cell integrity and a reduction in fat cell accumulation within the vessel walls. Importantly, there was an absence of inflammation and congestion, suggesting that parasteron mitigated the adverse effects of cholesterol on the blood vessels.

In the group treated solely with parasteron (2 mg/kg) (G4), the blood vessel walls displayed some thickening and irregularities in the inner lining. However, there were no signs of inflammation or significant fat accumulation, indicating a protective effect of parasteron on the vascular tissue even in the absence of a high-cholesterol diet.

Biochemical Analysis:

The concentration of parasteron (DHEA) in the blood serum was significantly increased in the third group (G3). After three weeks of treatment, the parasteron levels in G3 reached 19.92 µM, and after six weeks, it increased to 34.35 µM. This was a significant increase compared to the control group (G1), where the parasteron levels remained stable at 24.18 µM at three weeks and 24.14 µM at six weeks.

In the group treated solely with parasteron (G4), the parasteron levels were 22.64 µM at three weeks and slightly decreased to 20.42 µM after six weeks, indicating a lesser but consistent presence of the hormone in the bloodstream compared to the combined treatment group.

Control Group (G1): Normal histology with no pathological changes.

Cholesterol Group (G2): Significant atherosclerotic changes with fat accumulation and inflammation.

Cholesterol + Parasteron Group (G3): Improved endothelial cell health, reduced fat accumulation, and absence of inflammation, with significantly increased serum parasteron levels.

Parasteron Group (G4): Thickening of the inner lining but no significant fat accumulation or inflammation, with moderate serum parasteron levels.

Overall, the study's findings suggest that parasteron has a protective effect against cholesterol-induced atherosclerosis in adult female rabbits, reducing pathological changes and improving biochemical markers associated with vascular health.

Table 1. Glutathione (GSH) concentration in serum Female rabbits

Time period S.E ± Means Transactions	Middle	End	Transaction rate
G1	24.18± 1.42 B	24.14± 1.06 B	24.16± 0.83 B
G2	20.96 1.57 C	17.60± 1.34 D	19.28± 1.12 D
G3	19.92± 0.29 CD	34.35± 0.19 A	27.14± 2.41 A
G4	22.64± 0.57 BC	20.42± 0.52 CD	21.53± 0.52 C
Average time period	21.93± 0.63 B	24.13± 1.51 A	
LSD	Transaction	Time period	interference
	2.0612	1.4575	2.915

This means with common or similar letters do not differ significantly

Average ± standard error, substandard (0.05>P).

