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RESEARCH ARTICLE

The Anti-Inflammatory Effects of Anacardic Acid on a TNF-α - Induced Human Saphenous Vein Endothelial Cell Culture Model

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Abstract: ***Background and Objective*:** Coronary bypass operations are commonly performed for the treatment of ischemic heart diseases. Coronary artery bypass surgery with autologous human saphenous vein maintains its importance as a commonly used therapy for advanced atherosclerosis. Vascular inflammation-related intimal hyperplasia and atherosclerotic progress have major roles in the pathogenesis of saphenous vein graft disease.

***Methods*:** In our study, we investigated the effect of anacardic acid (AA), which is a bioactive phytochemical in the shell of *Anacardium occidentale*, on atherosclerosis considering its inhibitory effect on NF-κB. We observed relative ICAM-1 and NF-κB mRNA levels by qRT-PCR method in a TNF-α-induced inflammation model of saphenous vein endothelial cell culture after 0.1, 0.5, 1 and 5 μM of AA were applied to the cells. In addition, protein levels of ICAM-1 and NF-κB were evaluated by immunofluorescent staining. The results were compared between different concentrations of AA, and also with the control group.

***Results*:** It was found that 5 μM, 1 μM and 0.5 μM of AA had toxic effects, while cytotoxicity decreased when 0.1 μM of AA was applied both alone and with TNF-α. When AA was applied with TNF-α, there was a decrease and suppression in NF-κB expression compared with the TNF-α group. TNF-α-induced ICAM-1 expression was significantly reduced more in the AA-applied group than in the TNF-α group.

***Conclusion*:** In accordance with our results, it can be said that AA has a protective role in the pathogenesis of atherosclerosis and hence in saphenous vein graft disease.

Keywords: Coronary bypass, anacardic acid, saphenous vein, atherosclerosis, inflammation, NF-κB.

1. INTRODUCTION

Atherosclerosis is defined as a chronic inflammatory disease that is characterized by narrowing of the vessel lumen by atherosclerotic plaques as a result of thickening and hardening of the mid-sized or large-sized arteries and to which cellular and humoral immune responses contribute. Ross claims that even local inflammation due to a rupture in endothelial injury can be important at the onset of atherosclerosis [1]. Intercellular Adhesion Molecule-1 (ICAM-1), one of the adhesion molecules, plays an important role in this pathway and facilitates the adherence of leukocytes to endothelial cells [2]. In cell culture studies, it has been shown that the expression of many adhesion molecules associated with blood cells and endothelia, such as ICAM-1 and Vascular Cell Adhesion Molecule-1 (VCAM-1) is regulated by Nuclear Factor-Kappa B (NF-κB).

Activation of NF-κB in endothelial cells plays an important role especially in the early stages of atherosclerosis [3, 4]. Tumor Necrosis Factor-α (TNF-α) is a proinflammatory cytokine that stimulates the ICAM-1 expression in different types of cells by activation of the NF-κB pathway [5].

6-pentadecyl salicylic acid, also known as Anacardic Acid (AA), is a natural product found in cashew peel shells [6]. AA is predicted to provide good protection for many pathophysiological disorders, from oxidative damage to

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cancer [7]. AA has been reported to show its effect by inhibiting NF-κB kinase, which is the inhibitory subunit of NF-κB that is activated by inflammation, cytokines or tumor promoters. This inhibition leads to the suppression of gene products regulated by NF-κB. AA can also induce the inhibition of gene products, such as MMP-9 and ICAM-1, associated with invasion [8].

Coronary artery bypass surgery using an autologous human saphenous vein is a commonly used treatment method of advanced atherosclerosis [9]. Although the outcomes of the internal thoracic artery patency rate are better, the saphenous vein is still widely preferred in bypass surgery because of being easy to work with, being available in many patients, being convenient for most of the anastomoses, and, being easy to obtain [10, 11]. The most dominant lesion outcome of the coronary artery bypass graft in the following year is atherosclerosis, which causes vein occlusion and leads to the recurrence of ischemic symptoms [12].

In our study, inflammatory atherosclerosis in human saphenous vein endothelial cells (HSaVECs) was established by TNF-α-induced stimulation, and thus the possible effects of AA on inflammation were investigated. In addition, we aimed to determine the expression levels of ICAM-1 and NF-κB that is associated with atherosclerosis and demonstrate the location and levels of target proteins by immunofluorescent labelling.

2. MATERIALS AND METHODS

2.1. Cells and Reagents

In our study, HSaVECs (C-12231) and growth medium (C-22010) were used (Promocell Heidelberg, GE). Type-I collagen and TNF-α were obtained from Thermo Scientific (Waltham, MA, USA), and AA was obtained from SCBT (Dallas, TX, USA). Anti-ICAM-1 (ab20144) and anti-NF-κB (ab32536) that were used in immunofluorescent staining were obtained from Abcam (Cambridge, UK). The Life Tech TaqMan® Gene Expression Cells-to-CT™ (Carlsbad, CA, USA) was used for the qRT-PCR process. Cytotoxicity measurements were performed using the cytotoxicity detection kit (Roche Applied Science Mannheim, GE).

2.2. Human Saphenous Vein Endothelial Cell Culture

The HSaVECs, purchased in cryo-tubes, were dissolved in Promocell endothelial cell growth medium. Once the 25 cm^2-culture flasks were pre-coated with Type 1 collagen and washed with PBS; the flasks were prepared for culture after adding the 4.5 mL of the medium into each flask. Then, the cell suspension was transferred to the flasks at 10^4 cells per cm^2 each. The cells were allowed to multiply in an incubator containing 5% CO_2 at 37°C. The proliferated cells were counted using a hemocytometer before passaging and were transferred into collagen-coated 6-, 12- or 24-well plates at 6,000 cells per cm^2.

2.3. Applications on the Human Saphenous Vein Endothelial Cells

Different concentrations of AA and 10 ng/mL of TNF-α were added to the wells. No drug administration was applied to the control group. TNF-α was applied to the TNF-α group for 6 hours. 5, 1, 0.5 and 0.1 μM of AA were applied for 1 hour to the groups that were treated with only AA. For the groups that were induced by TNF-α after AA administration; 5, 1, 0.5 and 0.1 μM of AA were applied, and after incubation of those cells for 1 hour, 10 ng/mL of TNF-α was applied to each group for 6 hours. 5 μM of DMSO was applied to the last group as a vehicle.

