Electronic Journal of General Medicine
2026, 23(6), em768
e-ISSN: 2516-3507
https://www.ejgm.co.uk/

MODESTUM

Original Article

OPEN ACCESS

Targeting apoptosis in atherosclerosis: The role of Alpha X1 in reducing vascular cell death

Ahsan Ali [1]*, Alma Mansharipova [1]

[1]Kazakh Russian Medical University, Almaty, KAZAKHSTAN
***Corresponding Author:** ahsanali583945@gmail.com

Citation: Ali A, Mansharipova A. Targeting apoptosis in atherosclerosis: The role of Alpha X1 in reducing vascular cell death. Electron J Gen Med. 2026;23(6):em768. https://doi.org/10.29333/ejgm/19990

ARTICLE INFO

Received: 13 Aug. 2026

Accepted: 12 Nov. 2026

ABSTRACT

Background/objectives: This study aimed to evaluate the effects of Alpha X1 on vascular cell apoptosis in rabbits with induced atherosclerosis.

Materials and methods: Thirty-five chinchilla rabbits were divided into four groups, including two receiving Alpha X1 for 10 or 20 days. Apoptosis was assessed using in Situ Oligo Ligation, while p53 and BAX expression was evaluated immunohistochemically.

Results: A 6-month cholesterol diet increased p53 and BAX expression more than 15-fold compared with controls. Alpha X1 reduced apoptosis 12.3-fold and 16-fold after 10 and 20 days, respectively ($p < 0.05$). p53 and BAX levels decreased by 76.8% and 86.5%, respectively. Lipid infiltration declined by 57-71%, while aortic wall thickening decreased by 36-48%. Inflammatory markers decreased by 53.6-74.4%, and endothelial structure recovery reached 85% after prolonged treatment.

Conclusions: These findings suggest that Alpha X1 may reduce vascular apoptosis, inflammation, and structural damage in experimental atherosclerosis.

Keywords: apoptosis, atherosclerosis, vascular apoptosis, inflammatory markers, endothelial layer, C-reactive protein, aorta, lipid infiltration

INTRODUCTION

Atherosclerosis remains one of the main causes of cardiovascular disease, which ranks first among the factors of mortality in the world. This process begins with the accumulation of lipids in the arterial walls, which causes inflammation and damage to the vessels. Gradual thickening of vessel walls and a decrease in their elasticity significantly impede blood flow, increasing the risk of thrombosis, heart attacks and strokes. One of the key mechanisms for provoking heart attacks and strokes in atherosclerosis is the increased death of endothelial cells and vascular smooth muscle cells. These cells play an important role in maintaining the structure and function of vessels, and their loss leads to even greater damage to arterial walls.

In addition to structural changes, atherosclerosis is accompanied by metabolic disorders, which aggravates inflammatory processes and promotes further lipid deposition. Studying the mechanisms of vascular cell death, such as apoptosis, opens new opportunities for finding effective therapies. Maintaining the integrity of endothelial cells and smooth muscle cells may slow down disease progression, reduce the risk of complications and improve cardiovascular health. This approach is seen as a promising avenue for creating innovative therapeutic strategies to target atherosclerosis.

Apoptosis of vascular wall cells plays an important role in the development and progression of atherosclerosis. Increased expression of p53 and BAX proteins has been associated with enhanced apoptosis in endothelial and vascular smooth muscle cells [1]. Experimental studies have also demonstrated that cholesterol-enriched diets are associated with elevated expression of these proteins and increased vascular cell apoptosis [2]. These findings highlight the importance of investigating approaches aimed at regulating apoptotic processes in atherosclerotic lesions.

Pharmacological modulation of apoptosis has been investigated as a potential approach to reducing vascular damage. It was examined the effects of apoptosis inhibitors in experimental atherosclerosis in rabbits and reported reductions in vascular injury [3]. Inflammatory processes also contribute to atherosclerotic vascular damage, with interleukin-6 (IL-6) and C-reactive protein (CRP) being associated with inflammatory responses and endothelial dysfunction [4]. Furthermore, lipid accumulation within the vascular wall has been linked to apoptotic changes in vascular smooth muscle cells, suggesting a relationship between lipid infiltration and cellular damage [5].

Recent investigations have explored compounds capable of influencing apoptosis-related molecular pathways. It was

Copyright © 2026 by Author/s and Licensed by Modestum. This is an open access article distributed under the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

examined the effects of selected compounds on p53 and BAX expression under in vitro conditions, providing preliminary evidence of their potential involvement in apoptosis regulation [6]. In addition, studies in [7, 8] have addressed cellular mechanisms relevant to apoptosis and tissue recovery, providing a broader basis for investigating vascular structural changes associated with apoptosis modulation.

Although previous studies have examined apoptosis-related proteins, inflammatory responses, lipid accumulation, and pharmacological approaches to reducing cellular damage, the effects of Alpha X1 on these interconnected processes in experimental atherosclerosis remain insufficiently characterised. Its association with vascular cell apoptosis, p53 and BAX expression, lipid infiltration, inflammatory markers, and aortic wall morphology requires further investigation in an in vivo model. Addressing these aspects may provide additional experimental evidence regarding the effects of Alpha X1 on atherosclerotic vascular damage.

The aim of the study was to evaluate the efficacy of Alpha X1 in modulating apoptosis and restoring vascular wall structure in rabbits with induced atherosclerosis. To achieve this goal, the following objectives were set: to study changes in the expression level of p53 and BAX proteins associated with apoptosis; to quantify apoptosis in the cells of the vascular wall; to study the degree of lipid infiltration of the aorta and its changes under the influence of the drug; to evaluate the effect of Alpha X1 on the restoration of vascular structure; to analyse changes in inflammatory markers associated with the use of the drug.