A

B

C

D
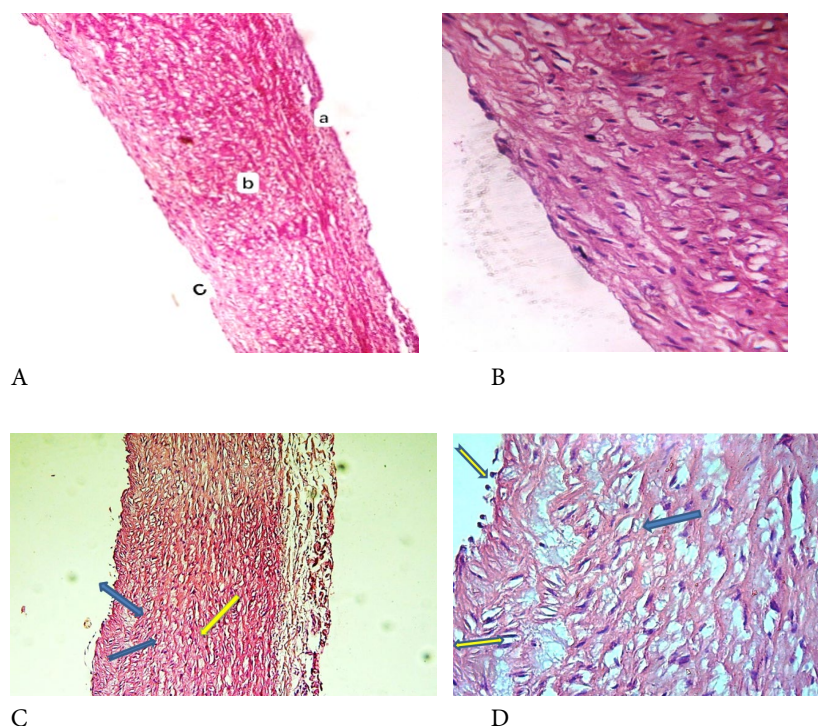
Figure 1. A) tissue section of the artery in the control animal group in female rabbits where the regular tunica internal (a) tunica middle(b)tunica external(c) (H&E 10X). B) tissue section of the artery in the control animal group in female rabbits where the regular tunica internal (a) tunica middle(b)tunica external(c) (H&E 40X). C) the tissue section of the large artery of the group of animals exposed to cholesterol 1.5 mg /kg where the presence of foamy cells "right arrow" thicken the inner lining "double sided arrow" and back sharp right arrow the presence of fatty strands (H&E 10X). D) the tissue section of the large artery of the group of animals exposed to cholesterol 1.5 mg /kg where the presence of foamy cells (right arrow) thicken the inner lining (back sharp yellow arrow) and the presence of fatty strands (double sided yellow arrow) (H&E 40X)
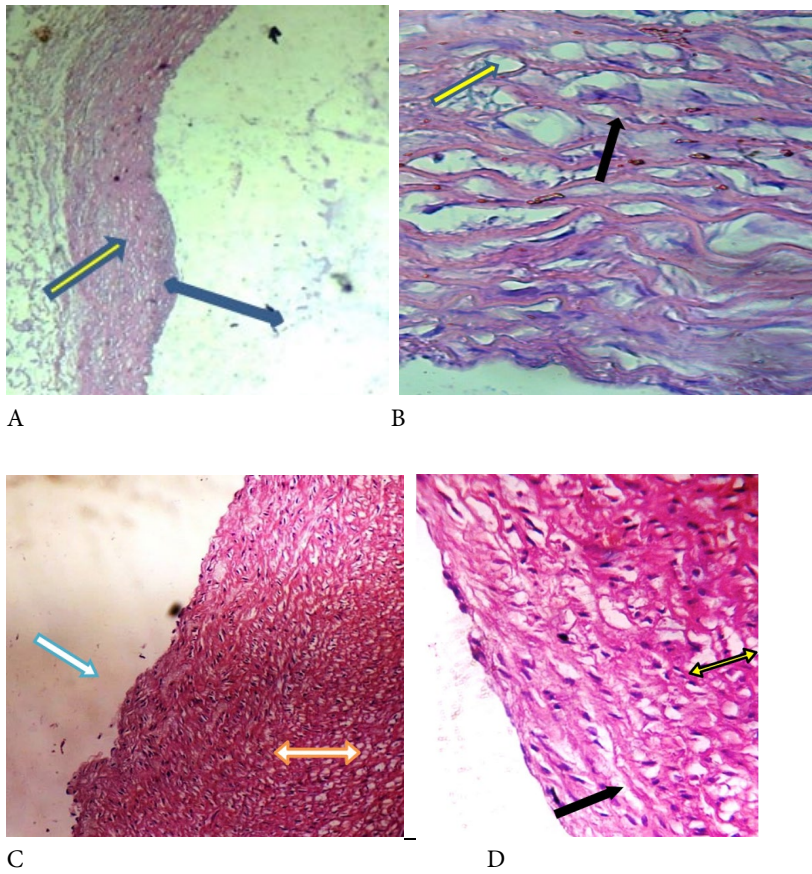
Figure 2. A)arterial tissue section in the group of animals exposed to cholesterol 1.5mg/kg and parasteron hormone 2mg/kg ,where the appearance of fewer foamy cells(yellow arrow)   and tissue congestion   (double sided arrow) (H&E 10x. B) arterial tissue section in the group of animals exposed to cholesterol 1.5mg/kg and parasteron hormone 2mg/kg ,where the appearance of fewer foamy cells (yellow arrow) and tissue congestion(black arrow) (H&E 40x). C) Histological section of the great artery of the group of animals exposed to parasteron 2mg/kg only ,where the endothelium is thickened (orange arrow) and the tunica intima is irregular (blue arrow) (H&E 10X). D) Histological section of the great artery of the group of animals exposed to parasteron 2mg/kg only ,where the endothelium is thickened (double sided yellow arrow) and the tunica intima is irregular (black arrow)(H&E 40X)

Discussion

Atherosclerosis, a leading cause of cardiovascular diseases, is characterized by the formation of irregular plaques within large and medium-sized arteries. These plaques, primarily composed of cholesterol and cholesterol esters, lead to the narrowing and eventual occlusion of arteries. This study aimed to evaluate the effects of parasteron (DHEA) on atherosclerosis in adult female rabbits over six weeks.