2.4. Cells and Reagents

A double staining procedure was applied for NF-κB and ICAM-1 as previously described [13]. For the secondary antibody application process of ICAM-1 staining, cells were incubated for 1 hour with goat anti-mouse IgG H&L secondary antibody (Alexa Fluor 488) (Abcam, Cambridge, UK). For the secondary antibody application step of NF-κB staining, the cells were incubated for 1 hour at 23°C with goat anti-mouse IgG H&L secondary antibody (Alexa Fluor 568) (Abcam, Cambridge, UK). The stained samples were observed under an inverted fluorescence microscope (Leica Microsystems, Heerbrugg, GE) after being covered with the occlusion media.

Comparisons of immunofluorescent intensities were made using ImageJ 1.44a software. Ten (10) pictures taken from random areas of each petri were analyzed for ICAM-1 and NF-κB expression and determined as corrected total cell fluorescence (CTCF) and calculated as CTCF=Integrated Density-(Area of selected cell X Mean fluorescence of background readings) [14] as previously described [15, 16].

2.5. Isolation of RNA from Human Saphenous Vein Endothelial Cells

Total RNA isolation from cells was performed using an RNA isolation kit. Before the isolation process, the culture media were aspirated, and the cells were washed with PBS before they were removed with trypsin/EDTA. After total RNA was isolated from the cells that were diluted as $2x10^5$ cells per μL, the amount and purity of the obtained RNA were determined by using a Qubit 2.0 fluorometer (ThermoFisher, Waltham, MA, USA).

2.6. cDNA Synthesis and qRT-PCR

qRT-PCR was performed as previously described [17]. For each group, cDNA synthesis from 30 ng/μL of total RNA was performed in the thermal cycler by using the commercial kit. Subsequently, changes in mRNA levels of target genes were detected by qRT-PCR technique with TaqMan probes. Experiments were repeated twice for each cDNA sample. The Actin Beta (ACTB) housekeeping gene was used as the internal control.

The qRT-PCR primers and TaqMan probes (4331182 Life Tech. Carlsbad, CA, USA) were Human ICAM-1 Hs00164932_m1; Human NF-κB Hs00765730_m1 and Human ACBT Hs01060665_g1.

2.7. Cytotoxicity Measurement

Using the cytotoxicity kit, cytotoxicity levels were measured by determining the amount of lactate dehydrogenase (LDH) released from the damaged or dead cells in the media. The culture flask containing only Promocell endothelial cell growth medium was used as the negative control group to

determine the average LDH activity in the medium. Control groups without any substance applied were identified as low-concentration control groups to determine LDH release under normal conditions. The high-concentration control group, which was given 2% Triton X-100 in the culture medium and formed at the end of the 24-hour incubation, was prepared to determine the highest amount of LDH that could be released.

2.8. Statistical Analysis

SPSS 22.0 software (IBM Corp., Armonk, NY, USA) was used in the analyses, and $p<0.05$ was considered significant. Mean, standard deviation, median lowest, and median highest values were used in the descriptive statistics of the data. The distribution of the variables was determined by the Kolmogorov-Smirnov test. The Kruskal-Wallis and Mann-Whitney U tests were used in the analysis of quantitative data.

3. RESULTS

In the present study, a TNF-α-induced inflammatory model with HSaVECs was generated followed by anacardic acid (AA) administration. Changes in mRNA levels of ICAM-1 and NF-κB genes were then detected by the qRT-PCR method. Immunofluorescent staining for ICAM-1 and NF-κB proteins was also performed on the endothelium. The lactate dehydrogenase (LDH) assay was performed on cell culture media.

3.1. Microscopic Evaluation of HSaVECs

The endothelial cells were inspected microscopically as they reached the required maturity. Dil-Ac-LDL, vWF and CD31-positive cells were purchased, and no further fluorescence or immunohistochemical validation was required before the administration.

3.2. Cytotoxicity Measurement

In the HSaVEC culture, the cytotoxicity measurements were performed by measuring the amount of LDH released by the cells. As given in Fig. (**1**), the LDH level in the control group was not significantly different when compared with those in both the DMSO and TNF-α groups ($p>0.05$). However, when 0.5, 1 and 5 μM of AA were applied on HSaVECs, the cytotoxicity levels significantly increased with respect to the control group ($p<0.05$). The results were similar when those concentrations were administered to the cells before TNF-α induction. On the other hand, the LDH levels in both 0.1 μM AA and 0.1 μM AA + TNF-α groups were significantly lower than those in the control group ($p<0.05$).

3.3. Changes in ICAM-1 mRNA Levels of TNF-α-induced HSaVECs After AA Administration

HSaVECs were treated with AA for 1 h, followed by TNF-α application for 6 h, and relative mRNA expression levels of ICAM-1 were determined by qRT-PCR. The results obtained are shown in Fig. (**2**).

As expected, the relative level of mRNA expression of ICAM-1 significantly increased in TNF-α-induced HSaVECs compared with that in the control group ($p<0.05$). In addition, ICAM-1 mRNA expression levels were significantly higher in the 5 μM AA + TNF-α, 1 μM AA + TNF-α, 0.5 μM AA + TNF-α and 0.1 μM AA + TNF-α groups than that in the 5 μM AA, 1 μM AA, 0.5 μM AA and 0.1 μM AA groups, respectively. These results support that TNF-α induces the expression of ICAM-1 in HSaVECs. Then, to de-

