Obesity And Inflammation Induces By High Fat Diet Concomitant With Mild Fatty Streak In Coronary Artery: Immuno-Histopathological Study.

Jasem Hannon Hashim Al-Awadi[1], Alaa Jawad Hassen[2],Karem Hammed Rashid[3].

1-College of Science, Al-Qadesiya University. 2-College of Science, Babylon University. 3-College of Agriculture, Karbala University.

Keywords:Obesity,Inflammation,HighFatDiet,CoronaryArtery,Atorvastatin

(Received: May2013, Accepted: Dec2013)

Abstract:

This research was designed to study the obesity which induced by rats fed on high fat diet which characterized by gain in body weight and elevation of some immunological parameters which often concomitant with mild to moderate fatty streak in coronary artery and the effects of atorvastatin treatment of male albino rats, the result showed:

High significant increase($p<0.0005$)in total and gain body weight of positive control rats, while atorvastatin treatment caused significant decrease($p<0.005$) in both total and gain body weight of hyperlipidemic rats as compared with positive control group, also atorvastatin reduce gain body weight in normolipidemic rats as compared with negative control group.

There were high significant increase($p<0.0005$)in C3,C4 in normolipidemic rats which treated with atorvastatin, while there were significant increase($p<0.0005$)in CRP,C3,C4 in hyperlipidemic rats of positive control group, whereas the treatment by atorvastatin result in returned the concentration of CRP,C3,C4 to its normal values as compared to negative control rats.

The histological sections of coronary arteries and its some branches were revealed the presence of fatty streak, infiltration of fat laden cells(foam cells)in subintimal layer of coronary arteries of positive control rats ted on HFD,also smooth muscle cell proliferation, vacuolar of tunica media were observed in the coronary arteries of these animals as a signs of onset of atherosclerosis, whereas atorvastatin reduced completely the presence of all these changes after three months of treatment**.**Thus our conclusions that atorvastatin reduced the body weight gain in hyperlipidemic animals, induce inflammatory changes in normolipidemic animals and reduced all signs of atherosclerosis in coronary arteries.

السمنة والالتهاب المستحثة بالغذاء عالي الدهن تتزامن مع تراكم خفيف للدهون في الشريان التاجي: دراسة مناعية-مرضية نسجية.

جاسم حنون هاشم[1] علاء جواد حسن[2] كريم حميد رشيد[3]

1-كلية العلوم-جامعة القادسية. 2-كلية العلوم- جامعة بابل. 3-كلية الزراعة- جامعة كربلاء.

مفتاح البحث:السمنة,الالتهاب,الغذاء مرتفع الدهن,الشريان التاجي,اتورفاستاتين.

الخلاصة:

صمم هذه البحث لغرض دراسة السمنة المستحثة بالغذاء عالي الدهن والتي تتصف بزيادة الوزن المكتسب وارتفاع المعايير المناعية الالتهابية والتي غالبا مايرافقها تراكم خفيف الى معتدل للدهون في الشريان التاجي وتاثير العلاج بعقار الاتورفاستاتين على ذكور الجرذ الامهق. وقد اوضحت نتائج الدراسة مايلي:

حدوث زيادة معنويةعالية جدا ($p<0.0005$) في وزن الجسم المكتسب والكلي في مجموعة السيطرة الموجبة,فيما تسبب عقار الاتوتورفاستاتين في حصول انخفاض معنوي عالي($p<0.005$) في وزن الجسم المكتسب والكلي في الجرذان الطبيعية الدهن, وحدوث انخفاض معنوي عالي جدا($p<0.0005$) في وزن الجسم المكتسب و الكلي في الجرذان مفرطة الدهن والتي عولجت بالعقار المذكور اذا ماقورنت بمجموعة السيطرة الموجبة.

ارتفع معنويا($P<0.0005$) كل من مكوني المتمم C3,C4 في الجرذان طبيعية الدهون والمعاملة بعقار الاتورفاستايتن,فيما ارتفع بروتين الطور الحاد ومكوني المتمم C3,C4 في الجرذان مفرطة الدهن-مجموعة السيطرة الموجبة ,فيما ادى العلاج بعقار الاتورفاستاتين الى اعادة التركيز الطبيعي لبروتين الطور الحاد وكذلك تركيز مكوني المتمم C3,C4الى مستوياتها الطبيعية اذا ماقورنت بالسيطره السالبة.

اوضحت المقاطع النسجية للشريان التاجي وبعض فروعه وجود تراكم وارتشاح للخلايا المحملة بالدهن تحت الطبقة البطانية للشريان التاجي للجرذان في مجموعة السيطرة الموجبة والتي تغذت على غذاء عالي الدهن طيلة فترة الدراسة,كما كثرت الخلايا العضلية الملساء في الغلالة الوسطى وكثرت الفجوات في هذه الطبقة ,كعلامات لبدء تصلب هذه الشرايين في هذه الحيوانات.فيما ازال العلاج بعقار الاتورفا ستاتين معظم الاثار السلبية المترتبة على فرط الدهون والتي ظهرت بشكل تصلبات خفيفة في بعض الشرايين التاجية.

وعليه يمكن الاستنتاج ان عقار اتورفاستاتين قد خفض وزن الحيوانات مفرطة الدهن واستحث تغيرات التهابية في الحيوانات طبيعية الدهن واختزل جميع العلامات الدالة على تصلب الشرايين التاجية.

Introduction:

Diets containing high amount of fats or cholesterol lead to both hypercholesterolemia and hypertriglyceridemia which are major prognosis for cardiovascular diseases CVD[**1**]; and leading cause of death in developing and developed countries[**2**].

