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L-arginineameliorates atherosclerosis by eliciting anti-inflammatory/antioxidant activity in rabbits

Hajir Karim Abd-Ulhussein[1], Fadhil A. Rizij[2], Ali M. Janabi[3*], Najah R. Hadi[4]

Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Babylon, Babel, Iraq[1]
Department of Clinical Pharmacy, Faculty of Pharmacy, University of Kufa, Najaf, Iraq[2]
Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Kufa, Najaf, Iraq[3]
Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Kufa, Najaf, Iraq[4]

Corresponding Author: 3*

ABSTRACT— The study was designed to investigate the effect of L-arginine on atherosclerosis particularly by identification of some elements of inflammatory and oxidative cascades. Eighteen local domestic rabbits (male) were introduced and allocated into 3 groups (6 rabbits per group) and the duration of the study was 12 weeks. The first one was considered as a negative control group in which animals received normal diet only. The second one was the group in which rabbits received dietenriched with cholesterol (5 %). The last group was the one in which received dietenriched with cholesterol (5 %) together with oral L-arginine (1.5%). At the end of the study, animals were sacrificed, and blood sampleswere to identify serumlevels endothelin-1(ET-1) and tumor necrosis factor-alpha (TNF-α). Lipid parameters (TC, TG, HDL-C, LDL-C and VLDL-C) were also determined in the serum. Aorta was isolated to detect total antioxidant capacity (TAC) and intimal thickness. Treatment of rabbit with L-arginine was associated withsignificant elevation of serum level of TC, HDL-C and LDL-C but insignificant reduction in TG and VLDL-C level when compared with untreated group. Treatment with L-arginine was associated withinsignificant elevation ofboth serum TNF-α level and aortic TAC andinsignificant reduction in serum ET-1andaortic intima thickness when compared with untreated rabbits. L-argininehas a potential ameliorating effect on atherosclerosis. This was mainly achieved by its inhibitory effects on some inflammatory and oxidative cascades.

KEYWORDS: Atherosclerosis, L-arginine, oxidative stress, TNF-α, ET-1.

1. INTRODUCTION

Atherosclerosis is a vascular wall disease in which arteries are associated with fat deposition in their inner layers with subsequent plaque generation (1). The preciserisk factors of atherosclerosisremain to be elucidated. Main risk factors includediabetes, hypertensionand obesity which can be modified; whereatherosclerosis can be delayed or prevented (2). However, age, genetic predispositionand male gender are the non-modifiable factors (3). Inflammation has been considered to play an important role in the initiation and development of atherosclerosis.As a result of that, various inflammatory mediators have been considered as factors for evaluation of cardiovascular risk(4). TNF-α is considered as the most important inflammatory marker that maintains lowlevel systemic inflammation. Several mechanisms were mentioned to interpret the proatherogenic effects of TNF-α on the endothelium, including its action on reactive oxygen species formation, reduction of the bioavailability of NO and increasing permeability of endothelium to circulating blood components and cells (5). Endothelin-1 (ET-1) is a peptide responsible for pathophysiological changes associated withmicrovascular and macrovascular disease (6).ET-1 may lead to atherosclerosis by its action as a mitogen on smooth muscle cells of vessels and activating of vascular inflammation and cytokine release in atherosclerotic lesions. ET-1 levels of coronary artery and plasma are

high in early and late atherosclerosis in humans (7). Oxidative stress contributes to the development of atherosclerosis (8). Antioxidant is any substance that prevents or removes oxidative damage and inhibits process of oxidation to a target molecule (9). The total antioxidant capacity is a mirror of the activity of the antioxidant system (10). L-arginine is a semi-essential amino acid necessary for most cellular activities. L-arginine is transformed to nitric oxide (which acts as a vasodilator) and citrulline. In recent years, it has been found that in hypercholesterolemia patients, L-arginine-rich foods were inversely associated with endothelial dysfunction. It has also been found that cardiovascular complications are reduced with long-term administration of L-arginine (11).

2. Materials and Methods

Eighteen local domestic rabbits (male) were introduced and allocated into 3 groups (6 rabbits per group) and the duration of the study was 12 weeks. The first one was considered as a negative control group in which animals received normal diet only. The second one was the group in which rabbits received diet enriched with cholesterol (5 %). The last group was the one in which received diet enriched with cholesterol (5 %) together with oral L-arginine (1.5 %). After 12 weeks, blood samples were obtained for measurement of serum level of total cholesterol (TC) triglycerides (TG), ET-1, TNF-α, HDL-C, LDL-C, and VLDL-Caccording to the manufacturer's instructions. Aorta of each rabbit was removed for measurement of TAC level and aortic intima thickness. Samples were homogenized using Sonicator after washing with phosphate buffer saline. Triton X was then added to each sample, then sonicated in ice box and centrifuged at 6000 rpmfor 30 min, and supernatants were analyzed to measure aorticTAC.