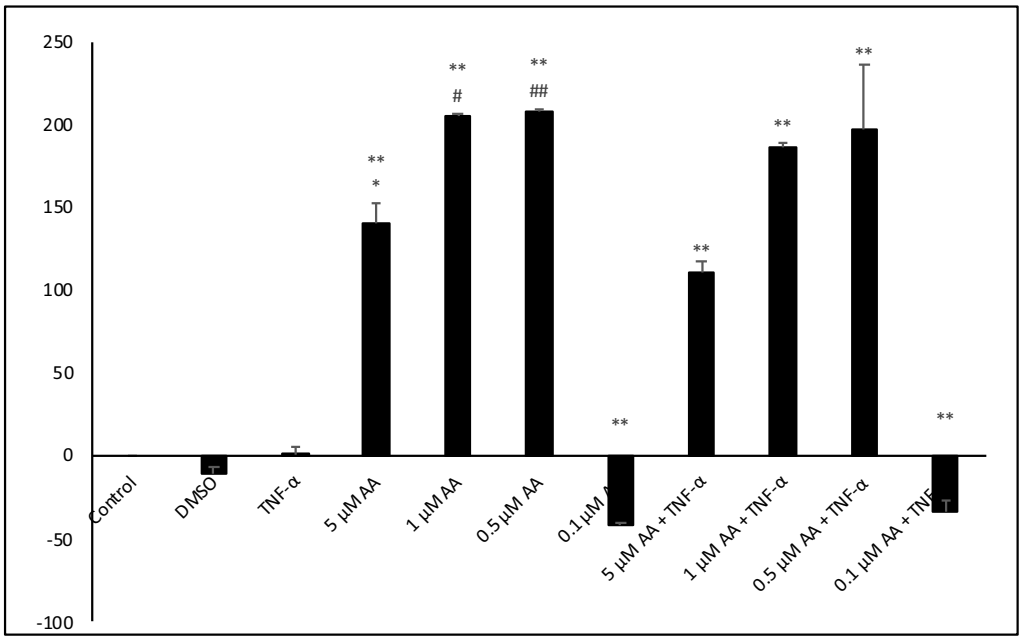

Fig. (1). The cytotoxicity levels of the groups were detected by measuring LDH levels. The groups were treated with AA for an hour followed by incubation for 6 hours after TNF-α application. The levels of LDH in the control group were considered baseline. * $p<0.05$ *vs*. both 5 μM AA and TNF-α treated group, # $p<0.05$ *vs*. both 1 μM AA and TNF-α treated group, ## $p<0.05$ *vs*. both 0.5 μM AA and TNF-α treated group, ** $p<0.05$ *vs*. untreated cells and cells treated with TNF-α alone.

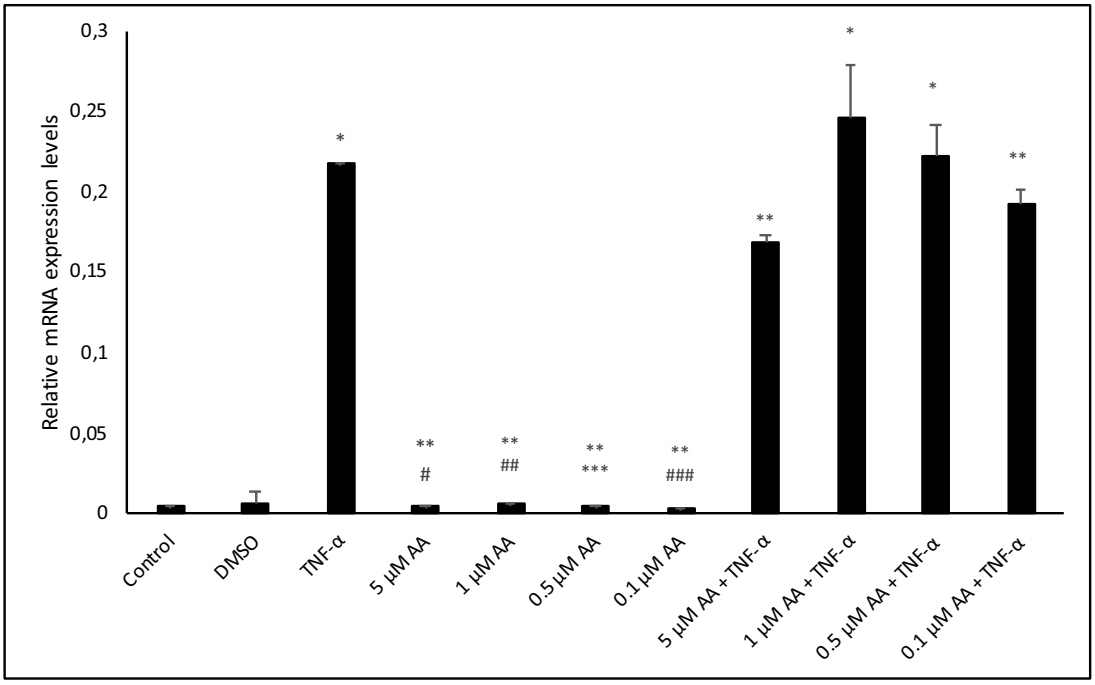

Fig. (2). Relative mRNA expression levels of ICAM-1 determined by qRT-PCR technique. * p<0.05 *vs*. untreated cells, # p<0.05 *vs*. both 5 µM AA and TNF-α treated group, ## p<0.05 *vs*. both 1 µM AA and TNF-α treated group, *** p<0.05 *vs*. both 0.5 µM AA and TNF-α treated group, ### p<0.05 *vs*. both 0.1 µM AA and TNF-α treated group, ** p<0.05 *vs*. untreated cells and cells treated with TNF-α alone.

termine the effect of AA on relative mRNA expression levels of ICAM-1 in TNF-α-induced cells, different concentrations of AA were applied to HSaVECs. 0.5 and 1 µM of AA applications after induction with TNF-α did not cause any significant difference compared with only TNF-α-induced HSaVECs (p>0.05). However, the obtained results suggest that in both 0.1 µM AA + TNF-α and 5 µM AA + TNF-α groups, relative levels of mRNA expression of ICAM-1 were significantly lower than that in the TNF-α group (p<0.05) and the decrease was observed as higher when 5 µM of AA was applied on TNF-α-induced HSaVECs.

3.4. Changes in NF-κB mRNA Levels of TNF-α-induced HSaVECs After AA Administration

HSaVECs were treated with AA for an hour followed by TNF-α application for 6 h, and relative mRNA expression levels of NF-κB were determined by using the qRT-PCR technique.

The given results suggest that TNF-α induces NF-κB expression in HSaVECs because relative mRNA levels of NF-κB were found significantly higher in all TNF-α-induced groups than that in the control group (p<0.05). In addition, NF-κB expression was significantly higher in 5 µM AA + TNF-α, 1 µM AA + TNF-α, 0.5 µM AA + TNF-α and 0.1 µM AA + TNF-α groups when compared with that in 5 µM AA, 1 µM AA, 0.5 µM AA and 0.1 µM, respectively (p<0.05). To test the effect of AA on TNF-α-induced NF-κB expression, different concentrations of AA were applied on HSaVECs before TNF-α induction. Based on the results, 1, 0.5 and 0.1 µM of AA administration before TNF-α application significantly decreased the relative mRNA levels of NF-κB when compared with the TNF-α group (p<0.05). TNF-α-induced NF-κB expression significantly increased in the 5 µM AA group when compared with the TNF-α group (p<0.05) (Fig. **3**).