HFDs are known as a risk factor for coronary vascular diseases (CVD), insulin resistance and obesity accompanied by systemic inflammation, the features of metabolic syndrome **[3].**

Obesity is a severe metabolic disorder, characterized with increases in energy intake and a decrease in energy output concerning body weight and glucose metabolism **[4].** Obesity is associated with many important complications such as diabetes and coronary heart disease **[5].**

The obesity and diabetes are often associated with Hyperlipidemia in a condition known as metabolic syndrome, which is characterized by a low serum HDL-C and elevated TG. Levels, blood pressure and blood glucose levels following a 12 hour fast **[6].**

Hyperlipidemia (mainly increased level of cholesterol or low density lipoprotein (LDL)-cholesterol) is an important risk factor in the initiation and progression of atherosclerotic lesions **[7]**.

Atherosclerosis is a common condition in both developed and developing countries and is now recognized to be an inflammatory condition leading to the development of ischemic heart diseases, cerebrovascular diseases and peripheral vascular disease. Atherosclerosis and its attendant morbidity and mortality remain a global health issue, with more than 17.5 millions death worldwide yearly attributable to the cardiovascular complication of this disease. The common risk factors for developing atherosclerotic cardiovascular disease, hyperlipidemia, hypertension, tobacco use, diabetes mellitus, and family history, alone or in combination, are associated with pathophysiological vascular phenotype **[8]**. One of the most important imitial events in the development of atherosclerosis is the accumulation of cells containing excess lipids within the arterial wall which mostly macrophages transformed monocytes that engulfment oxidized-LDL to become foam cells or fat laden macrophages. **[9]**.

Walt illustrated that the pathogenesis of atherosclerosis includes a highly complex series of inflammatory, metabolic, thrombotic and other known, possibly also some as yet unidentified mechanisms; moreover, the feared end points of atherosclerosis are cardiovascular disease (CVD)

which have the main causes of death and disability in developed countries**[10]**. Traditionally atherosclerosis has been thought to be solely a lipid accumulation disease in the arteries **[11]**; also inflammation plays a substantial role in the development and aggravation of atherosclerosis. On the other hands certain studies revealed that all the branches of the immune system are known to participate in the pathogenesis of atherosclerosis **[12]**.

Till now many efforts were performed to find out good medications against hyperlipidemia and its consequence coronary artery disease which collectively called Hypocholesterolemic drugs, Such as statins. Statins are widely prescribed medications effective to reduce blood lipids level. Atorvastation are members of an important class of lipid-lowering drugs, inhibitors to HMG-COA reductase. It's competitive inhibition of HMG-COA reductase reduce the amount of HMG-COA converted to Mevalonate, the rate-limiting step of cholesterol biosynthesis **[13]**. Thus this study was done to investigate the role of high amount of fat in diet which induces obesity and inflammatory process that eventually lead to atherosclerosis, before and after treatment with atorvastatin.

Materials and Methods:

Animals and groups:

Eighty male albino rats (*Rattus norvegicus*) was purchased from animal care center college of Medicine, University of Baghdad, Iraq, their ages ranged between (3.5-4) months, while weight were between (200- 250 g).Animals were housed in controlled condition of temperature (25 ± 3C°), and 12 hours light-dark cycles. Rats was acclimatized for two weeks and access to drink water *add libitum* and standard chow diet, then divided in to two major groups, each of forty animals.

1-Normolipidimic group: This group consists of two sub groups each of ten animals. The animals were caged in two large polypropylene cages (five rats for each cage). The animal in this groups maintained on standard chow for four months, then administered orally once daily for three months of the following treatments:

1-1-Subgroup (1): Normal control group (negative control). This group was administered orally by gastric gavages 1ml normal Saline /Animal/ day. (N.NS).

1-2-Subgroup (2) :(soluble albumin treated animals) Immunized group: each animal in this group was administered orally of 2% soluble albumin prepared in normal saline (PH=7.4).

1-3-Subgroup (3): Atorvastatin treated animals: each animal in this group was administered orally of 1 ml of atorvastatin 5mg/kg/day. (N.ATO.). phosphate buffered). 1ml of 2% soluble albumin/Animal/day. (N.NS+S.alb.).

1-4-Subgroup (4) : Atorvastatin and soluble albumin treated animals : each animal in this group was administered orally of 5mg/kg/day of atorvastatin concomitant with 2% soluble albumin.(N.ATO+S.alb.).

2-Hyperlipidemic group: Animals in this group were fed on hyperlipidemic diet(HFD) for four months, Which were maintained on cholesterol-rich diet (HFD) for four months to induce Hyperlipidemia in this group. At the end of this period rats were divided in to four subgroups each of ten rats which were caged in two poly propylene cages (each cage contained five rats). And treated as following:

2-1-Subgroup (1): Hyperlipidemic control rats: Each rat was administered once daily orally 1ml of normal saline by gastric gavages. (H.NS.).

2-2-Subgroup (2): Soluble albumin treated rats: Each rat in this group was administered orally 1ml of 2% soluble albumin prepared in normal saline. (H.NS+S.alb.).

2-3-Subgroup (3): Atorvastatin treated rats: Each rat in this group was administered orally 5mg/kg/ day of atorvastatin.(H.ATO).

2-4-Subgroup (4): Atorvastatin and Soluble albumin treated rats: Each rat in this group was administered orally 1ml of 5mg/kg/day of atorvastatin with 2% soluble albumin (atorvastatin and Soluble albumin were prepared in phosphate buffer PH = 7.4).(H.ATO+S.alb.).

At the end of second period of experiment which called treatment period which continuous for three months, all animals in the normolipidemic and hyperlipidemic groups were weighted and scarified after overnight fasting.