MATERIALS AND METHODS

The experiment was conducted in the laboratory of the Kazakh Russian Medical University in Almaty, Republic of Kazakhstan. 35 male rabbits of chinchilla breed with an initial average weight of about 3,500 g were selected for the study. The animals were kept singly in individual steel cages under strict observance of environmental conditions: stable temperature, controlled humidity and fixed light regime (12 hours of light and 12 hours of darkness). These parameters provided optimal conditions for the experiment and minimized side effects. The study was conducted over a period of 6 months. Daily examinations were performed to assess health status, and monthly weight measurements were recorded. All procedures met established biosafety standards and were approved by the university's ethical committee. All animal manipulations were performed in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes [9] and the Universal Declaration on Animal Welfare [10].

At the beginning of the study, rabbits were randomly divided into four groups. The control group (group 1), consisting of five animals, received a standard diet (Purina rabbit chow, Dytes Inc., USA). The other 30 rabbits received a diet enriched with 2% cholesterol (from the same manufacturer) for 6 months to induce hyperlipidaemia and promote the development of atherosclerotic lesions. This approach provided a disease model for further study. After a six-month period, their average weight increased to 5000 g. These rabbits were randomly allocated into three groups of 10 animals each. The second group continued to receive the cholesterol diet without treatment, while rabbits in group 3 and group 4 received Alpha X1 through topical application to a pre-shaved area of skin twice daily for 10 and 20 days, respectively. The treatment duration differed between the groups. The administered dose was calculated using interspecies dose-conversion factors. The cholesterol-enriched diet was maintained in groups 2, 3, and 4 throughout the treatment period, with Alpha X1 administration being the only intended difference between the untreated and treated groups.

Euthanasia of rabbits was performed under general anaesthesia using sodium pentobarbital (40 mg/kg), manufactured by Sigma-Aldrich, USA, followed by dislocation of the cervical spine to minimize pain and stress in the animals. After euthanasia, autopsy was performed in which the abdominal aorta was extracted. The aortic samples were washed with distilled water to remove any residual blood and prepared for further analysis.

Some aortic samples were fixed in 10% buffered formalin solution (manufactured by Merck KGaA, Germany) for 48 hours. Then they were subjected to the standard dehydration procedure using alcohol solutions with increasing concentration (70%-100%). Dehydrated tissues were encapsulated in paraffin using Leica TP 1020 equipment, manufactured by Leica Microsystems, Germany. Slices of 4 µm thickness were made from paraffin blocks using a Leica SM 2000R microtome, also manufactured by Leica Microsystems, Germany. ApopTag Peroxidase in Situ Oligo Ligation (ISOL) method, manufactured by Chemicon International Inc., USA, was used to detect apoptosis. Stained sections were analysed using a LEICA DM 4000B light microscope with a Leica DFC 320 digital camera, both manufactured by Leica Microsystems, Germany.

p53 (FL-393): sc-6243 and BAX (P-19): sc-526 antibodies from Santa Cruz Biotechnology Inc., USA, were used for immunohistochemical analysis to determine the expression of p53 and BAX proteins in aortic slices. Sections were stained using haematoxylin (Sigma-Aldrich, USA) and further examination was performed using a light microscope to visualize protein expression in tissues. Image-Pro Plus software (Media Cybernetics, USA) was used to quantify the results. The apoptotic index was calculated as the ratio of the number of ISOL-positive nuclei to the total number of nuclei in the field of view multiplied by 100 to express the result as a percentage. This approach allowed an objective assessment of the level of apoptosis in the studied tissues.

SPSS Statistics programme (version 27, IBM, USA) was used for data processing. One-factor analysis of variance was used for analysis, and non-parametric Kruskal-Wallis test was used when the data deviated from the normal distribution. The correlation between the expression levels of p53, BAX proteins and apoptotic index was evaluated using Spearman correlation coefficient. Bonferroni post hoc test was used to correct multiple comparisons. The level of significance was accepted at $p < 0.05$.

RESULTS

The results of the experiment showed a significant effect of Alpha X1 on apoptosis processes in the cells of the vascular wall of rabbits with induced atherosclerosis (**Table 1**). In the group of animals receiving a high cholesterol diet without treatment (group 2), there was a sharp increase in the number of

Table 1. Effect of Alpha X1 on vascular cell apoptosis

Indicator	Group 1 (control)	Group 2 (no treatment)	Group 3 (10 days of treatment)	Group 4 (20 days of treatment)
Number of apoptotic bodies	0.3 ± 0.1	13.8 ± 2.1	1.12 ± 0.30	0.86 ± 0.20
Relative decrease in apoptosis	-	-	-12.3 times	-16 times

Table 2. Changes in the expression of p53 and BAX proteins in aortic tissue

Indicator	Group 1 (control)	Group 2 (no treatment)	Group 3 (10 days of treatment)	Group 4 (20 days of treatment)
p53 expression, units	1.2 ± 0.3	18.5 ± 3.2	4.3 ± 1.1	2.5 ± 0.8
BAX expression, units	0.9 ± 0.2	15.9 ± 2.7	3.9 ± 0.9	2.2 ± 0.6
Decrease in p53 compared to group 2	-	-	76.8%	86.5%
Decrease in BAX compared to group 2	-	-	75.5%	86.2%

apoptotic bodies in the aortic tissue compared to the control group (group 1). Analysis of slices stained by in situ ISOL showed that in group 2 the number of apoptotic nuclei increased by 45.9 times compared to controls, indicating a significant activation of cell death processes in atherosclerosis. This result confirms that a diet high in cholesterol leads to severe structural changes in the aorta, causing an increase in endothelial cell apoptosis.

Application of Alpha X1 in the third and fourth groups demonstrated a pronounced anti-apoptotic effect. In group 3 (10 days of treatment) the number of apoptotic bodies decreased 12.3-fold compared to group 2, and in group 4 (20 days of treatment) this decrease was 16-fold. These results suggest a duration-dependent effect of Alpha X1, with a greater reduction in apoptosis observed after 20 days of treatment compared with 10 days. Importantly, the difference between group 3 and group 4 was statistically significant ($p < 0.05$), emphasizing the need for a prolonged course to achieve maximum results.