3.5. Immunofluorescent Staining Results of ICAM-1

In the cytoplasm of the control and DMSO groups, weak immune positivity for ICAM-1 was observed. In the 5 µM AA + TNF-α group, strong immune staining for ICAM-1 was observed, which was most visibly localized around the nuclei, and the cytoplasmic regions showed a weaker immune reaction although a few cells with dense immune staining in the cytoplasm near the cell surface were detected (Fig. **4**). In the 1 µM AA + TNF-α group, immune positivity for ICAM-1 decreased compared with the 5 µM AA + TNF-α group, and immune positivity was observed on the cell surface (Fig. **5**). In the 0.5 µM AA + TNF-α group, immune positivity of ICAM-1 decreased compared with the levels in the 5 µM AA + TNF-α and 1 µM AA + TNF-α groups and was limited with only a small number of point-local immune-stimulatory regions (Fig. **6**). In the 0.1 µM AA + TNF-α group, the immune positivity of ICAM-1 was observed to be weaker compared with that of the 5 µM AA + TNF-α and the 1 µM AA + TNF-α groups. On the cell surface, no staining was observed as the cells did not adhere to the base and only a spindle-shaped morphology was shown (Fig. **7**). The strongest ICAM-1 positivity was observed in the TNF-α group. Immune staining was apparent mainly on the cell surface, and a few nuclei also showed immune staining. The endothelial cells of the TNF-α group showed a different morphology in which most of the staining was scattered and adhered strongly to the base of the cell. In the 5, 1, 0.5 and 0.1 µM AA groups, no immune staining of ICAM-1 was observed.

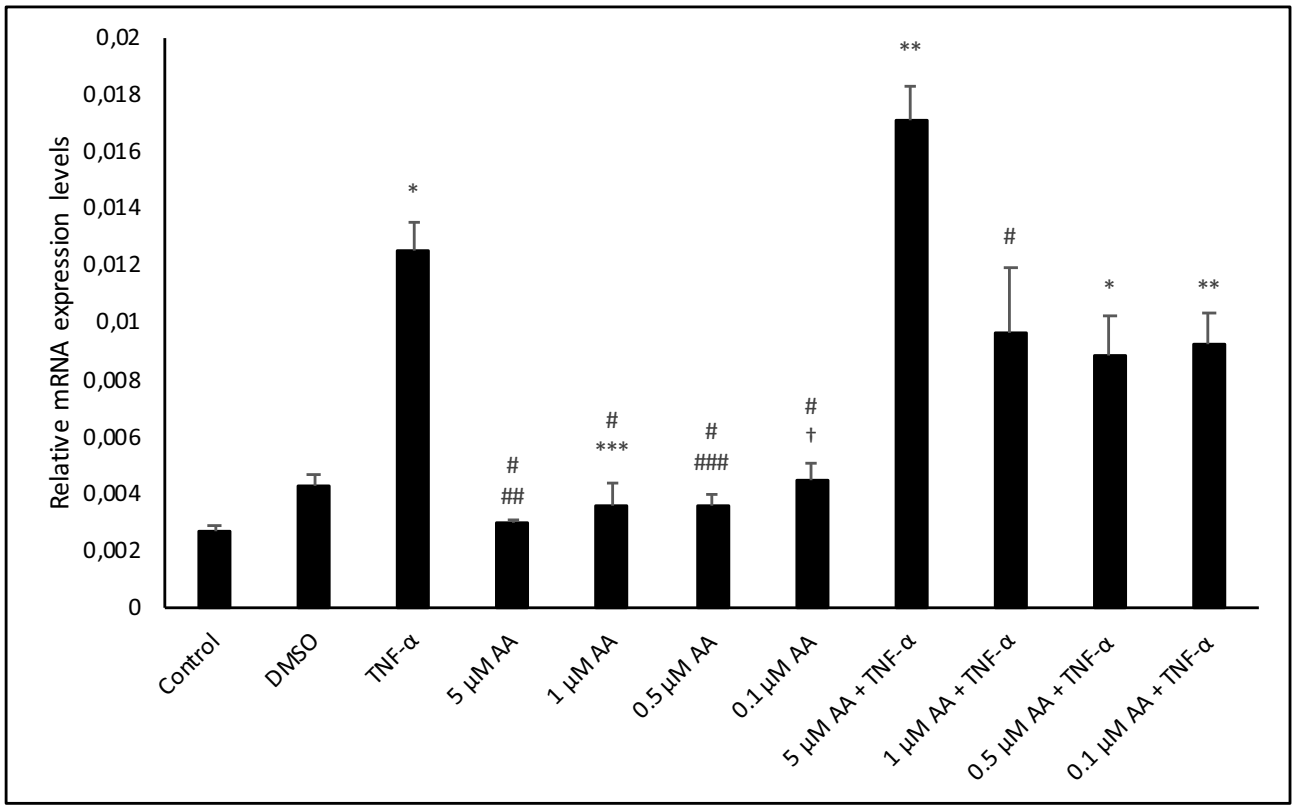

Fig. (3). Relative mRNA expression levels of NF-κB determined by qRT-PCR technique. * p<0.05 *vs*. untreated cells, # p<0.05 *vs*. cells treated with TNF-α alone, ** p<0.05 *vs*. both untreated cells and cells treated with TNF-α alone, ## p<0.05 *vs*. both 5 μM AA and TNF-α treated group, *** p<0.05 *vs*. both 1 μM AA and TNF-α treated group, ### p<0.05 *vs*. both 0.5 μM AA and TNF-α treated group, † p<0.05 *vs*. both 0.1 μM AA and TNF-α treated group.