Blood Sampling:

A bout 5ml of Blood was collected by direct heart puncture after overnight fasting at end of three months (second period of study), and after anesthetized of animal with chloroform and ketamin hydrochloride injection, blood was placed in gel test tube and left to stand for 30 minutes at room temperature to allowing clotting. The serum samples were prepared by centrifugation at 3000 rpm for 10 minutes to estimate the levels of complement components C3,C4,and C-reactive protein CRP (Immunological assays).

C-reactive protein (CRP):

The reagents were supplied by Spinreact and serum C-Reactive protein (CRP) was measured according to **[14].**

Determination of the C3,C4 proteins:

The reagents were supplied by LTA-Bussero (Milan). Italy, and serum C3,C4 protein was measured according to **[15].**

Histopathology

According to**[16]** processing and staining technique was as follow: Tissue (heart with 5mm of aorta which contain the origin of coronary artery) obtained from all experimental groups were washed immediately with saline and then fixed in 10% buffered neutral formalin solution. After fixation, the tissue was processed by embedding in paraffin. Then, the tissue was sectioned and stained with hematoxylin and eosin (H&E) and examined under high power microscope (200,400X) and photomicrographs were taken.

Statistical analysis:

Statistical Package for Social Science (SPSS) system/ version 13 was used to analyze our results. The analysis of variance (ANOVA) and the paired sample T test were used for this purpose.

Results:

Body weight measurement:

As shown in table 2,results of this study showed body weight changes in experimental animals and the changes represented by a gain in body weight between the final and initial body weight .There was significant decrease ($p<0.005$) in body weight gain in group (1-3)N.ATO. which treated with atorvastatin (154.07±11.825) compared to negative control group(1-1) normolipidemic rats treated with normal saline (192.33± 34.54),but there was insignificant changes($p>0.05$) in body weight gain in both groups (1-2,1-4)which treated with normal saline supplemented with 2%

soluble albumin ,or atorvastatin with 2% soluble albumin respectively compared with negative control rats. By comparison of negative control ,positive control rats(2-1.H.NS.) have significant increase ($p<0.0005$) in body weight gain (277.5± 33.53).There was no significant difference in body weight gain ($p>0.05$) in group (2-2)hyperlipidemic rats treated with normal saline and 2%soluble albumin compared to hyperlipidemic rats(2-1 H.N.S.) positive control,but there were significant decreases($p<0.0005$) in body weight gain in hyperlipidemic rats treated either with atorvastatin alone or supplemented with 2%soluble albumin (205.71±20.84 ,211.74±10.41)respectively. All experimental groups haven´t any significant differences in their initial body weight as compared with each others.

Final body weight decrease significantly ($p<0.05$) in normal rats treated with atorvastatin ,whereas there is insignificant changes($p>0.05$) in final body weight in both groups (1-2,1-4) as compared with negative control(1-1).

Hyperlipidemic rats (positive control) have significant increase in their final body weight ($p<0.0005$) compared to negative control (normolipidemic rats).whereas there are significant decreases ($p<0.005$) in the final body weight of both groups(2-3,2-4), hyperlipidemic rats treated either with atorvastatin only or supplemented with 2%soluble albumin compared with positive control.

Table (1):changes of body weight in study groups:

Groups	Body weight(mean±S.D)		
	Initial body weight (gm.)	Final body weight (gm.)	Gain in body weight (gm.)
1-1-N.N.S.	273.29±19.68[a]	465.62±47.37	192.33±34.54
1-2-N.N.S+S.alb.	274.91±16.94[a]	452.93±21.17[a]	178. 02±10.53[a]
1-3-N.ATO	271.1±18.0017[a]	425.98±20.42[*b]	154.07±11.825 [**b]
1-4-N.ATO+S.alb.	280.3±13.94[a]	483±27.43[a]	202.7±16.91[a]
2-1-H.N.S.	276.5±19.43[a]	544±45.60[***c]	277.5±33.53[***c]
2-2-H.N.S+S.alb.	273.06±17.042[a]	524.41±28.75[a]	251.37±22.44[a]
2-3-H.ATO.	274.97±16.088[a]	480.68±35.25[**d]	205.71±20.84 [***d]
2-4-H.ATO.+S.alb.	272.91±16.41[a]	484.65±24.22[**d]	211.74±10.41 [***d]

All values represent mean±S.D(N=10),* Significant differences ($p<0.05$), **Significant differences ($p<0.005$)),***Significant differences ($p<0.0005$), a=no significant differences, b=significant differences when compare group(1-3)with negative control group(1-1)., c=significant differences when compare positive control group(2-1)to negative control group(1-1), d=significant differences when compare hyperlipidemic treated groups(2-3,2-4),with its respective positive control group(2-1).

Changes in an Immunological Parameters in Different Study Groups:

Complement components C_3 ,C_4 , and C-reactive protein plasma concentration results were illustrated in table(3). There was high significant increase($p<0.0005$)in C_3 concentration (268.09±28.894) in normal rats treated with atorvastatin(5mg/kg/day) and less significant increase ($p<0.005$) in normal rats treated with atorvastatin(5mg/kg/day) supplemented with 2%soluble albumin compared to negative control (214.110±24.041).

High fat diet(HFD)(2%cholesterol)result in high significant increase ($p<0.0005$)in C_3 concentration in hyperlipidemic rats (positive control) compared to negative control rats, whereas atorvastatin significantly decrease ($p<0.0005$) C_3 concentration in hyperlipidemic rats

treated with this drug compared to positive control rats, as well as there was improvement in C_3 concentration supported by high significant decrease ($p<0.0005$) in hyperlipidemic rats treated with atorvastatin supplemented with 2%soluble albumin compared to hyperlipidemic positive control rats.