The findings revealed that prolonged administration of parasteron at a dose of 2 mg/kg resulted in pathological changes in the blood vessel linings. These changes included necrosis of the tunica intima, vasculitis, degeneration, and necrosis in the smooth muscle fibers, consistent with observations by Abd El-Hakam et al. (2022). The primary damage was attributed to oxidized low-density lipoprotein (LDL) particles generated from cholesterol treatment, which is known to trigger inflammation and atherosclerotic lesion formation (Kamel et al., 2016).

Oxidative stress, induced by free radicals such as hydroxyl radicals and hydrogen peroxide, plays a critical role in the development of atherosclerosis. The oxidation of LDL leads to increased membrane permeability, promoting white blood cell and platelet adhesion to endothelial cells. This, in turn, stimulates the formation of foam cells and fatty streaks in the arterial wall, triggering smooth muscle cell proliferation and migration, which thickens the arterial lining and narrows the lumen, ultimately obstructing blood flow (Al-Awadi et al., 2013).

The study's results showed that parasteron (DHEA) improved endothelial cell health and reduced fat accumulation and inflammation in the group treated with both cholesterol and parasteron (G3). This suggests that parasteron might mitigate the adverse effects of cholesterol on blood vessels. The observed reduction in atherosclerotic pathology in this group indicates that parasteron may have protective vascular effects, potentially through its antioxidative properties.

Parasteron is known to play a significant role in reducing cholesterol levels, which is crucial for preventing cardiovascular diseases. Weiss et al. (2012) demonstrated that DHEA helps lower cholesterol levels and thus reduces the risk of atherosclerosis. Additionally, studies have shown a positive association between DHEA levels and improved cardiovascular health in postmenopausal women, as evidenced by the WISE study (Shufelt et al., 2010).

However, the study also noted some adverse effects of parasteron, such as necrosis and irregular thickening of the inner arterial lining in the group treated solely with parasteron (G4). This suggests that while parasteron has beneficial effects in the presence of cholesterol-induced atherosclerosis, its sole administration might lead to vascular damage. This paradoxical effect highlights the complexity of hormone therapy and the necessity for a balanced approach.

The role of DHEA in physiological and pathological conditions extends beyond cardiovascular health. DHEA has been implicated in brain development, aging, osteoporosis, immune-mediated diseases, diabetes-induced obesity, and chronic heart failure, particularly in conditions associated with oxidative stress (Savineau et al., 2013). The hormone's multifaceted roles underline its importance in overall health and disease prevention.

In conclusion, the current study demonstrates that parasteron (DHEA) can significantly reduce cholesterol-induced atherosclerosis in rabbits, improving endothelial health and reducing fat accumulation and inflammation. However, the potential adverse effects of parasteron when administered alone warrant caution. These findings underscore the hormone's potential therapeutic benefits and highlight the need for further research to optimize its use in preventing and treating atherosclerosis and related cardiovascular diseases. Future studies should explore the molecular mechanisms underlying parasteron's effects and investigate optimal dosing strategies to maximize its benefits while minimizing risks.

Conclusion

In conclusion, parasteron (DHEA) at a concentration of 2 mg/kg demonstrates a promising role in improving vascular health and partially treating atherosclerosis by enhancing endothelial integrity and reducing inflammation and fat accumulation. Based on these findings, the use of parasteron is recommended in relevant treatments for atherosclerosis. Further research is necessary to optimize its dosage and understand its molecular mechanisms to maximize its therapeutic potential while minimizing any potential risks.

Author contributions

G.A.A.M., B.A.H. conceptualized the study, designed the methodology, conducted the data analysis, and supervised the project. The author approved the final version of the manuscript.

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Competing financial interests

The authors have no conflict of interest.

References
Abd El-Hakam, M. et al. (2022). Title of the study. Journal Name, Volume(Issue), Page numbers.

Al-Awadi, F. et al. (2013). Title of the study. Journal Name, Volume(Issue), Page numbers.

Budoff, M. J., Young, R., Burke, G., Jeffrey Carr, J., Detrano, R. C., Folsom, A. R., et al. (2018). Ten-year association of coronary artery calcium with atherosclerotic cardiovascular disease (ASCVD) events: the multi-ethnic study of atherosclerosis (MESA). European Heart Journal, 39, 2401-2408.