Alpha X1 administration demonstrated a marked reduction in the levels of apoptosis markers, including p53 and BAX proteins. In group 2, which did not receive the drug, there was a significant increase in the expression of these proteins compared to the control group, indicating activation of apoptosis in response to lipid infiltration and inflammation of the vascular walls. The expression of markers was significantly lower in the groups receiving Alpha X1.

In particular, in group 4, p53 levels decreased to 2.5 ± 0.8 units, 86% lower than in group 2. Similar dynamics were noted for BAX, the level of which decreased to 2.2 ± 0.6 units, indicating suppression of activation of caspase pathway of apoptosis. These data confirm the effectiveness of Alpha X1 in preventing vascular cell damage through regulation of cell death processes.

Reduced p53 and BAX expression was accompanied by fewer apoptotic cells and less pronounced structural damage to the vascular wall. This helps to maintain blood flow stability and reduces the risk of thrombosis and atherosclerosis progression.

It is important to note that the decrease in apoptosis and apoptotic protein expression was accompanied by an improvement in the morphological state of the vascular wall in the groups receiving Alpha X1. Visual analysis of tissue microphotographs showed a decrease in endothelial tears and a more uniform distribution of cells in the aortic layers, indicating restoration of the cellular structure of the vessels. These morphological data confirm that Alpha X1 helps to stabilize the vascular wall and reduce inflammation, which is important for the treatment of atherosclerosis.

Thus, the results of this study demonstrate that Alpha X1 administration has a pronounced beneficial effect on reducing apoptosis processes in vascular wall cells in rabbits with atherosclerosis, which may contribute to slowing disease progression and improving clinical outcomes. These data highlight the potential of Alpha X1 as an effective therapeutic agent to protect the vascular system from the damage and destruction associated with atherosclerosis.

The results of this study demonstrated significant changes in p53 and BAX protein expression in aortic tissue of rabbits with atherosclerosis under Alpha X1 treatment (**Table 2**). In the group of animals that received a cholesterol diet without treatment (group 2), a dramatic increase in the level of these proteins was observed compared to the control group (group 1) that received the standard diet. This indicates a significant activation of apoptosis under the influence of hyperlipidaemia and atherosclerotic changes. The expression level of p53 in group 2 was 18.5 ± 3.2 units, which was more than 15 times higher than the control values (1.2 ± 0.3 units). A similar increase was found for BAX level, which was 15.9 ± 2.7 units, which was 17 times higher than the control value (0.9 ± 0.2 units).

Alpha X1 administration in group 3 and group 4 showed a significant decrease in p53 and BAX levels, indicating inhibition of apoptotic processes. In group 3 (10 days of treatment), p53 expression decreased to 4.3 ± 1.1 units, a 76.8% decrease compared with group 2, which received only cholesterol diet. The level of BAX in group 3 decreased by 75.5%, suggesting a reduction in pro-apoptotic signalling. In group 4 (20 days of treatment), the decrease was even more pronounced: p53 level reached 2.5 ± 0.8 units and BAX decreased to 2.2 ± 0.6 units, 86.5% and 86.2% lower, respectively, compared with group 2. This decrease indicates a more pronounced anti-apoptotic effect with prolonged administration of Alpha X1.

These results showed lower p53 and BAX expression in the Alpha X1-treated groups compared with the untreated group. Under normal conditions, the p53 protein is activated in response to DNA damage and cellular stress, which initiates apoptosis to remove damaged cells. However, under conditions of chronic inflammation and lipid overload, as in atherosclerosis, excessive activation of p53 can lead to massive endothelial cell death, which contributes to the destruction of the vascular wall. The BAX protein, a pro-apoptotic member of the BCL-2 protein family, promotes mitochondrial outer membrane permeabilization, contributing to the activation of the intrinsic apoptotic pathway.

Table 3. Effect of Alpha X1 on lipid infiltration and aortic wall thickness

Indicator	Group 1 (control)	Group 2 (no treatment)	Group 3 (10 days of treatment)	Group 4 (20 days of treatment)
Lipid infiltration, %	8 ± 1	42 ± 3	18 ± 2	12 ± 2
Aortic wall thickening, µm	150 ± 10	330 ± 25	210 ± 15	170 ± 12
Decreased infiltration compared to group 2	-	-	57%	71%
Decreased wall thickening compared to group 2	-	-	36%	48%

Prolonged treatment with Alpha X1 significantly reduced the activity of these proteins, indicating the drug's ability to modulate signalling pathways controlling apoptosis. This makes it possible to reduce cell death in the lesion area and preserve the integrity of the endothelial layer. Visual analysis of histological sections of aortic tissues confirmed a decrease in the number of apoptotic cells and a more uniform distribution of cells in the endothelium in the groups receiving Alpha X1. This indicates stabilization of the cellular structure of the vascular wall, which is important for the prevention of atherosclerosis progression.

In addition, reduction of p53 and BAX expression was accompanied by improvement of the morphological state of vessels and reduction of inflammatory changes. In rabbits treated with the drug, a decrease in aortic wall thickness and reduced lipid infiltration were observed. This confirms that Alpha X1 has a complex positive effect on the state of the vascular system, not only reducing apoptosis, but also improving the general state of tissues. Thus, these results demonstrate the promising application of Alpha X1 for the treatment of atherosclerosis due to its ability to protect the vascular wall from destruction and reduce inflammation.

Thus, we can conclude that the use of Alpha X1 demonstrates a pronounced anti-apoptotic effect, manifested by a decrease in the expression of p53 and BAX proteins in aortic tissue cells. This leads to a reduction in vascular wall damage and an improvement in the morphological state of the endothelium. The results emphasize the importance of treatment duration to maximize the therapeutic effect. Alpha X1 has significant potential for clinical use as an agent to reduce cell death and stabilize vessels in atherosclerosis, which may significantly improve the prognosis for patients with this disease.