Normal rats which treatment with atorvastatin (5mg/kg/day)for three months showed highly significant increase ($p<0.0005$)in C_4 concentration (111.02±5.268)compared to respective value(82.640±4.126) which observed in negative control rats, as well as atorvastatin supplemented with 2%soluble albumin result in significant increase($p<0.05$)in C_4 concentration (92.15±6.04) compared to negative control rats.

Rats feed on high fat diet (2%cholesterol)for seven months observed to have high significant increase ($p<0.0005$)in C_4 concentration (106.750±10.257) compared to negative control rats, whereas these rats treated either with 2%soluble albumin or atorvastatin (5mg/kg/day) alone were observed to undergo improvement with significant decrease ($p<0.05$) in C_4 concentration compared to positive control rats , but there was high significant decreases($p<0.0005$) in C_4 concentration in hyperlipidemic rats treated with atorvastatin(5mg/kg/day)together with 2%soluble albumin.

Hyperlipidemic rats in positive control group were observed to have significant increase($p<0.0005$)in CRP concentration(11.4±1.9) compared to negative control(6.0±0.0). Atorvastatin alone(5mg/kg/day)and supplemented with 2%soluble albumin significantly($p<0.0005$)reduced CRP concentration to its normal value in hyperlipidemic rats treated by this drug compared to positive control rats.

Table(2):changes in an immunological parameters in different study groups:

Groups	C_3 mg/dl±S.D	C_4 mg/dl±S.D	CRP mg/l±S.D
1-1-N.N.S	214.110±24.041	82.640±4.126	6.0±0.0
1-2-N.N.S+S.alb.	232.07±39.95^{a}	72.32±15.71^{a}	7.2±2.53^{a}
1-3-N.ATO.	268.09±28.894 ^{***b}	111.02±5.268 ^{***b}	7.8±2.9^{a}
1-4-N.ATO.+S.alb	259.76±23.184 ^{**b}	92.15±6.04^{*b}	6.6±1.9^{a}
2-1-H.N.S.	270.580±15.914^{***c}	106.750±10.257^{***c}	11.4±1.9^{***c}
2-2-H.N.S.+S.alb.	201.360±17.199^{***d}	94.17±10.60^{*d}	10.8±2.53^{a}
2-3-H.ATO.	212.890±31.171^{***d}	97. 960±4.627 ^{*d}	7.2±2.53^{**d}
2-4. H.ATO.+S.alb.	183.840±19.610^{***d}	92. 15±6.04^{***d}	6.6±1.9^{***d}

All values represent mean±S.D from(n=10)rats.*Significant differences ($p<0.05$),**Significant differences ($p<0.005$), ***Significant differences ($p<0.0005$). a=no significant differences , b=significant differences between group (1-1) and groups(1-2,1-3,1-4). c=significant differences between group(1-1) and group(2-1). d=significant differences between group(2-1) and groups (2-2,2-3,2-4).

Histopathology of coronary artery:

Microscopic examination of cross-section of normolipidemic heart muscle rats (group 1-1)stained with heamatoxylin-eosin stain shows well defined very thin intimal layer, consist of endothelium layer, distinguish media, and adventitia figure (1). In all normolipidemic rats treated either with atorvastatin alone or supplemented with soluble albumin there were no changes in its coronary arteries and similar to normal control rats figure (1).

Compared with negative control group, microscopic examination of hyperlipidemic positive control rats showed mild intimal wall thickness and increase of the total thickness of the

coronary artery wall in all ten rats fig(2,3). Moreover fatty streak characterized by foam cells appeared in sub intimal layer , as well as irregular thickened media with mild degree of smooth muscle cells(SMC) proliferation in some cases and vacuolar of tunica media as showed in figure(4). 15%of these animals developed atheromatus plaque in this group figure(5). Hyperlipidemic rats treated either with torvastatin(5mg/kg/day) alone or supplemented with soluble albumin2% revealed no fatty streak nor atheromatus plaque in any of twenty rats of these two groups ,only mild thickness of intimal and total wall thickness in some cases, about two of ten in group(2-3) and one of ten in group (2-4) as observed in figure (6).

Discussion:

Development of atherosclerotic disease is a complicated process involving accumulation of lipid-containing particles in the walls of coronary arteries and other major arteries within the body. A high-fat diet causes cholesterol levels to increase in susceptible people, which leads to obesity. The weight gain in high cholesterol diet (HCD) of rats was significantly higher than control rats reflecting the influence of high cholesterol diet**[17]**. Similarly, in present study there was significant weight gain in positive control group which fed on (HCD) for seven months as compared to normal control groups.

Cholesterol 2%supplemented diet in positive control rats caused significant increase in both final and gain body weight compared with negative control rats. It has been shown that High fat diet (HFD) induce body weight gain and adiposity in animals and humans **[18].** Similarly, we found that 2%cholesterol fed animals for seven months caused significant increases in body weight gain and adiposity may be due to increased intra-abdominal fat pad mass, this increase may be attributed to increase in food intake as well as to increase of subcutaneous, visceral and abdominal adiposity(such as abdominal, perirenal, epididymal, retroperitoneal white adipose tissues)**[19].**

Normolipidemic and hyperlipidemic rats treated by atorvastatin for three months had undergone decrease in the body weight gain as well as final body weight compared to their respective control group. Atorvastatin, a recently introduced statin produces pronounced lipid lowering via 3-hydroxy3-methylglutarylCoenzymeA reductase inhibition (HMG-CoA). Atorvastatin lead to greater decreases in LDL-C,TG and apo-B levels than other statins**[20]**,which lead to decrease of deposition of lipid in body organs and reduce body weight.