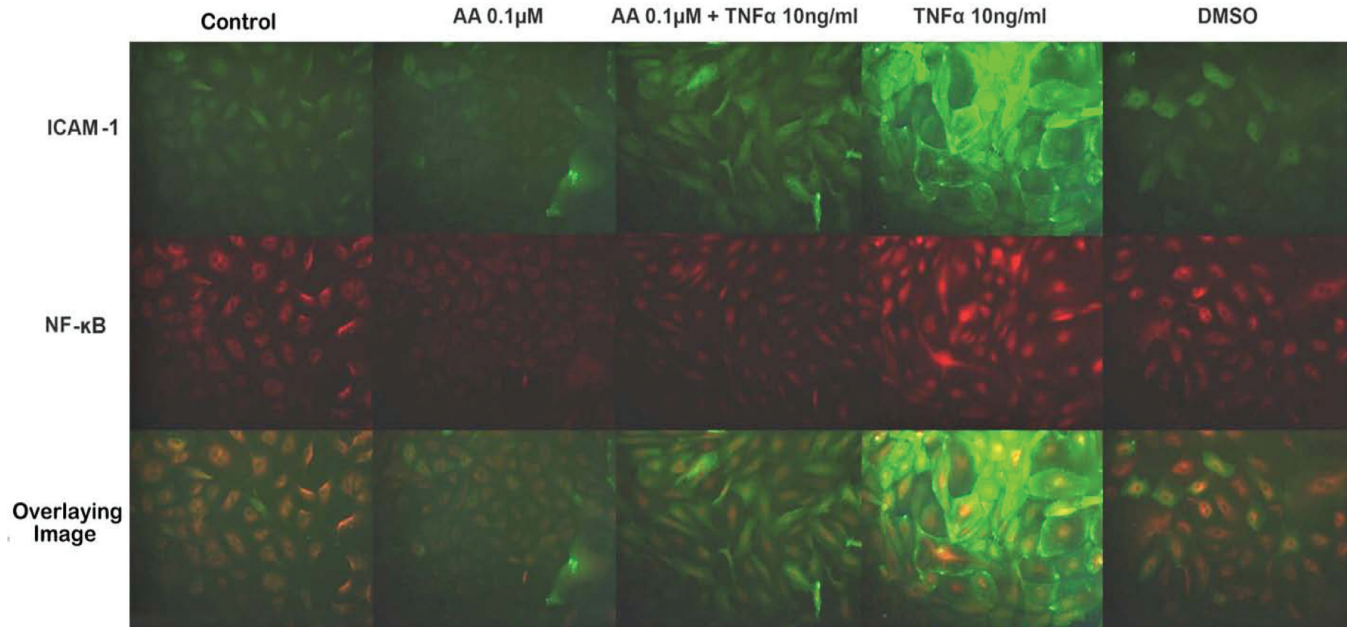

Fig. (4). Immunofluorescent staining images for ICAM-1 and NF-κB. AA and TNF-α were applied as indicated. In the overlaying image, the cell compartments, where both ICAM-1 and NF-κB were localized, are shown in orange (20X magnification, scale bar = 100 μm).

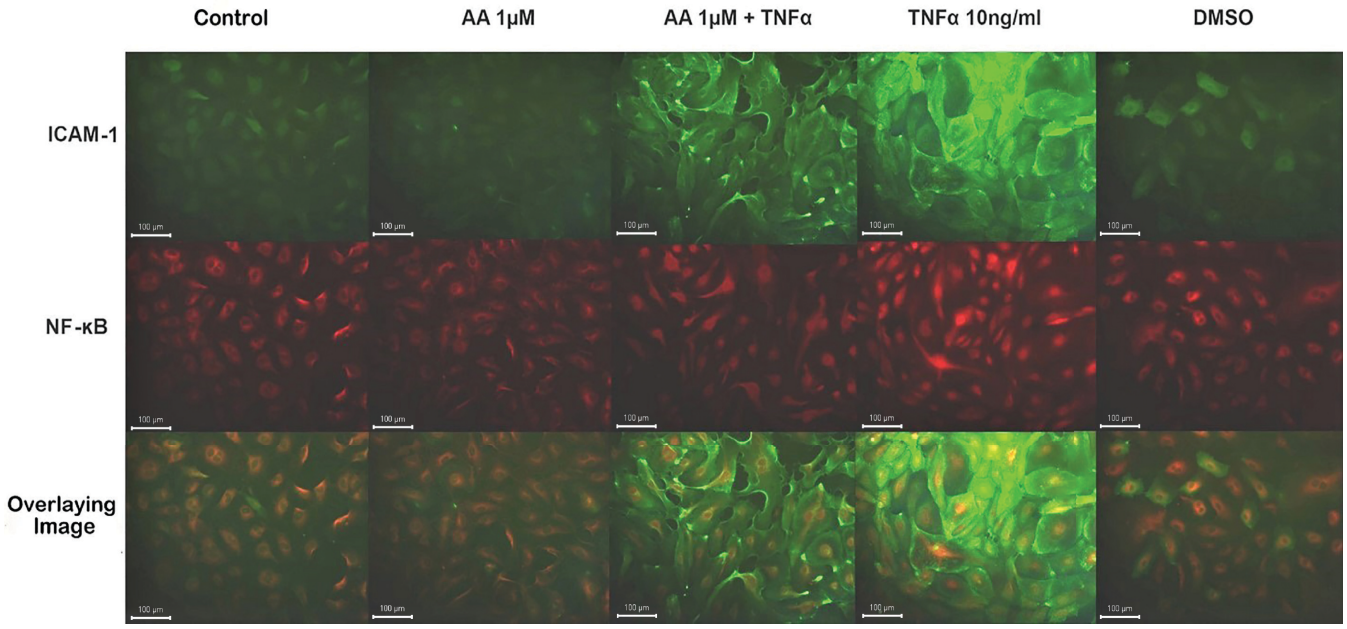

Fig. (5). Immunofluorescent staining images for ICAM-1 and NF-κB. AA and TNF-α were applied as indicated. In the overlaying image, the cell compartments, where both ICAM-1 and NF-κB were localized, are shown in orange (20X magnification, scale bar = 100 μm).

