The effects of candesartan on atherosclerosis in hyperlipidemic male rabbit induced by atherogenic diet

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مرض تصلب الشرايين هو مرض يصيب الشرايين الكبيره و سببه ارتفاع مستوى الدهون بالدم، أن الكانديسارتان هو من الأدوية الأساسية التي تستخدم في علاج ضغط الدم و من الأدوية الواعدة التي تستخدم في علاج تصلب الشرايين أربعة وعشرون أرنبا محليا من الذكور أدخلت في هذه الدراسة. تم توزيع هذه الأرانب بصورة عشوائية إلى ٣ مجاميع: المجموعة الأولى هي مجموعة السيطرة الطبيعية وأعطيت غذاء قياسي طبيعي لمدة اثنا عشر أسبوعا. المجموعة الثانية أعطيت غذاء عالي الدسم يحتوي ١% كولسترول لمدة اثنا عشر أسبوعا. المجموعة الثالثة أعطيت غذاء عالي الدسم يحتوي 1% كولسترول لمدة ستة أسابيع، بعد ذلك أعطيت عقار الكانديسارتان 0.5ملغم/ كغم لكل يوم عن طريق الفم بالموازاة مع الغذاء العالي الدسم لمدة ستة أسابيع أخرى. تم سحب عينات الدم أولا عند بداية الدراسة، وعند ستة أسابيع من فترة الدراسة ثم في نهاية المعالجة البالغة أثنا عشر أسبوعا . تم قياس مستوى الدهون بالدم وهي الكولسترول الكلي، الكلسيريدات الثلاثية، الكولسترول واطئ الكثافة، الكولسترول واطئ الكثافة جدا الكولسترول عالي الكثافة كذلك تم قياس مستوى الانترليوكين-٦ و تيومرنكروتك فاكتر الفا والسي رئاكتف بروتين في الدم ,مقياس درجة التصلب، ومستوى ألإجهاد التاكسدي في نسيج الابهر المتمثل بمستوى(MDA and GSH). تم قياس عرض وسُمك جدار الشريان الأبهر بواسطة جهاز الهستومور فومتري عند نهاية الدراسة. كذلك تم فحص المقاطع النسيجية للشريان الابهر البطني عند نهاية الدراسة. سبب الغذاء العالي الدسم الذي يحتوي ١% كولسترول زيادة معنوية ($P<0.05$) في مستوى الكولسترول الكلي، الكلسيريدات الثلاثية، الكولسترول واطئ الكثافة، الكولسترول واطئ الكثافة جدا، الكولسترول عالي الكثافة، وسبب زيادة معنوية ($P<0.05$) في مقياس درجة التصلب. كما سبب زيادة معنوية ($P<0.05$) في مستوى ألاجهاد التاكسدي في الدم المتمثل (بارتفاع مستوى MDA المصاحب لانخفاض مستوى GSH). كما سبب زيادة معنوية في مستوى السايتوكاينات في الدم كان هناك أيضا زيادة معنوية في عرض وسُمك الشريان الابهر. سبب العلاج بالعقار الكانديسارتان انخفاضا معنويا ($P<0.05$) في الكولسترول الكلي، الكلسيريدات الثلاثية، الكولسترول واطئ الكثافة، وزيادة في الكولسترول العالي الكثافة، وانخفاضا في الكولسترول واطئ الكثافة جدا في الدم. بينما سبب العقاران زيادة معنوية ($P<0.05$) في مستوى (GSH) وانخفاضا معنويا في مستوى (MDA) في نسيج الابهر. كما سبب العلاج بالعقار انخفاضا معنويا ($P<0.05$) في عرض وسُمك جدار الشريان الابهر البطني.

Summary:
Atherosclerosis is a disease of large and medium-sized muscular arteries and is characterized by endothelial dysfunction, vascular inflammation, and the build up of lipids, cholesterol, calcium, and cellular debris within the intima of the vessel wall. This buildup results in plaque formation, vascular remodeling, acute and chronic luminal obstruction, abnormalities of blood flow, and diminished oxygen supply to target organ. Candesartan exert a potent anti inflammatory activity.

Objectives:
The objective of present study was to assess the effect of candesartan on atherosclerosis via interfering with inflammatory and oxidative pathways.