3. Statistical analyses

Data are expressed as mean + SEM. Statistical comparisons were performed using one-way ANOVA followed by Bonferroni's test for multiple comparisons using Prism (GraphPad 7 Software Inc., San Diego, CA, USA). Statistical significance was considered for $P < 0.05$.

4. Results

There was statistically insignificant difference ($P>0.05$) in body weight levels among study groups on suggesting that cholesterol and L-argininedid not impact body weight.In comparison with rabbits fed with normal diet, levels of total cholesterol, triglycerides, HDL-C, LDLC,VLDL-C, TNF-α, ET-1 and aortic intima thickness were significantly increased while aortic TAC insignificantly lowered($P>0.05$) in rabbits fed high cholesterol diet.Treatment withL-argininesshowedsignificant increase in total cholesterol,HDL-C and LDL-C butinsignificant decreased TG and VLDL-C level when compared with induced untreatedgroup ($P<0.05$). L-arginine had no significant effect on serum TNF-α and serum ET-1 level at week 12 of study when compared with atherogenic diet group. L-arginine significantly decreased aorticintimathickness ($P <0.05$) and insignificantly increased aortic TAC level whencompared with untreatedrabbits (Table 1, 2, 3).

Table 1: Serum lipid profile (mg/dl) of experimental animals after 12 weeks Data are expressed as mean ± SEM (N=6 per group) using one-way ANOVA followed by Bonferroni's test

Group	Total cholesterol	Triglyceride	HDL-C	LDL-C	VLDL-C
Control	95.5±2.59	73.02±2.32	10.38±0.44	51.9±2.8	14.6±0.46
Induced	796.57±43.7*	127±18.9*	15.6±0.93*	723.2±45.6 *	25.4±3.8*
L-arginine	896.2±3.4†	103.3±2.3	26.1±1.49†	823.2±2.57†	20.6±0.46

* $P<0.05$ compared to control group

†$P<0.05$ compared to induced group (untreated)

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Table 2: Serum TNF-α and ET-1 of experimental animals. Data were expressed as mean ± SEM (N=6 per group) using one-way ANOVA followed by Bonferroni's test.

Group	TNF-α (pg/ml)	ET-1(pg/ml)
Control	140.59±20.47	20.62±0.67
Induced	184.49±8.3*	30.51±1.53*
L-arginine	185±2.4	28.75±1.55

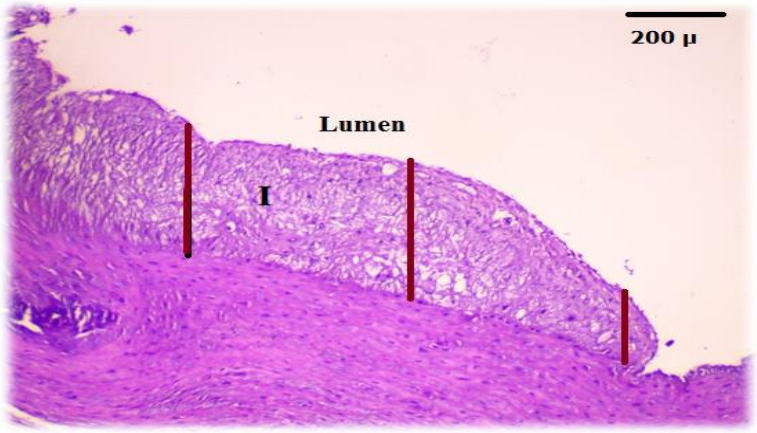
*p<0.05compared to control group

Table 3: The means of aortic TAC and intima thickness of experimental animals at the end of study The data was expressed as Mean ±SEM (N=6 per group) using one-way ANOVA followed by Bonferroni's test.

Group	Aortic TAC (mmole/gm)	Aortic intima thickness (µm)
Control	0.64± 0.05	39.8± 1.2
Induced	0.62 ± 0.03	275.46 ± 33.2*
L-arginine	0.72 ± 0.03	156.44 ± 3.13†

* P<0.05 compared to control group
†P<0.05 compared to induced group (untreated)

a)



b)