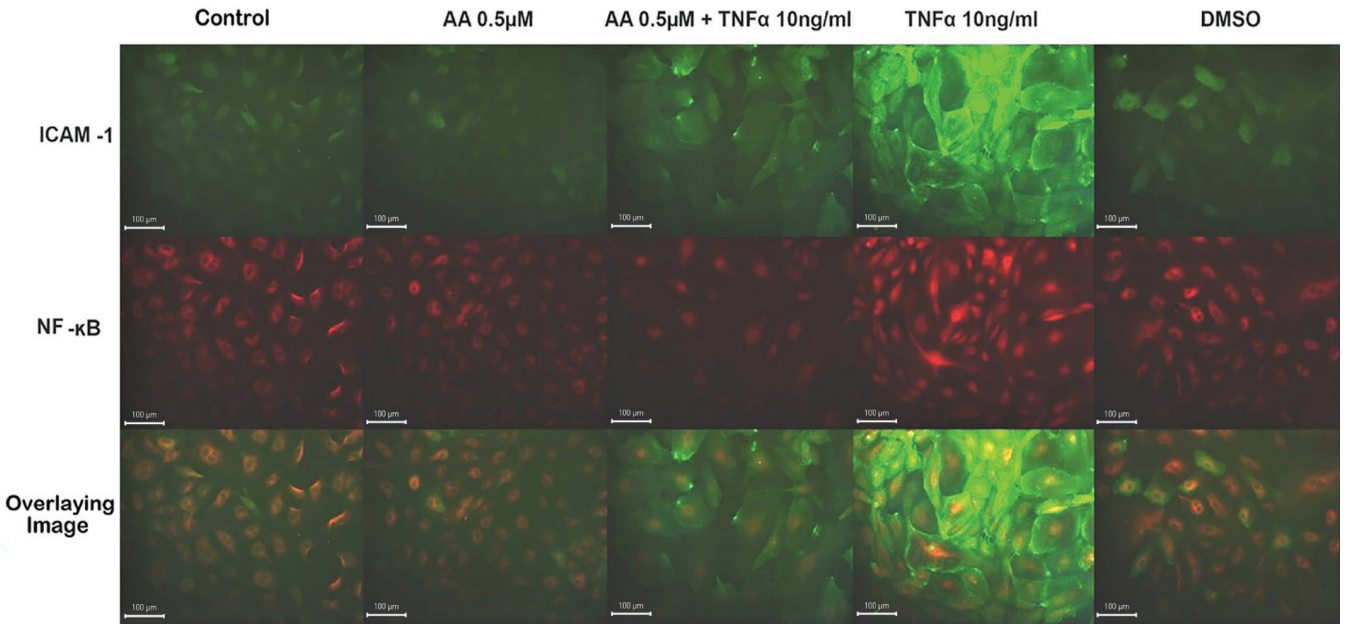

Fig. (6). Immunofluorescent staining images for ICAM-1 and NF-κB. AA and TNF-α were aplied as indicated. In the overlaying image, the cell compartments, where both ICAM-1 and NF-κB were localized, are shown in orange (20X magnification, scale bar = 100 μm).

3.6. Quantitative Analysis of Immunofluorescent Intensities of ICAM-1 Staining

The results of the quantitative ICAM-1 analysis of the images for HSaVECs are given in Fig. (**8**). ICAM-1 fluorescence intensity values significantly increased in all the TNF-α-induced HSaVEC groups when compared with that in the control group ($p<0.05$). In addition, ICAM-1 levels were detected as significantly lower in the 5 μM AA, 1 μM AA, 0.5 μM AA and 0.1 μM AA groups than in the 5 μM AA + TNF-α, 1 μM AA + TNF-α, 0.5 μM AA + TNF-α and 0.1 μM AA + TNF-α groups, respectively ($p<0.05$). These results support the TNF-α-induced ICAM-1 expression in HSaVECs, which is concordant with the relative mRNA expression results of ICAM-1. If the effect of AA on ICAM-1 intensity results in Fig. (**8**) are considered, 0.5 and 0.1 μM of AA application caused significant decreases in TNF-α-induced HSaVECs when compared with the TNF-α group ($p<0.05$). However, there was no significant difference in both 5 μM AA + TNF-α

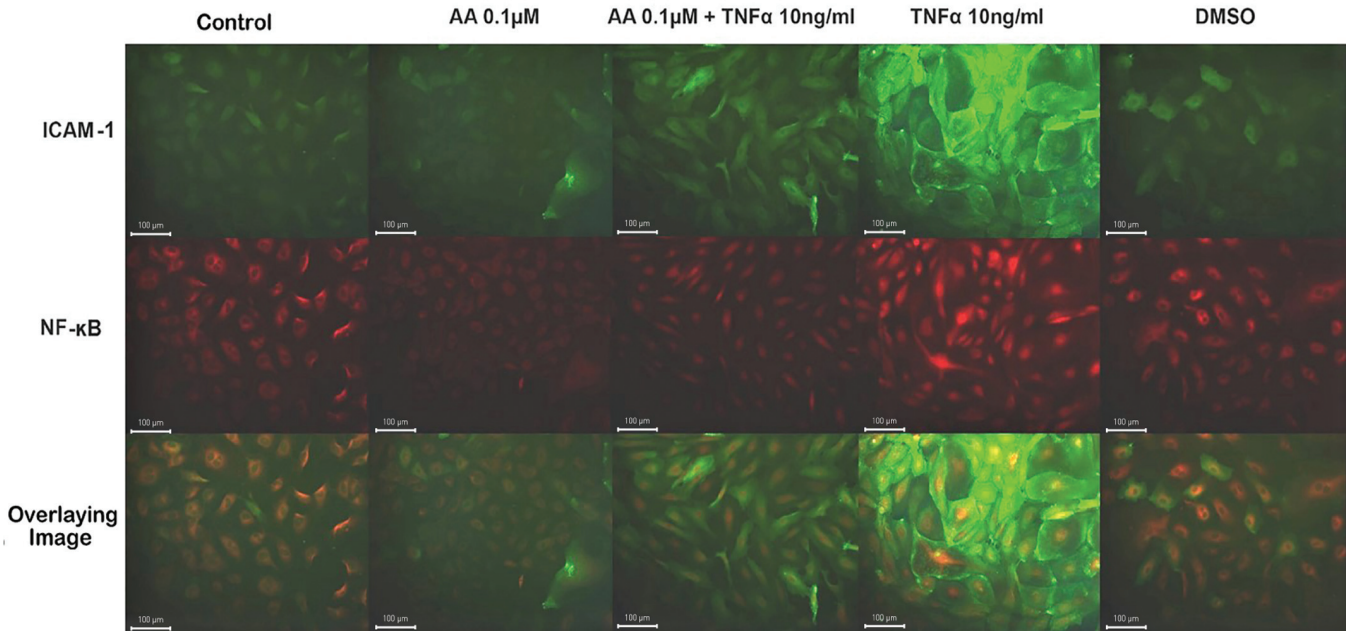

Fig. (7). Immunofluorescent staining images for ICAM-1 and NF-κB. AA and TNF-α were applied as indicated. In the overlaying image, the cell compartments, where both ICAM-1 and NF-κB were localized, are shown in orange (20X magnification, scale bar = 100 μm).