Method:
24 local domestic rabbits were assigned to 3 groups: Group I (*n* = 8), control; Group II (n = 8), Rabbit fed 1% cholesterol-diet (induced untreated group); Group III (*n* = 8), 1% cholesterol-diet +candesartan (0.5mg/kg/daily orally). Blood samples were collected at (0 time), after 6 and after 12 weeks on experimental diets for measurement of serum triglycerides (TG), total cholesterol (TC), HDL-C and serum high sensitive C-Reactive Protein (hsCRP), serum IL-6 and serum TNF-a. At the end of 12 weeks the aorta was removed for histopathology and histomorphometry to assess the atherosclerotic change according to American Heart Association classification of atherosclerosis phases and for aortic intema-media thickness also for aortic malondialdehyde (MDA)and reduced glutathione (GSH).

Results: Compared with the control, levels of TC, TG, LDL-C, VLDL-C, atherogenic index, hsCRP ,IL-6 ,TNF-a and aortic MDA were increased and aortic GSH and serum HDL-C were decreased in the animals with a high-fat diet ($P < 0.01$). Histologically all induced-untreated rabbit showed significant atherosclerosis lesions ($P < 0.05$). Candesartan treated groups showed significant effects on lipid parameters in comparing with induced untreated group ($P < 0.05$).Candesartan counteract the change in hsCRP, IL-6, TNF-a and MDA in compare with induced untreated group ($P < 0.01$). Candesartan prevent decrease tissue GSH level ($P < 0.01$) Morphologic analysis revealed that candesartan markedly reduced ($P < 0.05$) the severity of atherosclerotic lesion in the aorta compared with rabbits on a high-fat diet alone and decreases aortic intima-media thickness in histomorphometric measurements.

Conclusion: The results of the present study reveal that Candesartan prevented atherosclerosis in hypercholesterolemic rabbit via inhibition of inflammatory and oxidative pathways and reduced level of lipid parameters and decreased aortic intima-media thickness.

Key words:
Candesartan, inflammatory markers, oxidative stress, atherosclerosis.

Introduction:

Atherosclerosis is a chronic inflammatory, fibroproliferative disease of large and medium-sized arteries fulled by lipids, it is mostly associated with hyperlipidemia and other several risk factors[(1)].

There are many risk factors of atherosclerosis either :

1.Nonmodifiable which include age, gender and genetic.

2.Modifiable which include dyslipidaemia, hypertension, diabetes, infection, smoking and systemic autoimmune disease and others[(2)].

Atherosclerotic lesions are composed of three major components:

1. the cellular component comprised predominately of smooth muscle cells and macrophages.
2. the connective tissue matrix and extracellular lipid.
3. intracellular lipids that accumulate within macrophages, thereby converting them into foam cells.[3]

Among the many cardiovascular risk factors, elevated plasma cholesterol level is probably unique in being sufficient to drive the development of atherosclerosis, even in the absence of other known risk factors . If all adults had plasma cholesterol levels <150 mg/dl, symptomatic disease would be rare. The other risk factors, such as hypertension, diabetes, smoking, male gender, and possibly inflammatory markers (e.g., C-reactive protein, cytokines, and so on), appear to accelerate a disease driven by atherogenic lipoproteins, the first of which being low-density lipoprotein (LDL)[4].

The endothelium becomes activated by atherogenic and proinflammatory stimuli, and the expression of adhesion molecules, primarily vascular cell adhesion molecule-1 (VCAM-1), are up-regulated, and monocytes and T cells are recruited. Besides VCAM-1, other adhesion molecules, such as intercellular adhesion molecule-1, E selection, and P selection, probably contribute to the recruitment of blood-borne cells to the atherosclerotic lesion [5,6].

Multiple cytokines and acute phase reactants have been studied having possible parts to predict cardiovascular events in healthy men as well as for risk stratification in established cases of CAD[7].

The only blood biomarkers currently recommended for use in cardiovascular risk prediction by the adult treatment Panel are LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), and triglycerides[8]. However, plasma total cholesterol concentrations alone poorly discriminate risk for coronary heart disease, as more than half of all vascular events occur in individuals with below-average total cholesterol concentrations[9].