In experimental field, this result consistent with other study of **[21]**who noticed that hyperlipidemic rats treated for eight weeks with atorvastatin significantly reduced body weight and attenuated HFD-induced hyperlipidemia and liver steatosis in rats.

Complement component (c_3, c_4) concentrations showed significant increases in positive control rats as compared with negative control rats, as well as their values remained without any change in rats treated with normal saline supplemented with 2% soluble albumin as compared to positive control rats. The possible explanation of our result that the increases of complement component plasma concentrations may be due to its production by different types of cells such as adipocytes, hepatocytes, macrophages, vascular smooth muscle cells VSMC which stimulated by c-reactive protein as mechanism defense during hyperlipidemia and atherosclerosis,CRP is able of activating adaptive immune response via activating complement system (classical pathway), in which C1q is one of the binding sites of CRP, thus, CRP stimulate early phase of complement cascade , one of which C4**[22]**.

C_3 is also formed in different cells, such as hepatocytes and macrophages,it could be hypothesized that the C_3 increase is not adipocyte-derived but it may have its origin in the liver. Persistently increased concentrations of chylomicron remnants may lead to enhanced C_3

production by the liver, as has been demonstrated for cytokines **[23]**. Alternatively, chylomicron remnants are taken up by macrophages, and the late C_3 increase may originate from these cells.

The link between C_3 and lipid metabolism is implied by the association of serum C_3 concentration with serum lipid levels. There are some evidences which referred to higher serum C_3 levels in hypertriglyceridemic subjects as compared to normolipidemic control subjects. Serum C3 also seems to be elevated in obesity **[24]**.

The relationship between serum C_3 and various CHD risk factors may therefore reflect the degree of atherosclerosis, which is enhanced by the CHD risk factors. Atherosclerosis is a complex inflammatory disease triggered by several cardiovascular risk factors (e.g., hyperlipidemia, hypertension, obesity, diabetes, smoking), which promote endothelial damage and neointimal accumulation of various cell types (e.g. VSMCs, monocytes/macrophages, T-cells and dendritic cells), and non-cellular material, such as modified lipids and extracellular matrix components**[25].**

Hyperlipidemic rats induced by fed on HFD (2%cholesterol supplemented diet)for seven months showed significant increases in c-reactive protein levels (both positive control and immunized groups). Our finding could be attributed to that the CRP is produced basically in response to any kind of inflammatory stimulus. It is produced mainly by liver in response to inflammatory signals, most prominently IL-1ß, IL6 and TNF-α . The most important cells that secrete IL6 , IL1ß and TNF-α is stimulated monocyte and macrophages, endothelial cells, fibroblast cells and plasma cells all of which were contributed in hyperlipidemia and early atherosclerosis events **[26].** CRP can also be directly synthesized by the cells at the site of inflammation e.g. monocytes, endothelial cells, fibroblasts and adipocytes **[27]**. CRP recognizes particles that express phospocholine, phospocholine is located between phospholipids in cell membranes and in normal conditions it is coated so that CRP cannot interact with it. but, when the cell membrane is damaged, CRP is able to interact with the phospocholine and initiate inflammatory response. This could be an important mechanism in the apoptotic and necrotic cells elimination, as well as, CRP can be bound to nuclear material such as histones, chromatins and ribonucleic acid particles. From all these interactions we can concluded that the function of CRP could be the removal of disposable materials,which can be lead to aggravate of athersclerosis in absence of CRP **[28].**

atorvastatin alone or in combination with 2% soluble albumin was decreased significantly the CRP concentration to its normal value in hyperlipidemic rats (both H.ATO and H.ATO+S.alb. groups) as compared to negative control rats. This result could be attributed to the actions of atorvastatin which observed to reduces tissue injury as well as reduced atherosclerosis plaque , therefore reduces the inflammatory markers(CRP,C_3,C_4).

Another possible reason for decreased of CRP concentration in hyperlipidemic rats treated with atorvastatin was detected by **[29]**who demonstrate that Atorvastatin reduces the number of intimal macrophages, monocyte-chemoattractant protein-1 (MCP-1) and the activation of nuclear factor NFkB in hypercholesterolemic rabbits . Cytokines receptors are coupled to GTP-bound proteins, and the binding of leukocytes to the endothelium is regulated by G protein. Statins can affect small GTP-ases or trimeric G proteins, by preventing their prenylation and thus reducing the inflammatory response**[30].** Statins diminish leukocytes recruitment in postcapillary venules, stimulated by a lipid mediator (platelets activation factor-PAF or leukotriene B4) in hypercholesterolemic rats**[31].** Another antiinflammatory effect of statins on monocytes and macrophages was the decrease of the expression of intercellular adhesion molecule -1 and the secretion of interleukine-6 (IL-6), induced by lipopolysacharides (LPS) **[32]**, by these effects atorvastatin may acts to decrease the inflammatory markers(CRP,C_3,C_4)which used in our study which elevated after induced hyperlipidemia in our model.