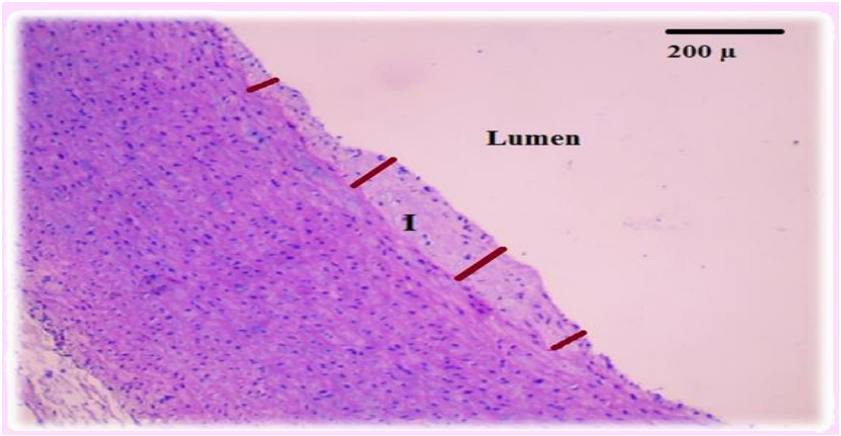


Figure 1: a) Photomicrograph of the transverse section of aorta of rabbit fed atherogenic diet for 12 weeks shows diffuse intima thickening and lipids deposition in intima. b) Photomicrograph of the transverse section of aorta of rabbit fed atherogenic diet with L-arginine for 12 weeks (L-arginine group) shows significant decrease ofintima thickness.Sections were stained with haematoxylin and eosin (x10). I: intima

5. Discussion

Results of the present study indicated that 12 week-consumption of atherogenic diet with 0.5% cholesterol increased serum lipid profile and activated atherosclerosis lesions formationinvolving the intimal thickening. As well as, data of the present study revealed that the level of TNF-α and ET-1 were significantly elevated in the atherosclerotic rabbits (induced untreated group) and L-arginine treatment affects them insignificantly. The present study revealed that treatment with L-argininesshowed different effects on serum lipid profile.Presence of L-arginine showed significant elevation in TC, HDL-C and LDL-C level but insignificant reduction in TG and VLDL-C level when compared with atherogenic diet group (Table 1). Data obtained in this study were found to be in disagreement with most studies reported that L-arginine is advantageous due to reduction of serum TC as in clinical study conducted by Pahlavani and colleagues. They illustrated that L-arginine taken for 45 days diminished TC, TG and LDL-C concentrations significantly and improved HDL-C levels when compared with control group (11). However, Kumar and co-workers clarified that administration of L-arginine to hypercholesterolemic rabbits for 16 weeks increased serum cholesterol level. It apparently shows that L-arginine caused elevation in TC may possibly be important for normal vasculature that is accountable for the conservation of blood pressure. The mechanism by which L-arginine rises cholesterol subsequent to administration necessitates promote study. These results advocate that L-arginine supplementation necessitates an additional precaution (12). No effect on serum TNF-α level was seen in rabbits treated with L-argininewhen compared with atherogenic diet fed rabbits. Likewise, L-arginine reduced serum ET-1 level insignificantly when compared with atherogenic diet group (Table 2). Although, numerous studies showed that acute or temporary treatment with L-arginine improves vasodilation or reduces blood pressure in experimental animals or human cardiovascular diseases (13), many studies revealedthat L-arginine lacked persistent effects on endothelial action (14). Xiongand colleagues revealed that the effect of L-arginine on vascular endothelial cells is time-dependent. When human endothelial cells were exposed to L-arginine for 7 days (chronic), an over production of ICAM, VCAM and uncoupling eNOS were linked with upregulation of arginase-II and S6K1 vitalization (15). Arginase-II has been found to occupy a critical responsibility in diverse diseases include atherosclerosis (16,17).

Therefore, the reason for elevated level of TNF-α may be due to long duration of treatment with L-arginine (12 weeks). This study revealed that L-argininetreatment increased aortic total antioxidant capacity (TAC) level insignificantly in atherosclerosis model of hypercholesterolemic rabbit when compared with untreated rabbits (see Table 3). Jabłecka and colleagues confirmed that there was a statistically significant increase in serum TAC level of patients with lower extremities atherosclerotic peripheral arterial disease after two months of treatment with L-arginine suggesting that the ant oxidative fuction of L-arginine may be due to the inhibitory effect of L-arginine on superoxide radicals. Furthermore, L-arginine increased antioxidants, which were protecting the native forms of LDL from oxidation through diminishing creation of O2(18). In the present study, L-arginine showed a significant decrease in the intimal thickness of aorta in comparison with rabbits untreated with L-arginine figure (1) and table (3). Saleh and co-workers established that administration of L-arginine to hypercholesterolemic rabbits for 28 days led to significantly decreased aortic intima thickness compared to atherosclerotic untreated group (19). Li and Förstermann showed that chronic use of L-arginine restrains atherosclerotic lesion creation in atherosclerosis animal, for example diet-induced atherosclerosis in rabbits (20). In hypercholesterolemia, generation and/or liberation of NO is markedly

weakened leading to weakened vasodilation mediated by endothelium and enhanced intimal thickening. Administration of L-arginine can increase NO synthesis in cardiovascular tissue so can reduce atherosclerosis lesion and intima thickness (19).

6. Conclusion

The results of this study indicate thatL-arginine has a potential ameliorating effect on atherosclerosis. This was mainly achieved by its inhibitory effects on some inflammatory and oxidative cascades.

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