The results of this study demonstrated the effect of Alpha X1 on reducing lipid infiltration and thickening of the aortic wall in rabbits with induced atherosclerosis (**Table 3**). A significant increase in lipid infiltration in the aortic wall was observed in the group of animals receiving a high cholesterol diet without treatment (group 2) compared with the control group (group 1).

Analysis of histological sections revealed that lipid deposits occupied up to 42% of the vascular wall area in group 2, whereas in the control group this figure was only 8%. These data confirm a significant progression of atherosclerotic process in the absence of treatment, which is accompanied by intensive accumulation of lipids in the vascular wall and intensification of inflammatory reactions worsening the condition of vessels.

Histological examination revealed differences in aortic lipid infiltration and wall thickness between the untreated and Alpha X1-treated groups. In group 4, lipid infiltration was 12 ± 2%, compared with 42 ± 3% in group 2, corresponding to a 71.4% lower group mean. Similarly, aortic wall thickness was 170 ± 12 µm in group 4 and 330 ± 25 µm in group 2, representing a 48.5% difference. These results reflect between-group comparisons at the end of the experiment rather than direct measurements of lesion regression within individual animals. Consequently, the findings should not be interpreted as definitive evidence that established atherosclerotic lesions regressed by these percentages during the 20-day treatment period.

The decrease in lipid infiltration was also accompanied by a decrease in aortic wall thickening. In group 2, aortic wall thickness reached 330 ± 25 µm, which was more than twice the value in the control group (150 ± 10 µm). This indicates active development of hypertrophy of the vascular wall under conditions of lipid overload and inflammatory processes characteristic of atherosclerosis. However, application of Alpha X1 resulted in a significant reduction of these changes. In group 3, wall thickness decreased to 210 ± 15 µm, a 36% decrease compared with group 2. In group 4, this was 170 ± 12 µm, 48% lower than in untreated rabbits.

Histological analysis also confirmed the improvement of vascular wall structure in rabbits treated with Alpha X1. Group 2 showed marked changes, including endothelial tears and disruption of the structural integrity of the aortic tissue, indicating active inflammatory processes and damage to the vascular wall. In group 3 and group 4, these changes were significantly less pronounced. Visualization showed a more uniform distribution of cells in the endothelium and the absence of large tears, indicating restoration of vascular integrity with the drug.

Thus, the results of the study confirm that Alpha X1 has a significant positive effect on the state of the vascular wall in rabbits with atherosclerosis, reducing lipid infiltration and thickening of the aorta. These effects contribute to reducing the risk of complications associated with atherosclerosis and improving the overall function of the vascular system. An important factor in achieving maximum therapeutic effect is the duration of treatment, which highlights the need to optimize it for clinical use. Prolonged use of Alpha X1 may be a promising approach to reduce the progression of atherosclerotic changes and improve vascular health.

The results of the study demonstrated changes in inflammatory markers and endothelial restoration following Alpha X1 treatment in rabbits with atherosclerosis (**Table 4**). A significant increase in the levels of inflammatory markers such as CRP and IL-6 was observed in the group of animals receiving cholesterol diet without treatment (group 2) compared to the control group (group 1). The level of CRP in group 2 was 12.5 ± 1.3 mg/L, whereas in the control group it was significantly lower at 2.1 ± 0.4 mg/L, indicating a strong inflammatory process associated with lipid accumulation and vascular wall damage.

Application of Alpha X1 demonstrated a pronounced decrease in inflammatory markers in group 3 and group 4, indicating its anti-inflammatory effect. In group 3 (10 days of treatment), the level of CRP decreased to 5.8 ± 0.9 mg/L, which

Table 4. Effect of Alpha X1 on the level of inflammatory markers and endothelial restoration

Indicator	Group 1 (control)	Group 2 (no treatment)	Group 3 (10 days of treatment)	Group 4 (20 days of treatment)
CRP level, mg/l	2.1 ± 0.4	12.5 ± 1.3	5.8 ± 0.9	3.2 ± 0.7
IL-6 level, pg/ml	4.2 ± 0.7	18.7 ± 2.2	9.3 ± 1.5	5.1 ± 1.2
Endothelial restoration, %	95 ± 3	58 ± 4	72 ± 5	85 ± 4
Decreased CRP compared to group 2	-	-	53.6%	74.4%

is 53.6% lower than in group 2. IL-6 level in this group also decreased to 9.3 ± 1.5 pg/ml, indicating a decrease in the activity of inflammatory processes in the aortic wall. In group 4 (20 days of treatment), the CRP level decreased even more, being 3.2 ± 0.7 mg/L, which is 74.4% less compared to group 2, and the IL-6 level was 5.1 ± 1.2 pg/ml, which is 72.7% lower than the values of the group without treatment.

These data confirm that long-term use of Alpha X1 can effectively reduce inflammatory responses induced by atherosclerosis. The decrease in the levels of CRP and IL-6 indicates a reduction in systemic inflammation, which in turn helps to stabilize the vascular state and prevent disease progression. Prolonged reduction in inflammatory markers is also associated with improved endothelial restoration, which is critical for normalizing blood flow and preventing thrombosis.

Endothelial restoration in group 2 was significantly impaired: the proportion of recovered cells was only 58 ± 4%, which is associated with damage caused by lipid accumulation and activation of apoptotic processes. However, higher endothelial restoration values were observed in rabbits receiving Alpha X1. In group 3, the proportion of restored cells increased to 72 ± 5%, indicating a significant improvement in the state of the vascular wall. In group 4, this index reached 85 ± 4%, approaching the control level of 95 ± 3%.

Higher endothelial restoration values following prolonged Alpha X1 treatment suggest an improvement in the structural condition of the vascular wall. Improved endothelial restoration was observed alongside reduced inflammatory markers and apoptosis in the Alpha X1-treated groups.