Histology of coronary artery and its branches in hyperlipidemic rats fed on HFD(2%cholesterol)for seven months showed mild to moderate degrees of atherosclerotic

lesions which represented by fatty streak, subintimal foam cell infiltration, mild smooth muscle cell proliferation, vacuolar of medial layer, all these events represent the first steps of atherosclerosis onset in the coronary arteries. These changes in coronary vessels of hyperlipidemic rats could be attributed to high levels of cholesterol in both serum and tissues particularly in the arteries, these conditions could induces oxidized-LDL, by oxidative stress that result from ROS which lead to transforming of monocytes to macrophage, with phagocytosis of ox-LDL, these cells become macrophage laden fats(foam cells) which filled subintimal layer and form fatty streak in the coronary arteries**[33]** The earliest type of lesion, the so-called fatty streak, which is common in infants and young children, is a pure inflammatory lesion, consisting only of monocyte-derived macrophages and T lymphocytes, whereas in persons with hypercholesterolemia, the influx of these cells is preceded by the extracellular deposition of amorphous and membranous lipids**[34].**

Atherosclerosis is an inflammatory disease. Because high plasma concentrations of cholesterol, in particular those of low-density lipoprotein-cholesterol(LDL-c), are one of the principal risk factors for atherosclerosis, the process of atherogenesis has been considered by many to consist largely of the accumulation of lipids within the artery wall**[33].** The endothelial dysfunction that results from the injury leads to compensatory responses that alter the normal homeostatic properties of the endothelium. Thus, the different forms of injury increase the adhesiveness of the endothelium with respect to leukocytes or platelets, as well as its permeability. The injury also induces the endothelium to have procoagulant instead of anticoagulant properties and to form vasoactive molecules, cytokines, and growth factors. the inflammatory response stimulates migration and proliferation of smooth-muscle cells that become intermixed with the area of inflammation to form an intermediate lesion. If these responses continue unabated, they can thicken the artery wall, which compensates by gradual dilation **[45].** As for the inflammatory cells, granulocytes are rarely present during any phase of atherogenesis. Instead, the response is mediated by monocyte-derived macrophages and specific subtypes of T lymphocytes at every stage of the disease**[36].**

Continued inflammation results in increased numbers of macrophages and lymphocytes, which both emigrate from the blood and multiply within the lesion. Activation of these cells leads to the release of hydrolytic enzymes, cytokines, chemokines, and growth factors, which can induce further damage and eventually lead to focal necrosis. Thus, cycles of accumulation of mononuclear cells, migration and proliferation of smooth-muscle cells, and formation of fibrous tissue lead to further enlargement and restructuring of the lesion, so that it becomes covered by a fibrous cap that overlies a core of lipid and necrotic tissue-a so-called advanced, complicated lesion. At some point, the artery can no longer compensate by dilation; the lesion may then intrude into the lumen and alter the flow of blood**[33].**

Coronary arteries in hyperlipidemic rats treated with atorvastatin showed improvement in the walls of these arteries, there were no fatty streak nor atheromtous plaque, this improvement may be attributed to the actions of atorvastatin which exerts its actions through different mechanisms. It reduce total cholesterol as a competitive inhibitor of HMG-CoA reductase. As well as increase receptors of LDL in the hepatocytes which lead to elimination of plasma LDL, a further reduction of cholesterol by activation of conversion of cholesterol to bile acids. Decreased lipid peroxidation another probable reason by which atorvastatin could use to reduce atherosclerosis and considered to be the best antihyperlipidemic agent**[37].**

Inhibition of several mechanisms involved in the formation of atherosclerotic lesion, including monocyte infiltration, smooth muscle cell migration and proliferation, activation of ox-LDL receptors and cell foam formation, could account for the reduction in intimal thickening induced by atorvastatin**[38].** Similar to that observed with atorvastatin ameliorated endothelial dysfunction and reduced intimal thickening in atherosclerotic rabbits**[39]**.

References:

[1]- Reiner, Z. and Tedeschi-Reiner, E.(2006).Atherosclerosis-a paradox of Eastern European countries. *Atherosclerosis*., 7/3 (suppl.): 461.

[2]-Jadeja, R.N.; Thounaojam, M.C.; Ansarullah, D.R.V. and Ramachandran, A.V. (2010).*Clerodendron glandulosum* Coleb., Verbonaceae, ameliorates high fat diet-induced alteration in lipid and cholesterol metabolism in rats. *Braz.J.Pharm*., 20 (1): 117-123.

[3]-Lopez-Garcia, E.; Schulze, M.B.; Meigs, J.B.; Manson, J.E.; Rifai, N.and Stampfer, M.J. (2005). Consumption of trans fatty acids is related to plasma biomarkers of inflammation and endothelial dysfunction. *J Nutr*., 135:562-566.

[4]-Akiyama, T.; Tachibana, I.; Shirohara, H.; Watanabe, N.and Otsuki M.(1996).High-fat hypercaloric diet induces obesity, glucose intolerance and hyperlipidemia in normal adult male Wistar rat. *Diabetes Res Clin Pract*., 1-3: 27-35.

[5]-Pi-Sunyer, F.X. (2002).The medical risks of obesity. *Obes Surg*., 1:6-11.

[6]-Rosenson, R. S. (2002). The rationale for combination therapy. *Am.J.Cordiol*., 90: 2k-7k.

[7]-Harrison, D.; Kathy, K.G.; Hornig, B. and Drexler H.(2003).Role of oxidative stress in atherosclerosis. *Am. J. Cardiol*., 91:7-11.

[8]-Leopoid, J. A. and . Loscalzo, J. (2009).Oxidative risk for atherothrombotic cardiovascular disease. *Fre.Rad.Bio.Med*., 47: 1673-1706.

[9]-Salim, S. A.; Hassan, D.R. and Mowafy, A.R. (2009).Comparative Impact of Different Types of a single Antioxidant Supplementation. (B-Carotene, a- Tocopherols and Ascorbic acid) on lipid Profile In Hyperlipidemic Rats. *Midd-Eas.J.Sci.Res*., 4(4): 354-360.

[10]-Walt, G. (2004). WHO's World Health report 2003. *BMJ*., 328 (7430):6.

[11]-Ross, R. and Glomset, J. A.(1976).The Pathogenesis of atherosclerosis (first of two parts). *N.Engl.J.Med*., 295(7): 369-377.