Method:

24 local domestic rabbits were assigned to 3 groups: Group I (n = 8), control; Group II (n = 8), Rabbit fed 1% cholesterol-diet (induced untreated group); Group III (n = 8), 1% cholesterol-diet +candesartan (0.5mg/kg/daily orally). Blood samples were collected at (0 time), after 6 and after 12 weeks on experimental diets for measurement of serum triglycerides (TG), total cholesterol (TC), HDL-C and serum high sensitive C-Reactive Protein (hsCRP) , serum IL-6 and serum TNF-a. At the end of 12 weeks the aorta was removed for histopathology and histomorphometry to assess the atherosclerotic change according to American Heart Association classification of atherosclerosis phases and for aortic intema-media thickness also for aortic malondialdehyde (MDA)and reduced glutathione (GSH).

Results:

Compared with the control, levels of TC, TG, LDL-C, VLDL-C, atherogenic index, hsCRP ,IL-6 ,TNF-a and aortic MDA were increased and aortic GSH and serum HDL-C were decreased in the animals with a high-fat diet ($P < 0.01$). Histologically all induced-untreated rabbit showed significant atherosclerosis lesions ($P < 0.05$). Candesartan treated groups showed significant effects on lipid parameters in comparing with induced untreated group ($P < 0.05$). Candesartan counteract the change in hsCRP, IL-6, TNF-a and MDA in compare with induced untreated group ($P < 0.01$). Candesartan prevent decrease tissue GSH level ($P < 0.01$) Morphologic analysis

revealed that candesartan markedly reduced ($P < 0.05$) the severity of atherosclerotic lesion in the aorta compared with rabbits on a high-fat diet alone and decreases aortic intima-media thickness in histomorphometric measurements.

Table 1: Represents lipid profile for three experimental groups

		TC mg/dl	TG mg/dl	HDL mg/dl
Normal control	**Zero time**	**47.8±0.9**	**40±0.6**	**17±0.42**
	12 weeks	**49.5±1.14**	**39±0.76**	**18±0.47**
Induced untreated	**Zero time**	**49±0.98**	**40±0.96**	**19±0.42**
	12 weeks	**883±45***	**225±9***	**12±0.36***
Candesartan 0.5 mg/kg	**Zero time**	**50.6±3.34**	**39±0.76**	**18.16±0.47**
	12 weeks	**330±18***	**100±4.2***	**16±0.33***

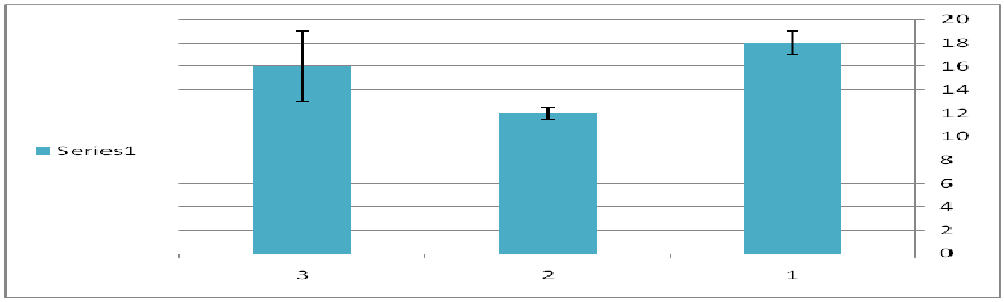


Figure 1: Represents HDL concentration for three experimental group 1.normal, 2.induced untreated, 3.Candesasrtan treated.

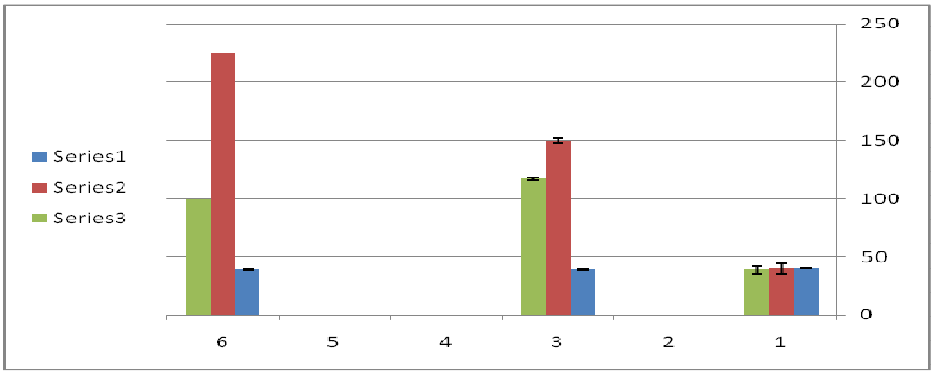


Figure 2: Represents TG concentration for three experimental group 1.normal, 2.induced untreated, 3.Candesasrtan treated.