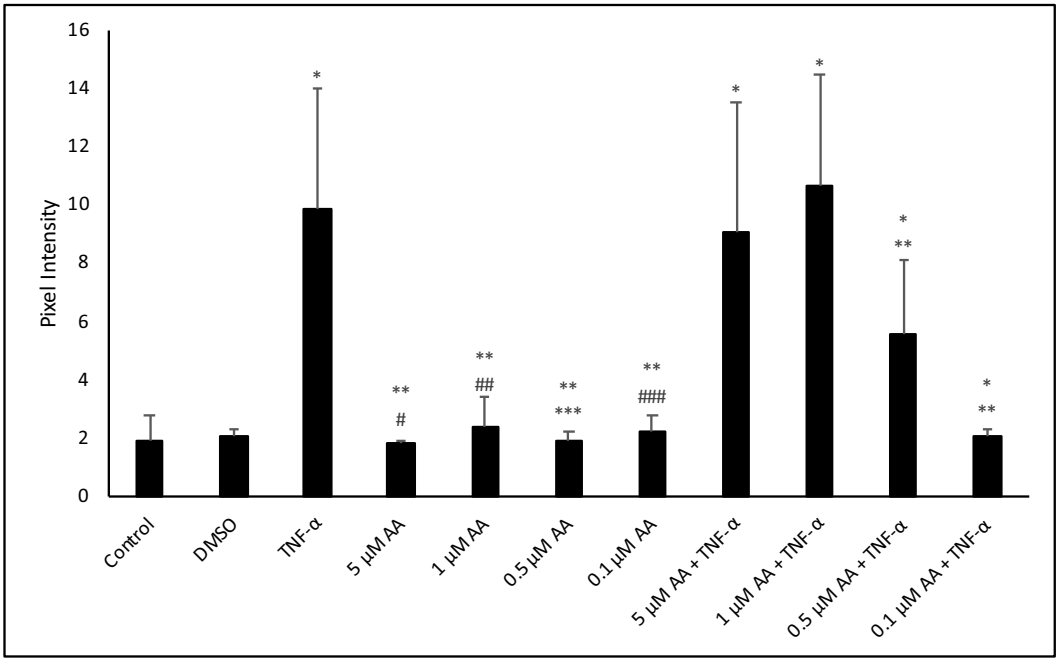

Fig. (8). Quantification of ICAM-1 fluorescent intensity. * p<0.05 *vs*. untreated cells, * p<0.05 *vs*. untreated cells, # p<0.05 *vs*. cells treated with TNF-α alone,** p<0.05 *vs*. both untreated cells and cells treated with TNF-α alone, ## p<0.05 *vs*. both 5 μM AA and TNF-α treated group, *** p<0.05 *vs*. both 1 μM AA and TNF-α treated group, ### p<*vs*. both 0.5 μM AA and TNF-α treated group.

and 1 μM AA + TNF-α groups when compared with the TNF-α group in terms of ICAM-1 intensity levels (p>0.05).

3.7. Immunofluorescent Staining Results of NF-κB

In the control and DMSO groups, immunofluorescence staining of NF-κB was observed in the cytoplasm and nuclei. In the 5 μM AA group, the immunoreaction of NF-κB was slightly weaker than that of the control group. In the 5 μM AA + TNF-α group, staining was localized in the nucleus and immune positivity for NF-κB increased compared to the control group (Fig. **4**). In the 1 μM AA group, immune competence and localization were almost equal to those in the control group, whereas NF-κB showed an increased positive effects in the 1 μM AA group. In the 1 μM AA + TNF-α group, strong NF-κB immune reactivity in the nuclei was observed, whereas weaker positive effects were seen in the cytoplasm (Fig. **5**). In the 0.5 μM AA group, immunofluo-

rescence for NF-κB was almost identical to that in the control group and was stronger than that in the 5 μM AA group. In the 0.5 μM AA + TNF-α group, NF-κB positivity was weaker than that in the 1 μM AA + TNF-α group (Figure 6). In the 0.1 μM AA group, immune positivity for NF-κB was weak when compared with the control group. In the 0.1 μM AA+TNF-α group, immunofluorescence positivity for NF-κB was high compared to that in the 0.1 μM AA group but weaker compared to the 5 μM AA + TNF-α and 1 μM AA + TNF-α groups (Fig. **7**). In the TNF-a group, localized immunoreaction in the nucleus was dominant, and there was also regional cytoplasmic staining. When images of immune staining samples for ICAM-1 and NF-κB were superimposed, the morphological features mentioned earlier were observed in all groups. The endothelial nuclei of all the groups that were treated with both AA and TNF-α and solely with TNF-α showed immunoreactivity for NF-κB.

3.8. NF-κB Intensity Results of Immunofluorescent Analysis

The results of the quantitative analysis of the NF-κB Image for HSaVECs are shown in Fig. (**9**). NF-κB fluorescence intensity was detected as significantly higher in the 5 μM AA + TNF-α, 1 μM AA + TNF-α and TNF-α groups when compared with the control group ($p<0.05$). Furthermore, 0.5 and 0.1 μM of AA application caused significant decreases in detected NF-κB levels in HSaVECs with respect to the control group ($p<0.05$).

4. DISCUSSION

We investigated relative ICAM-1 and NF-κB mRNA expression levels, cell survival, localization and fluorescence densities of target proteins by immunofluorescent staining after the administration of Anacardic Acid (AA) in the TNF-α-induced human saphenous vein endothelial cells (HSaVECs) inflammation model. Four different doses of AA were applied to detect the effect of AA on HSaVECs. It was determined that 0.1 μM of AA did not have any toxic effect; it rather reduced cell death observed under traditional cell culture conditions. In addition to the available concentrations in the literature, it was decided that the 5, 1 and 0.5 μM concentrations might stimulate HSaVEC death [18], and 0.1 μM was the best fit concentration with regard to toxicity.

AA, a phytochemical agent, is a salicylic acid analogue that can pass through the cell membrane and is used as a traditional therapeutic source around the world [7]. NF-κB modification is one of the mechanisms by which AA acts on inflammatory pathways. NF-κB is a potential proinflammatory nuclear transcription factor responding to the onset and induces inflammation [19].