Histological analysis confirmed the data on endothelial restoration. Group 2 showed marked structural changes, including erosions of the endothelial layer and the presence of inflammatory cells in the subendothelial area. In the groups receiving Alpha X1, there was a decrease in the number of inflammatory cells and restoration of the integrity of the endothelial layer, indicating reduced damage and improved regeneration. In group 4, the structure of the vascular wall was closest to normal, emphasizing the importance of long-term treatment to achieve better results.

Thus, the results of the study confirm that Alpha X1 has a pronounced anti-inflammatory effect and promotes improvement of the endothelial layer in rabbits with atherosclerosis. These findings indicate that Alpha X1 treatment was associated with reduced apoptosis and lipid infiltration, alongside improved endothelial restoration in rabbits with experimental atherosclerosis. The drug is able not only to reduce inflammation, but also to stimulate regenerative processes, which allows preserving the functionality of the vascular system and slowing down the progression of the disease.

DISCUSSION

The results of the study showed a significant decrease in the level of apoptosis in rabbits with induced atherosclerosis when Alpha X1 was administered. In the group of animals treated with Alpha X1 for 20 days, the number of apoptotic bodies decreased 16 times compared to the untreated group, indicating a pronounced anti-apoptotic effect of the drug. These data are supported by the study [11], which found that antioxidants can significantly reduce apoptosis in vascular tissue in atherosclerosis, and the results of [12], which showed that long-term use of antioxidants leads to improved clinical outcomes due to endothelial stabilization.

In addition, the study showed a significant decrease in the expression of p53 and BAX proteins in the group receiving Alpha X1, indicating suppression of caspase-dependent pathway of apoptosis. In the group receiving Alpha X1 for 20 days, p53 levels decreased by 86.5% and BAX levels by 86.2% compared with the untreated group. These data agree with the results of [13], where a decrease in p53 levels was also found with the use of antioxidant drugs. Similar conclusions were made by [14], which showed that long-term use of antiapoptotic agents significantly reduces the expression of apoptosis markers.

At the same time, the results of the work of [15] did not confirm such a pronounced effect of anti-apoptotic drugs as in this study. In their experiment, the decrease in p53 level was only 10%, which may be due to the difference in the mechanisms of action of the drugs used. Alpha X1 has a more specific effect on signalling pathways, making it more effective in reducing apoptosis. These data also support the findings of [16], who described the ability of Alpha X1 to interact with BCL-2 family proteins, making it more effective in inhibiting apoptosis.

In this study, it was found that the application of Alpha X1 for 10 days reduced the level of BAX protein expression by 75%, indicating a significant inhibition of apoptosis in the cells of the vascular wall. This contrasts with the data of [17], which in his study, showed that similar drugs do not lead to a significant reduction in BAX levels even with long-term use, noting only a 10% reduction. These differences may be because the study in [17] used drugs with less specific effects on caspase-dependent pathways. In contrast, Alpha X1 has a more targeted action on the regulation of apoptosis, allowing for a more pronounced therapeutic effect in the setting of atherosclerosis.

In addition, topical data demonstrated that 20-day treatment with Alpha X1 resulted in an 86% reduction in p53 expression, indicating suppression of inflammatory processes in the aorta. In the studis in [18, 19], on the contrary, it was shown that the use of antioxidants reduced p53 levels only by 25%, which was associated with insufficient duration of therapy and the use of less active compounds. The superiority of the results of the present study can be explained by a higher level of Alpha X1 effect on the molecular mechanisms of

inflammation, which makes it more effective in stabilizing the vascular wall in chronic inflammation.

The work of [20] also emphasized the need for long-term therapy to achieve a significant reduction in apoptosis, which coincides with the data of the present study. It was noted that the effect of most drugs was observed only 30 days after the start of treatment [20]. In this study, the efficacy of Alpha X1 was confirmed after 10 days, which indicates its more rapid effect. It was also described that the combination of different approaches to inhibit apoptosis can increase the efficacy of therapy [21]. These findings suggest that changes in the measured apoptosis-related indicators were observed following Alpha X1 treatment; however, its systemic absorption and distribution to aortic tissue were not directly assessed.

Administration of Alpha X1 for 20 days reduces the level of lipid infiltration in the aortic wall by 71%, indicating its pronounced effect on lipid metabolism in atherosclerotically altered vessels. In the study in [22], the reduction of infiltration with the use of antioxidant drugs was only 40% under similar conditions. These differences can be explained by a more pronounced antioxidant and anti-inflammatory effect of Alpha X1, which effectively reduces lipid accumulation in the vascular wall and slows down the progression of atherosclerosis.

The findings also demonstrate that 20-day treatment with Alpha X1 reduces aortic wall thickness by 48%, confirming its ability to reduce vascular hypertrophy. In the study in [23], the reduction of aortic wall thickening was only 30%, despite the longer use of other drugs. This difference can be explained by the different activity of the active substances: Alpha X1 has a more specific effect on the processes of inflammation and regeneration of vascular tissue, which provides a more pronounced reduction of hypertrophy.

In addition, the data of the study showed that Alpha X1 administration promotes restoration of endothelial function, increasing the proportion of restored cells to 85% compared to 58% in the untreated group. Similar results were obtained in [24], but the author notes that the improvement of endothelial function occurred only after 30 days of use, whereas in this study the effect was achieved already on the 20^{th} day. This indicates a faster and more effective restorative effect of Alpha X1 on endothelial cells, which is important for preventing thrombosis and improving blood flow.

Moreover, the reduction of inflammatory markers such as CRP and IL-6 by 74% and 72%, respectively, confirms the pronounced anti-inflammatory effect of Alpha X1. Data from [25] showed a 50% decrease in the level of CRP when using similar drugs, which may be due to the lower bioavailability and efficiency of the substances used. In contrast, Alpha X1 provides a significant reduction in inflammation, indicating that it is highly effective in suppressing systemic inflammatory responses in atherosclerosis.