[12]-Libby, P.; Ridker, P. M., and Hansson,G.K.,(2009).Inflammation in atherosclerosis:from Pathophysiology to Practs.,*J.Am.Coll.Cardiol*.,54(23):21292138.

[13]-Camerino, G. M.; Pellegrino, M.A.; Brocca, L.;Digennaro, C.; Camerino, D. C.; Pierno, S. and Bottinelli, R. (2011).Statin or fibrate chronic treatment modifies the proteomic profile of rat skeletal muscle. *Biochem.Pharm*.,81: 1054-1064.

[14]-Visser, M.; Bouter, L.M. and McQuillan, G.M. (1999).C-reactive protein levels in overweight and obese adult. *JAMA*., 282:2131-2135.

[15]-Fahey and Coll.(1965).*J Immunol*.,94:84.

[16]-Bancroft, J. D.; Layton, C. and Suvarna, S.K. (2013). Bancroft's theory and practice of histological techniques. 7th edition. Chrchill Livingstone Elsevier. Elsevier Limited.

[17]-Sethupathy, S.; Elanchezhiyan, C.; Vasudevan, K. and Rajagopal, G.(2002). Antiatherogenic effect of taurine in high fat diet fed rats. *Indian.J.Exp.Biol.Oct.*, 1169-1172.

[18]-Ramachandran, H.D.; Narasimhamurthy, K. and Raina, P.L. (2003). Modulation of cholesterol induced hypercholesterolemia through dietary factors in Indian desert gerbils (Merioneshurricinae). *Nutr.Res*., 23:245-256.

[19]-Akbay, E.;Ulusu, N.N.;Toruner, F.;Ayvaz, G.; Taneri, F.; Akturk, M.; Arslan, M. and Karasu, C.(2004).Effect of rosiglitazone treatment on the pentose phosphate pathway and glutathione-dependent enzyme in liver and kidney of rats fed a high fat diet;. *Curr.Therap Res*.,65(1):79-89.

[20]-Turley, S. D. (2004). Cholesterol metabolism and therapeutic targets: rational for targeting multiple metabolic pathways. *Clin.Cardilo*., 27:III 16-III 21.

[21]-Ji, G.; Zhao, X.; Liu, P. and Jiang, Z.(2011).Comparison of dietary control and Atorvastatin on high fat diet induced hepatic steatosis and hyperlipidemia in rats. *Lip Heal Dis*.,10:23.

[22]-Berman, S.; Gewurz, H. and Mold, C. (1986).Binding of C-reactive protein to nucleated cells leads to complement activation without cytolysis. *J.Immunol*., 136(4): 1354-1359.

[23]-Platel, D.; Bernard, A.; Mack, G.and Guiguet, M.(1996). Interleukin 6 upregulates TNF-alpha-dependent C3-stimulating activity through enhancement of TNF-alpha specific binding on rat liver epithelial cells. *Cytokine*., 8:895–899.

[24]-Koistinen, H.A.; Koivisto, V.A. and Ebeling, P.(1998). Serum complement protein C3 is a marker of insulin resistance, which is related to obesity, but not to hyperglycemia. *Diabetes*., 47(Suppl. 1):A311.

[25]-Binder, C.J,; Chang, M.K.; Shaw, P.X.; Miller, Y.I.; Hartvigsen, K.and Dewan, A. (2002). Innate and acquired immunity in atherogenesis. *Nat.Med*.,8:1218–1226.

[26]-Haarala, A.(2012): Inflammation and Early Atherosclerosis, University of Tampere school of Medicine, Finland, Msc. Thesis, 91 pp

[27]-Morley, J. J. and Kushner, I. (1982).Serum C-reactive protein levels in disease. *Ann. N.Y.Acad.Sci*., 389: 406-418.

[28]-Szalai, A.J.(2004). C-reactive protein (CRP) and autoimmune disease: facts and conjectures.*Clin.Dev.Immunol*., 11(3-4): 221-226.

[29]-Bustos, C.; Hernandez-Presa, H.; Ortego, M.; Tunon, J.; Ortega, L.and Perez, F.(1998). HMG-CoA reductase inhibition by atorvastatin reduces neointimal inflammation in a rabbit model of atherosclerosis. *J.Am.Coll. Cardiol.*, 32: 2057-2064.

[30]-Stancu, C.and Sima, A. (2001). Statins: mechanism of action and effects. *J.Cell.Mol.Med*., 5 (4):378-387.

[31]-Kimura, M.; Kurose, I.; Russell, J.and Granger, D.N.(1997). Effects of fluvastatin on leukocyte-endothelial cell adhesion in hyper cholesterolemic rats. *Arterioscler.Thromb.Vasc. Biol*., 17: 1521-1526.

[32]-Bellosta, S.; Ferri, N.; Bernini, F.; Paoletti, R.and Corsini, A.(2000). Non-lipid-related effects of statins. *Ann.Med*., 32: 164-176.

[33]-Ross,R.(1999).Atherosclerosis-An Inflammatory Disease.*New.Engl.J.Med*.,340(2):115-126.

[34]-Napoli,C.; D'Armiento, F.P.and Mancini F.P. (1997). Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia: intimal accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atherosclerotic lesions. *J. Clin. Invest*.,100:2680-2690.