In our study, NF-κB localized in nuclei has been elevated significantly in the TNF-α-applied group in comparison with the control and AA groups and hence ICAM-1 mRNA and protein levels were increased, in concordance with the pathogenesis of atherogenesis. Thus, it was understood through recent studies that NF-κB stimulated the inflammatory pathway of atherosclerosis by inducing ICAM-1 expression in a parallel manner [3, 4]. AA can inhibit TNF-α-induced NF-κB activation. Although it was observed in our study that different concentrations of AA did not have a suppressive effect on NF-κB. The TNF-α-induced NF-κB expression suppressed and kept at the control level when the same concentrations were administered with TNF-α. Therefore, AA might reduce the progression of vascular inflammation, which plays a role in the pathogenesis of saphenous graft disease and decreases the primarily important long-term patency of saphenous vein grafts. In addition, it was suggested that one of the basic mechanisms of AA reduction of TNF-α-induced NF-κB expression might be decreased in the formation of free oxygen radicals [20]. Accordingly, it is deduced that AA can be beneficial to prevent or treat saphenous vein graft disease by not only inhibiting NF-κB but also by reducing the formation of free oxygen radicals, leading to a decrease in oxidative stress.

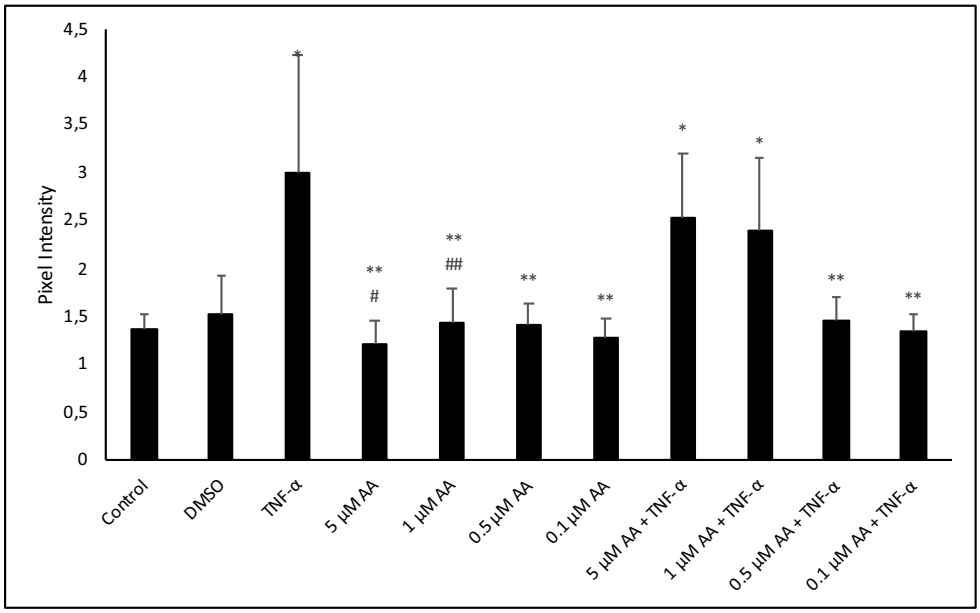

Fig. (9). Quantification of NF-κB fluorescent intensity. * $p < 0.05$ *vs*. untreated cells, # $p < 0.05$ *vs*. both 5 μM AA and TNF-α treated group, ** $p < 0.05$ *vs*. cells treated with TNF-α alone.

It was shown that the expression of adhesion molecules in saphenous vein graft endothelial cells increased due to exposure to high pressure after arterial anastomosis of the saphenous vein grafts [21, 22]. This causes atherosclerosis during and after the progression of inflammation in saphenous veins, leading to saphenous vein graft disease and hence, the loss of early and late period saphenous vein graft dilation [23-25]. Vascular inflammation-related intimal hyperplasia and atherosclerosis formation have major roles in the pathogenesis of saphenous vein graft disease [12, 26, 27]. Thus, better clarification of the pathogenesis of saphenous vein graft disease, prevention of its progress and its optimum medical treatment after progression come into prominence.

It has been suggested in recent studies that NF-κB is related to metabolic diseases as well as the formation of inflammation. There has been an indication that adipokines, such as visfatin, TNF-α and adiponectin, are regulated through NF-κB; in addition, several regulators of inflammation such as MCP-1, IL-6, IL-8, E-selectin, ICAM-1 and VCAM-1, which are decreased or increased by adipokines, have been indicated to be regulated through NF-κB as well. It has also been specified that TNF-α induces ICAM-1, VCAM-1 and NF-κB expressions [19]. In our study supporting these findings, ICAM-1 and NF-κB mRNA levels and protein expressions in the TNF-α-applied group were statistically higher than those in the control and AA groups, which is compatible with the pathogenesis of atherogenesis.

ICAM-1 provides firm adhesion, which has an important role during leukocyte transendothelial migration and antigen presentation and hence has a positive impact on atherogenesis [28]. It was observed in our study that AA reduced the inflammation-induced ICAM-1 expression, which is an important indication in the prevention of saphenous graft disease progression. It has been suggested in recent studies that NF-κB has a significant regulatory role in the expression of adhesion molecules [23-25]. Thus, reduction in NF-κB expression levels after AA administration might be the reason for the decrease in expression of adhesion molecules, playing important roles in both vascular inflammation and the pathogenesis of atherosclerosis. Based on our results, ICAM-1 expression levels were significantly lower in the group administered AA with TNF-α compared with the group received only TNF-α. It is thought that this decrease might be a result of the suppression on the NF-κB pathway. However, ICAM-1 expression could still not reach the levels in either the control group or the group to which only AA was applied. When these results were considered, it was deduced that TNF-α might increase the expression levels of adhesion molecules by inducing inflammation through not only the NF-κB pathway but also through some other transcription factors [29, 30]. A correct approach to determine this effect more accurately might be to extend administration time or give sequential administrations. In our study, ICAM-1 expression levels decreased, especially in the 0.1 μM AA + TNF-α group, proving that AA might have a positive impact on cell-cell communication. Thus, the NF-κB pathway may not be the only mechanism involved in this effect of AA; it needs to be considered that different cellular mechanisms might be included in this inflammatory pathway, and AA might be a more complex regulator.

CONCLUSION

The acute effect of AA on adhesion molecules was determined after short-term AA administration to HSaVECs in our study. Although there has been no available information on long-term applications yet, it is thought that AA might be effective in the inhibition of saphenous vein graft disease progression and in its treatment by lowering the expression of adhesion molecules *via* the NF-κB pathway. Therefore, new experimental and clinical studies are necessary to reveal both the activity of AA on saphenous vein graft disease and the molecular mechanisms of this effect on the pathogenesis of the disease.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

No human participants or animals were involved in the study.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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