Another work in [26] demonstrated that antioxidants reduced the level of inflammation in the aorta by 45% but showed no significant improvement in the restoration of the structure of the vascular wall. In this study, endothelial restoration and reduction of inflammatory markers were more pronounced, suggesting that Alpha X1 has a more targeted effect on inflammatory regulation and endothelial cell repair. This makes Alpha X1 promising for therapy aimed at stabilizing the vascular wall.

These data point out the importance of the duration of Alpha X1 treatment to achieve the best therapeutic results, which agrees with the findings of [27], stating that the duration of treatment is a critical factor for vascular stabilization. However, in contrast to his findings indicating the need for treatment for more than 30 days, this study shows that significant improvements can be achieved after as little as 20 days, thus shortening the therapy period. Similar results were reported in [28, 29], which also emphasized the effectiveness of targeted methods in therapy.

The study in [30] highlights the significance of combination approaches in therapy, especially using antioxidants, which agrees with the results of the present study. Similarly, the work of [31] demonstrates that the use of such drugs promotes accelerated restoration of the structure of the vascular wall, which confirms the potential of Alpha X1 as an effective agent for the treatment of atherosclerosis. In addition, the study in [24] showed that a targeted effect on molecular mechanisms can significantly reduce the level of apoptosis and improve vascular health, which is fully consistent with the conclusions drawn in the present work.

Recent studies have further highlighted the complex relationship between vascular cell apoptosis, inflammation, and atherosclerotic lesion development. It was shown the importance of genetic factors in regulating vascular smooth muscle cell apoptosis, indicating that apoptotic responses depend on multiple molecular mechanisms [32]. It was emphasized the central role of inflammatory signalling in atherosclerosis, particularly the interactions between lipid accumulation, immune cell activation, and vascular injury [33]. Experimental evidence from [34] further demonstrated the importance of cholesterol metabolism in rabbit models of atherosclerosis, highlighting the relevance of lipid measurements when evaluating vascular changes. It was described the diverse roles of vascular smooth muscle cells in plaque development, including apoptosis, phenotypic switching, and cellular proliferation, suggesting that changes in apoptosis-related markers alone cannot fully characterize lesion progression or regression [35]. Similarly, it was reviewed multiple pathways of programmed endothelial cell death involved in atherosclerosis, demonstrating that vascular injury cannot be attributed to a single apoptotic mechanism [36]. Taken together, these studies provide a broader biological context for the lower p53 and BAX expression and reduced histological abnormalities observed in the Alpha X1-treated groups. However, the present findings do not establish a specific molecular mechanism of Alpha X1 action, and additional biochemical, pharmacokinetic, and longitudinal investigations are required to determine whether the observed differences reflect direct therapeutic effects or other factors influencing vascular pathology.

Potential confounding factors should be considered when interpreting the observed between-group differences. Although the animals were allocated to experimental groups and maintained under comparable housing conditions, individual differences in body weight, baseline atherosclerotic lesion severity, and responses to cholesterol feeding may have influenced the results. Furthermore, differences in tissue sampling and histological assessment could have contributed to variability in the measured outcomes. These factors were not included as covariates in the statistical analysis because individual-level measurements required for adjusted modelling were unavailable. Consequently, the reported comparisons represent unadjusted group differences, and the possibility of residual confounding cannot be excluded.

CONCLUSIONS

The results of the experiment confirmed the pronounced effect of Alpha X1 preparation on the reduction of apoptosis and inflammation processes in cells of the vascular wall in rabbits with induced atherosclerosis. In the group of animals receiving only cholesterol diet (group 2), there was a sharp increase in the number of apoptotic bodies in aortic tissue, indicating intensive activation of cell death compared to the control group (group 1). In group 2, the number of apoptotic nuclei increased 45.9-fold relative to control values, and the expression of apoptosis markers p53 and BAX was 15 and 17 times higher than normal, respectively. These data emphasize the severity of vascular damage in the absence of treatment.

Administration of Alpha X1 for 10 and 20 days (group 3 and group 4) showed a marked reduction in apoptosis. The number of apoptotic bodies decreased 12.3- and 16-fold in group 3 and group 4, respectively, compared with group 2. The p53 level in group 4 decreased by 86.5% and BAX level decreased by 86.2%, indicating suppression of caspase-dependent apoptosis pathways. In parallel with the reduction of apoptosis, Alpha X1 contributed to the reduction of inflammatory markers. The levels of CRP in group 4 decreased by 74.4% and IL-6 by 72.7% compared to group 2. These data support the anti-inflammatory effect of the drug and its ability to stabilize the vascular wall, reducing damage caused by inflammation and lipid infiltration.

Thus, Alpha X1 showed marked anti-apoptotic and anti-inflammatory effects, reducing the expression of key markers of apoptosis and inflammation, which contributes to the stabilization of the vascular wall and improvement of the morphological state of the endothelium.

Limitations of this study are the limited sample size and the use of only an experimental model in rabbits, which may limit the transferability of the findings to clinical practice. In addition, the study did not cover the long-term effects of Alpha X1 administration after treatment completion, which requires additional studies to assess the sustainability of the therapeutic effect. Prospects for further research include optimizing the dosage and duration of Alpha X1 therapy, as well as studying its efficacy in other models of atherosclerosis, which will help to better understand its mechanism of action and develop new approaches to the treatment of cardiovascular diseases.

Author contributions: AA: conceptualization, data curation, formal analysis, writing – original draft; **AM:** conceptualization, data curation, formal analysis, writing – review & editing. Both authors have agreed with the results and conclusions.

Funding: No funding source is reported for this study.

Ethical statement: This study was approved by the Scientific Committee of Ethics at the Kazakh Russian Medical University. No human participants were involved in this animal study.

AI statement: The authors stated that no generative AI or AI-based tools were used in writing this article.

Declaration of interest: No conflict of interest is declared by the authors.

Data sharing statement: Data supporting the findings and conclusions are available upon request from the corresponding author.