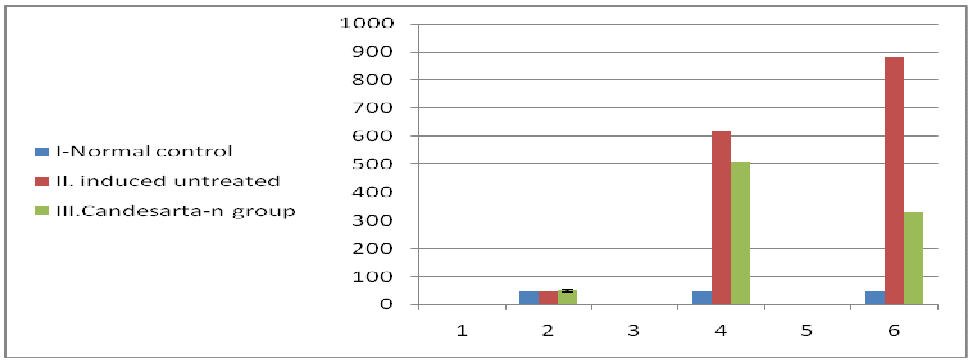


Figure 3: Represents TC for three experimental group.

Table 2: Represents changes of inflammatory biomarkers for three experimental groups:

		Hs-CRP(mg/l)	IL-6 (Pg/ml)	TNF-α (pg/ml)
Normal control	**Zero time**	**3.5±0.5**	**0.75±0.1**	**0.6±0.09**
	12 weeks	**4.3±0.7**	**1.1±0.09**	**1.05±0.06**
Induced untreated	**Zero time**	**4.2±0.9**	**1.06±0.05**	**0.77±0.1**
	12 weeks	**20.7±1.7***	**5.5±0.5***	**7.05±0.44***
Candesartan 0.5 mg/kg	**Zero time**	**4.1±0.5**	**0.9±0.08**	**0.85±0.05**
	12 weeks	**10.4±0.6***	**2.8±0.3***	**2.7±0.17***