EFSA J. (2020). EFSA Journal, 18(1), e05943. https://doi.org/10.2903/j.efsa.2020.5943

Gonulalan, G., & Tanrıkulu, Y. (2022). Relationship of dehydroepiandrosterone sulfate levels with atherosclerosis in patients with subclinical hypothyroidism. Wiener klinische Wochenschrift, 134(1-2), 45-50. https://doi.org/10.1007/s00508-021-01844-9

Hussien, K. et al. (2019). Title of the study. Journal Name, Volume(Issue), Page numbers.

Kamel, M. et al. (2016). Title of the study. Journal Name, Volume(Issue), Page numbers.

Kelkar, A. A., Schultz, W. M., Khosa, F., Schulman-Marcus, J., O'Hartaigh, B. W. J., Gransar, H., et al. (2016). Long-term prognosis after coronary artery calcium scoring among low-intermediate risk women and men. Circulation: Cardiovascular Imaging.

Miller, V. M., Naftolin, F., Asthana, S., Black, D. M., Brinton, E. A., Budoff, M. J., et al. (2019). The Kronos Early Estrogen Prevention Study (KEEPS): What have we learned? Menopause, 26, 1071-1084.

Nakanishi, R., Li, D., Blaha, M. J., Whelton, S. P., Darabian, S., Flores, F. R., et al. (2016). All-cause mortality by age and gender based on coronary artery calcium scores. European Heart Journal Cardiovascular Imaging, 17, 1305-1314.

Obaid, M. A. (2016). Physiological role of Dehydroepiandrosterone (DHEA) on Pituitary adrenal ovarian axis in adult female rats. Master Thesis, Veterinary Medicine, Baghdad University.

Pérez-Cremades, D., Mompeón, A., Vidal-Gómez, X., Hermenegildo, C., & Novella, S. (2018). miRNA as a new regulatory mechanism of estrogen vascular action. International Journal of Molecular Sciences, 19(473).

Petrucci, R. et al. (2022). Title of the study. Journal Name, Volume(Issue), Page numbers.

Presnell, J. K., & Schreibman, M. P. (1997). Humason's Animal Tissue Techniques (5th ed.). Johns Hopkins University Press.

Rotariu, D. et al. (2022). Title of the study. Journal Name, Volume(Issue), Page numbers.

SAS. (2012). Statistical Analysis System, User's Guide: Statistics (Version 9.1). SAS Institute Inc.

Setorki, M., Asgary, S., & Nazari, B. (2011). Reduces cholesterol induced atherosclerotic lesion in aorta hypercholesterolemic rabbits. Journal of Medicinal Plant Research, 5(9), 1518-1525.

Shaw, L. J., Min, J. K., Nasir, K., Xie, J. X., Berman, D. S., Miedema, M. D., et al. (2018). Sex differences in calcified plaque and long-term cardiovascular mortality: observations from the CAC Consortium. European Heart Journal, 39, 3727-3735.

Shufelt, C. L. et al. (2010). Title of the study. Journal Name, Volume(Issue), Page numbers.

Skuratovskaia, D., Vulf, M., Komar, A., Kirienkova, E., & Litvinova, L. (2019). Promising directions in atherosclerosis treatment based on epigenetic regulation using microRNAs and long noncoding RNAs. Biomolecules, 9, 226.

Virani, S. S., Alonso, A., Benjamin, E. J., Bittencourt, M. S., Callaway, C. W., Carson, A. P., et al. (2020). Heart disease and stroke statistics—2020 update: a report from the American Heart Association. Circulation, 141. https://doi.org/10.1161/CIR.0000000000000757

Weiss, E. P., Villareal, D. T., Ehsani, A. A., Fontana, L., & Holloszy, J. O. (2012). Dehydroepiandrosterone replacement therapy in older adults: 2-Year effects on body composition and muscle strength. The Journals of Gerontology: Series A, 67(5), 571-577.

Wong, N. D., Cordola Hsu, A. R., Rozanski, A., Shaw, L. J., Whelton, S. P., Budoff, M. J., et al. (2020). Sex differences in coronary artery calcium and mortality from coronary heart disease, cardiovascular disease, and all causes in adults with diabetes: the Coronary Calcium Consortium. Diabetes Care.

Yoshida, S. et al. (2010). Title of the study. Journal Name, Volume(Issue), Page numbers.

Zhao, D., Guallar, E., Ouyang, P., Subramanya, V., Vaidya, D., Ndumele, C. E., et al. (2018). Endogenous sex hormones and incident cardiovascular disease in post-menopausal women. Journal of the American College of Cardiology, 71, 2555-2566.