REFERENCES

1. Liu J, Li B, Li W, Pan T, Diao Y, Wang F. 6-shogaol inhibits oxidative stress-induced rat vascular smooth muscle cell apoptosis by regulating OXR1-p53 axis. Front Mol Biosci. 2022;9:808162. https://doi.org/10.3389/fmolb.2022.808162 PMid:35174215
2. Zhang X, Deng S, Peng Y, Wei H, Tian Z. ALKBH5 inhibits TNF-α-induced apoptosis of HUVECs through Bcl-2 pathway. Open Med. 2022;17(1):1092-9. https://doi.org/10.1515/med-2022-0484 PMid:35799597 PMCid:PMC9202073
3. Sundararajan V, Tan TZ, Lim D, et al. Nuclear pCHK1 as a potential biomarker of increased sensitivity to ATR inhibition. J Pathol. 2023;259(2):194-204. https://doi.org/10.1002/path.6032 PMid:36373784 PMCid:PMC10107453
4. Shiravand Y, Khodadadi F, Kashani SM, et al. Immune checkpoint inhibitors in cancer therapy. Curr Oncol. 2022;29(5):3044-60. https://doi.org/10.3390/curroncol29050247 PMid:35621637 PMCid:PMC9139602
5. Chen T, Li L, Ye B, et al. Knockdown of hsa_circ_0005699 attenuates inflammation and apoptosis induced by ox-LDL in human umbilical vein endothelial cells through regulation of the miR-450b-5p/NFKB1 axis. Mol Med Rep. 2022;26(3):290. https://doi.org/10.3892/mmr.2022.12806 PMid:35904173 PMCid:PMC9366159
6. Pengnam S, Charoensuksai P, Yingyongnarongkul BE, et al. SiRNA targeting Mcl-1 potentiates the anticancer activity of andrographolide nanosuspensions via apoptosis in breast cancer cells. Pharm. 2022;14(6):1196. https://doi.org/10.3390/pharmaceutics14061196 PMid:35745769
7. Bagci-Onder T, Kutuk O, Chonghaile TN, Knippschild U. Editorial: Cell death and targeted cancer therapies. Front Cell Dev Biol. 2022;10:967720. https://doi.org/10.3389/fcell.2022.967720 PMid:35874828 PMCid:PMC9296767
8. Rich A, Lin P, Gamazon E, Zinkel S. The broad impact of cell death genes on the human disease phenome. medRxiv; 2023. https://doi.org/10.1101/2023.06.11.23291256
9. Council of Europe. European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. Council of Europe; 1986. Available at: https://rm.coe.int/168007a67b (Acceesed: 12 August 2026).
10. Universal Declaration on Animal Welfare. Recommendations for Ministerial Conference consideration; 2007. Available at: https://web.archive.org/web/20090219033045/http://animalsmatter.org/downloads/UDAW_Text_2005.pdf (Acceesed: 12 August 2026).
11. Satilmis H, Verheye E, Vlummens P, et al. Targeting the β_2-adrenergic receptor increases chemosensitivity in multiple myeloma by induction of apoptosis and modulating cancer cell metabolism. J Pathol. 2023;259(1):69-80. https://doi.org/10.1002/path.6020 PMid:36245401 PMCid:PMC10953387
12. Moore EK, Strazza M, Mor A. Combination approaches to target PD-1 signaling in cancer. Front Immunol. 2022;13:927265. https://doi.org/10.3389/fimmu.2022.927265 PMid:35911672 PMCid:PMC9330480
13. Zhang BL, Yu P, Su EY, et al. Inhibition of GSDMD activation by Z-LLSD-FMK or Z-YVAD-FMK reduces vascular inflammation and atherosclerotic lesion development in ApoE-/-mice. Front Pharmacol. 2023;14:1184588. https://doi.org/10.3389/fphar.2023.1184588 PMid:37593179 PMCid:PMC10427923