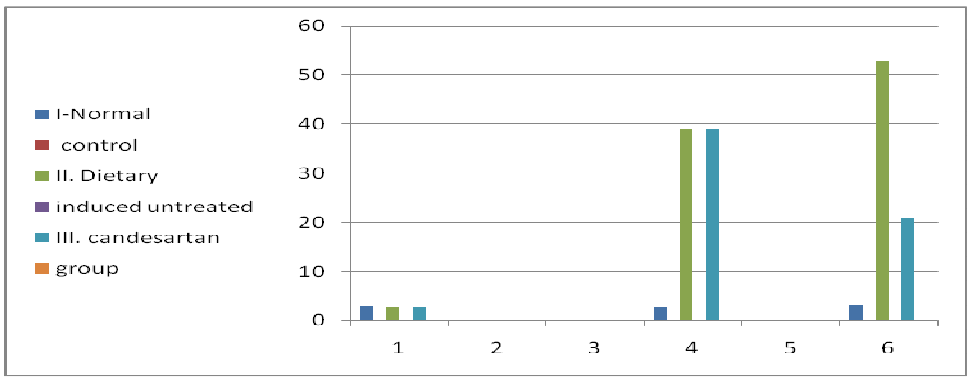


Figure4: Represents Hs-CRP for three experimental groups

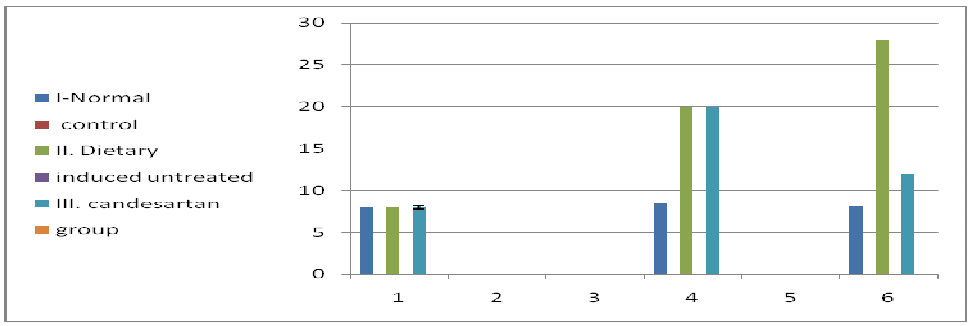


Figure5: Represents IL-6 for three experimental groups

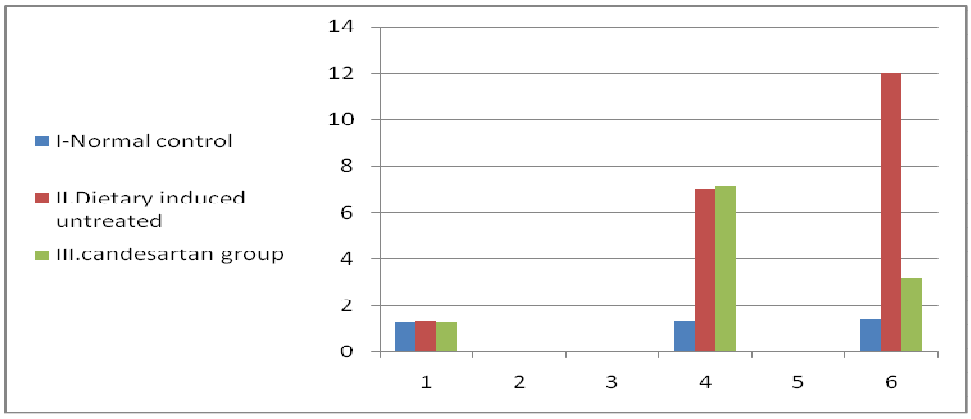


Figure6: Represents TNF-α for three experimental groups

Table (3): Changes of aortic MDA level µmole/gm of the 3 experimental group.

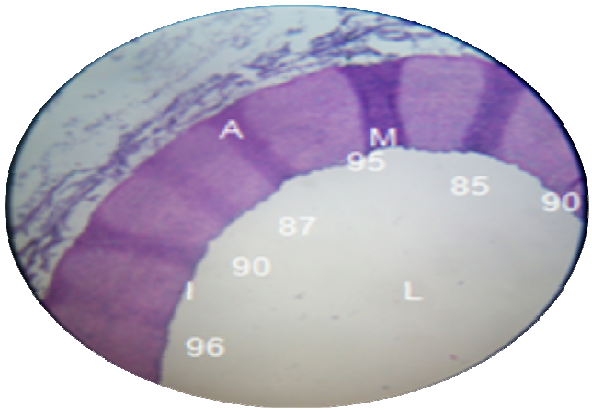
group	*MDA level(*µmole/gm aorta)
I-Normal control	*1.283±0.5*
II. Dietary induced untreated	*2.2±0.4**
III. candesartan group	*1.48±0.6**

Table (4): Changes of aortic GSH level nmole/mg of three experimental groups.

group	*GSH level(*nanomole*/mg aorta)*
I-Normal control	*37.16±0.67*
II. Dietary induced untreated	*24.5±0.67**
III. candesartangroup	*29.66±0.67**

Table (5): Changes of aortic intimal thickness level (µm) of three experimental groups.

group	*Aortic intimal thickness(*µm*)*
I-Normal control	*87.33±12*
II.Dietaryinduced untreated	*325.83±12**
III. candesartan group	*191.66±12**



Figur: (1) Photomicrograph of histophotometric section in aortic arch of rabbit fed on normal diet for 12 wks (normal control) show the normal intimal thickness and intact continuous endothelium. Stained with haematoxylin and Eosin (x10), where, I: intima of the aorta, M: media of the aorta, A: adventitia of the aorta and L: the lumen of the aorta.

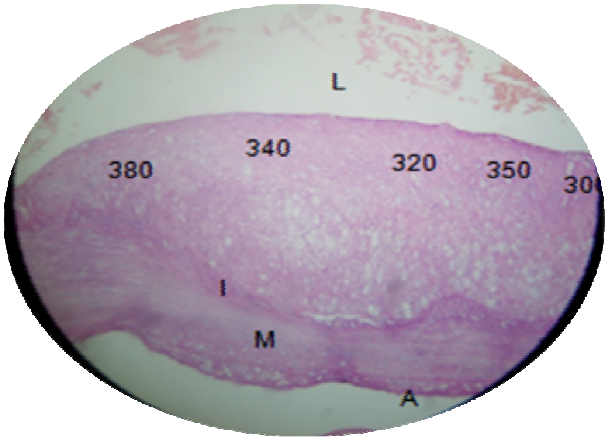


Figure (3): Photomicrograph of histomorphometric section in aortic arch of rabbits on atherogenic diet for 12 wks (induced untreated show diffuse intema thickening and in completely coalesced extracellular lipid underneath a layers of macrophages and smooth muscle cells. The section stained with haematoxylin and eosin (x10). where, I: intima of the aorta, M: media of the aorta, A: adventitia of the aorta and L: the lumen of the aorta.