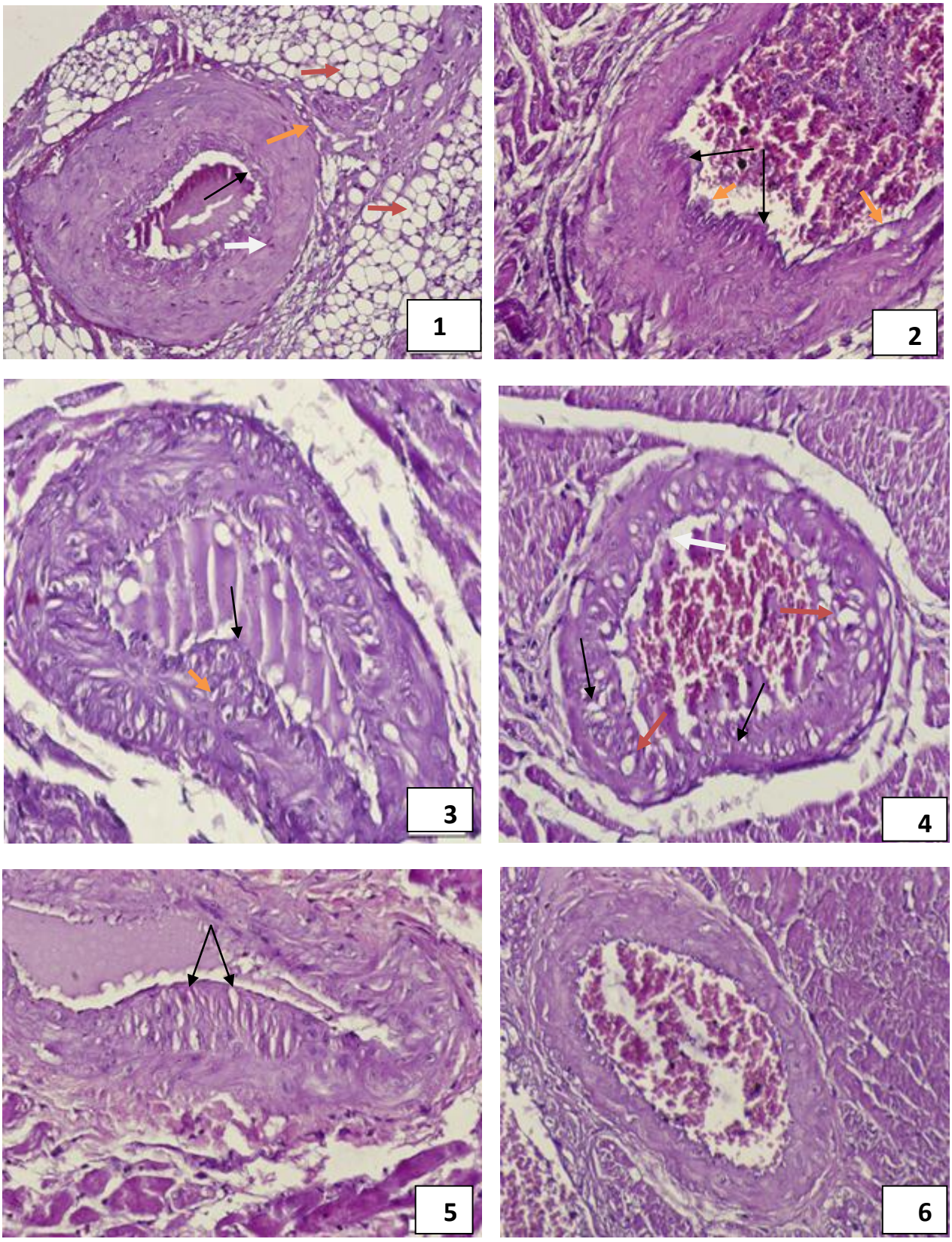
[35]-Glagov, S.;Weisenberg, E.;Zarins, C.K. ; Stankunavicius, R. and Kolettis, G.J.(1987). Compensatory enlargement of human atherosclerotic coronary arteries.*N.E.J.M*.,316:1371-1375.

[36]-Stary, H.C.(1996). The histological classification of atherosclerotic lesions in human coronary arteries. In: Fuster V, Ross R, Topol EJ, eds. Atherosclerosis and coronary artery disease. 1. Philadelphia: Lippincott-Raven, 463-474.

[37]-Murad, S.; Mahmood, G.; Bashir, A.; Asghar,J.; Asif S.; Ch, A. M. and Aslam, M.(2012). Single Blind Placebo Controlled Comparative Study Of Hypolipidemic Effects Of Kalonji And Atorvastatin. *IJPRD*., 3(12): 33-38.

[38]-Plana, J. C. and Jones, P. H. (2001).The use of statins in acute coronary syndromes: the mechanisms behind the outcomes. *Curr. Atheroscler. Rep*., 3:355–364.

[39]-Oubina, M. P .; Heras, N.; Cediel, E.; Sanz-Rosa, D.; Aragoncillo, P.;Diaz, C.; Hernandez G.; Lahera, V. and Cachofeiro, V.(2003). Synergistic effect of angiotensin-converting enzyme (ACE) and 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibition on inflammatory markers in atherosclerotic rabbits. *Clinical Science*., 105:655–662.



Figure(1)cross-section of coronary artery of group(1-1), intima (black arrow), media(white arrow), adventitia(yellow arrow)adipose tissue(orange arrow),200x.Figure(2)cross-section of coronary artery of group(2-2), mild fatty streak(black arrow),irregular thickness of coronary wall(yellow arrow)400x. Figure(3)cross-section of coronary artery of group(2-1) moderate fatty streak lesion(black arrow) proliferation of smooth muscle cells(yellow arrow),400x.Figure(4) cross-section of coronary artery ,proliferation of smooth muscle cells(black arrow) vacuolation of tunica media(yellow arrow), foam cell(white arrow),400x., Figure(5)cross-section of coronary artery of group(2-1) moderate fatty streak,400x., Figure(6)cross-section of coronary artery with normal architecture after treated by atorvastatin of group(2-3),400x.