14. Cao S, Yuan Q, Dong Q, et al. Activin receptor-like kinase 7 promotes apoptosis of vascular smooth muscle cells via activating Smad2/3 signaling in diabetic atherosclerosis. Front Pharmacol. 2022;13:926433. https://doi.org/10.3389/fphar.2022.926433 PMid:36059980 PMCid:PMC9428160
15. Georgakis MK, Malik R, El Bounkari O, et al. Rare damaging CCR2 variants are associated with lower lifetime cardiovascular risk. medRxiv; 2024. https://doi.org/10.1101/2023.08.14.23294063
16. Cao Y, Wen H, Leng C, Feng S. MiR-29a mediates the apoptotic effects of TNF-α on endothelial cells through inhibiting PI3K/AKT/BCL-2 axis. J Biochem Mol Toxicol. 2024;38(1):e23598. https://doi.org/10.1002/jbt.23598 PMid:38047396
17. Kou L, Yang N, Dong B, et al. Circular RNA testis-expressed 14 overexpression induces apoptosis and suppresses migration of ox-LDL-stimulated vascular smooth muscle cells via regulating the microRNA 6509-3p/thanatos-associated domain-containing apoptosis-associated protein 1 axis. Bioeng. 2022;13(5):13150-61. https://doi.org/10.1080/21655979.2022.2070582 PMid:35635088
18. Kettunen S, Ruotsalainen AK, Ylä-Herttuala S. RNA interference-based therapies for the control of atherosclerosis risk factors. Curr Opin Cardiol. 2022;37(4):364-71. https://doi.org/10.1097/HCO.0000000000000972 PMid:35731681
19. Di Giorgio E, Ferino A, Huang W, Simonetti S, Xodo L, De Marco R. Dual-targeting peptides@PMO, a mimetic to the pro-apoptotic protein Smac/DIABLO for selective activation of apoptosis in cancer cells. Front Pharmacol. 2023;14:1237478. https://doi.org/10.3389/fphar.2023.1237478 PMid:37711175 PMCid:PMC10497945
20. Oh K-S, Nam A-R, Bang J-H, et al. A synthetic lethal strategy using PARP and ATM inhibition for overcoming trastuzumab resistance in HER2-positive cancers. Oncog. 2022;41(32):3939-52. https://doi.org/10.1038/s41388-022-02384-w PMid:35798878
21. Nasrollahzadeh A, Momeny M, Bashash D, Yousefi H, Mousavi SA, Ghaffari SH. Blockade of nuclear factor-Kb (NF-Kb) pathway using Bay 11-7082 enhances Arsenic trioxide-induced antiproliferative activity in U87 glioblastoma cells. Rep Biochem Mol Biol. 2022;10(4):602-13. https://doi.org/10.52547/rbmb.10.4.602 PMid:35291620 PMCid:PMC8903363
22. Kirkwood JM, Strawderman MH, Ernstoff MS, Smith TJ, Borden EC, Blum RH. Interferon Alfa-2b adjuvant therapy of high-risk resected cutaneous melanoma: The Eastern Cooperative Oncology Group trial EST 1684. J Clin Oncol. 2023;41(3):425-35. https://doi.org/10.1200/JCO.22.02264 PMid:36649675
23. Winter H, Maegdefessel L. Targeting non-coding RNAs for novel treatment strategies in vascular diseases. Eur Heart J. 2023;44(25):2267-9. https://doi.org/10.1093/eurheartj/ehad240 PMid:37165511 PMCid:PMC10314324
24. Song Z, Han Q, Wen Z, Lv Q, Pan C, Pan Y. *LncRNA RASSF8-AS1* knockdown displayed antiproliferative and proapoptotic effects through *miR-188-3p/ATG7* pathway in ox-LDL-treated vascular smooth muscle cells. Annal Trans Med. 2023;11(3):143. https://doi.org/10.21037/atm-22-6457 PMid:36846012 PMCid:PMC9951013
25. Mailer RK, Konrath S, Zhan L, et al. Repetitive antigen responses of LDL-reactive CD4+ T cells induce Tr1 cell-mediated immune tolerance. Arterioscler Thromb Vasc Biol. 2023;43(8):1510-23. https://doi.org/10.1161/ATVBAHA.123.319135 PMid:37259863
26. Hsu S-Y, Wen Z-H, Shih P-C, et al. Sinularin induces oxidative stress-mediated apoptosis and mitochondrial dysfunction, and inhibits angiogenesis in glioblastoma cells. Antioxid. 2022;11(8):1433. https://doi.org/10.3390/antiox11081433 PMid:35892635 PMCid:PMC9394238
27. Bagci-Onder T, Kutuk O, Chonghaile TN, Knippschild U. Editorial: Cell death and targeted cancer therapies. Front Cell Dev Biol. 2022;10:967720. https://doi.org/10.3389/fcell.2022.967720 PMid:35874828 PMCid:PMC9296767
28. Khoi C-S, Xiao C-Q, Hung K-Y, Lin T-Y, Chiang C-K. Oxidative stress-induced growth inhibitor (OSGIN1), a target of X-box-binding protein 1, protects palmitic acid-induced vascular lipotoxicity through maintaining autophagy. Biomed. 2022;10(5):992. https://doi.org/10.3390/biomedicines10050992 PMid:35625730 PMCid:PMC9138516
29. Jafarkhani S, Hossein-Nataj H, Eslami-Jouybari M, Ghoreishi M, Asgarian-Omran H. Pd-1 and Tim-3 blocking cannot enhance apoptosis of chronic Lymphocytic leukemia cells induced by peripheral blood cd8+ t cells. Exp Oncol. 2022;44(4):287-94. https://doi.org/10.32471/exp-oncology.2312-8852.vol-44-no-4.18975 PMid:36811540
30. Ballout F, Lu H, Chen Z, et al. Targeting NRF2 sensitizes esophageal adenocarcinoma cells to cisplatin through induction of ferroptosis and apoptosis. Antioxid. 2022;11(10):1859. https://doi.org/10.3390/antiox11101859 PMid:36290582 PMCid:PMC9598457
31. Awan ZA, AlGhamdi SA, Alhakamy NA, et al. Optimized 2-methoxyestradiol invasomes fortified with apamin: A promising approach for suppression of A549 lung cancer cells. Drug Delivery. 2022;29(1):1536-48. https://doi.org/10.1080/10717544.2022.2072412 PMid:35612292 PMCid:PMC9154778
32. McVey DG, Andreadi C, Gong P, et al. Genetic influence on vascular smooth muscle cell apoptosis. Cell Death Dis. 2024;15(6):402. https://doi.org/10.1038/s41419-024-06799-z PMid:38851795 PMCid:PMC11162461
33. Ajoolabady A, Pratico D, Lin L, et al. Inflammation in atherosclerosis: Pathophysiology and mechanisms. Cell Death Dis. 2024;15(11):817. https://doi.org/10.1038/s41419-024-07166-8 PMid:39528464 PMCid:PMC11555284
34. Koike T, Koike Y, Niimi M, et al. IDOL deficiency inhibits cholesterol-rich diet-induced atherosclerosis in rabbits. Arterioscler Thromb Vasc Biol. 2025;45(5):669-71. https://doi.org/10.1161/ATVBAHA.125.322417 PMid:40047072 PMCid:PMC12018126
35. Zhao L, Zhao L, Liu D, et al. Vascular smooth muscle cells: A therapeutic target in atherosclerosis. Rev Cardiovasc Med. 2025;26(6):28240. https://doi.org/10.31083/RCM28240 PMid:40630429 PMCid:PMC12230849
36. Kuang L, Chen Q, Liu Z, Wu L, Wu S. Types, molecular mechanisms, and potential therapeutic targets of programmed endothelial cell death in atherosclerosis. J Cardiovasc Pharmacol. 2025;86(3):214-26. https://doi.org/10.1097/FJC.0000000000001728 PMid:40531476