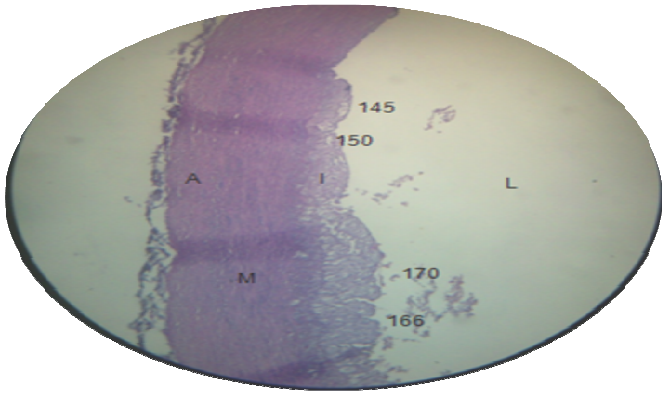


Figure (3): Photomicrograph of histomorphometric section in aortic arch of candesartan hyperlipidemic rabbits. The section stained with haematoxylin and eosin (x10). where, I: intima of the aorta, M: media of the aorta, A: adventitia of the aorta and L: the lumen of the aorta.

Discussion:

In the current study, feeding of atherogenic diet to rabbits for 12 weeks resulted in marked hypercholesterolemia in which serum TC, TG, LDL-C, VLDL-C and atherogenic index levels were found to be increased significantly. While, HDL-C level was found to be decreased significantly. These results were obtained by **(Romero *et al.*, 2000)[14] ; (Prasad *et al., 2007*)[15]** and (**Nigris *et al.*, 2008)[16]**

Significant changes in serum TC, TG, HDL-C, LDL-C, VLDL-C and atherogenic index levels in candesartan treated rabbits as compared with that in the control untreated rabbits. Our results were supported by **(Vasilios P ..et al., 2001)**[17], **(Michael T.et al,2004)**[18]. These results demonstrated that angiotensin receptor blockade attenuates the degree of atherosclerosis and reduces lipid profile. The AT_1 receptors was up regulated in the case of hypercholesterolemia and ANGII was involved in the progression of atherosclerosis. Therefore, treatment with Candesartan blocks AT_1 receptors and attenuates this process.

In this study, hs-CRP level was increased many folds compared with that of normal rabbits group. These results are in agreement with **(Ridker..et al 2004)** [19], **(Nissen..et al 2005)** [20]

In the present study, treatment with Candesartan leads to significant reduction in hs-CRP plasma level comparing with untreated rabbits and these results are in line with **(Carmine S.. et al 2007)** [21].

In this study, atherogenic diet causes significant increase in TNF-a level when compared with normal rabbits this is in agreement with **(Ross..et al; 1993)** [22]. TNF-a is an important modulator in the chronic inflammatory process of atherosclerosis. TNF-a elicits responses predominantly through the TNF-R1 (p55) receptor, including mediators of inflammatory processes **(Liu..et al; 1996)** [23]

In our study, treatment of hyperlipidemic rabbits with Candesartan leads to reduce TNF-a level compared with untreated hyperlipidemic rabbits, these results are supported by **(Larrayoz..et al 2009)** [24] who showed that the proinflammatory cytokines tumor necrosis factor alpha, interleukin-1 beta and interleukin-6 were reduced by Candesartan treatment.

In the present study, Candesartan treatment causes significant reduction of IL-6 level (**Mohan..et al, 2009)** [25] , **(Liu Y, et al , 2010)**[26]. In these studies, Angiotensin II type-1 receptor blockade (ARB), has been reported to have anti-inflammatory effects and reduce IL-6 level.

Conclusion: The results of the present study reveal that Candesartan prevented atherosclerosis in hypercholesterolemic rabbit via inhibition of inflammatory and oxidative pathways and reduced level of lipid parameters and decreased aortic intima-media thickness.

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