Journal of Kerbala University , Vol. 14 No.1 Scientific . 2016

Study The Effect of Iraqi Butter Ghee on Some Biochemical Parameters and Histopathological Changes on Aorta of Albino Male Mice.

دراسة تأثير التغذية على الدهون الحيوانية العراقية على بعض المعايير الكيموحيوية والتغييرات النسجية المرضية في الشريان الابهر لذكور فئران المهق

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Abstract:

This study designed to investigate the effects of Iraqi animal oils on aortal tissue as well as biochemical changes of lipid profiles of mice, which feeds, by these oils for six months, the result showed:

There were different degrees of significant increase ($p<0.05$), ($p<0.005$), ($p<0.0005$)in TC,TG,LDL,VLDL , and AI, but there was high significant decrease($p<0.0005$) in HDL in mice fed on Iraqi animal oils for six months if compared with control group,

The histological sections of aorta were revealed presence of severe histopathological changes, which represented by increase the thickness (89.7±21.3µm) of atherosclerotic plaque in intima which formed by accumulation of lipid droplets (cholesterol, oxidized-LDL), as well as increase the thickness and fibrosis of medial layer. The most severe changes were in aorta, showing significant atherosclerosis and marked luminal narrowing which appeared as semi blocked of blood stream of the aorta of some animals as well as fibrosis and thickness of some areas in some very severe cases.

Key words: Atherosclerosis, animal oils, Aorta.

الخلاصة:

صمم هذا العمل لغرض دراسة تاثير تناول الغذاء ذات النسبة العالية من الدهون الحيوانية العراقية على بعض المعايير الكيموحيوية لدهون الدم والتغييرات النسجية المرضية في الشريان الابهر على مجموعتين من ذكور الفئران المهق بعد استحداث توليد حالة فرط الدهن في حيوانات التجربة وذلك بتغذيتها على غذاء عالي الدهون العراقية ذات الاصل الحيواني لمدة ستة أشهر، وقد اوضحت نتائج الدراسة مايلي:

ارتفع كل من الكوليستيرول,الكليسيريدات الثلاثية,الدهون البروتينية واطئة الكثافة وواطئة الكثافة جدا وكذلك معامل تصلب الشرايين وبمختلف درجات المعنوية ($P<0.0005$),($p<0.005$), ($p<0.05$) فيما كان هناك انخفاض معنوي عالي جدا ($P<0.0005$) في الدهون البروتينية عالية الكثافة في الفئران التي تناولت غذاء عالي الدهون لمدة ستة اشهر اذا ما قورنت المتغيرات المذكوره اعلاه بمجموعة السيطرة السالبة, في الفئران اعتيادية الدهن .

اوضحت المقاطع النسجية للابهر الى حدوث تغيرات مرضية-نسجية شديدة وعديدة تمثلت بزيادة سمك الطبقة الدهنية المتصلبة(89.7±21.3µm)المتكونة نتيجة تراكم القطرات الدهنية والممثلة بالكولستيرل والدهون البروتينية واطئة الكثافة المؤكسدة وتحولها الى طبقة دهنية متصلبة(رغوة).كان اكثرها حدة في المقاطع النسجية لابهر الفئران مفرطة الدهون حيث وجد تضيق تجويف شريان الابهر بدرجة كبيرة بسبب اتساع الطبقة الدهنية المترسبة بشكل طبقة رغوة والتي أدت الى انسداد شبه كامل لمجرى الدم في الحالات الحادة جدا لبعض الحيوانات ,كما لوحظ وجود تليف وبدرجات مختلة لبعض الشريان الابهر لبعض الفئران.

مفتاح البحث: تصلب الشرايين، الدهن الحيواني ,الابهر

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]**.

Cardiovascular diseases, (CVD), particularly coronary heart disease (CHD), have become a growing problem, especially in developing countries. Hypercholesterolemia is widely known as a dominant risk factor for the development of cardiovascular diseases[**3]**.

Much research on hyperlipidemia has sought to identify which lipid parameters are most closely correlated with an increased risk of CVD. Elevated serum total cholesterol (TC) and low density lipoprotein-cholesterol (LDL-C) levels, low serum high-density lipoprotein cholesterol (HDL-C) levels, and high serum triglycerides (TG) levels have been correlated with increased incidences of hyperlipidemia and CVD **[4]**.

Hyperlipidemia which mostly induces Oxidative stress is now believed to be an important factor in the development of non-alcoholic fatty liver disease (NAFLD) **[5]**. NAFLD is the most common liver disorder in the world, and in obesity, type 2 diabetes and related metabolic diseases, its incidence reaches 70-90% **[6]**. The disease is characterized by the accumulation of triacylglycerols inside liver and aortal cells, and the condition can progress into more serious liver and aortal disease, such as atherosclerosis,non-alcoholic steatohepatitis, liver fibrosis, cirrhosis, and more rarely, liver carcinoma **[7]**. Previous works have shown that feeding animals a high fat diet induces atherosclerosis and aortal damage, which are stages of the disease **[8]**.thus this study was designed to investigate the most histopathological changes which induces by feeding of high amounts of animal oils particularly cholesterol and saturated fatty acids on the aortal tissue.

Materials and Methods:

Animals and groups:

Twenty male albino mice 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 (25- 35 g).Animals were housed in controlled condition of temperature (25 ± 3C°), and 12 hours light-dark cycles. mice 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 Ten animals.

1-control group: This group consists of ten animals. The animals caged in two large polypropylene cages (five mice for each cage). The animal in this group maintained on standard chow, for six months.

2-Hyperlipidemic group: Animals in this group were fed on Iraqi animal oils-rich diet (IAOD) for six months. Animals in this group were maintained on (IAOD) for six months to test the ability of this oil in induce Atherosclerosis in this group. Ten mice, which caged in two polypropylene cages (each cage contained five mice).

Preparation of Iraqi animal oils-rich diet (high fat diet) (HFD)10 g of Iraqi animal oil was mixed thoroughly with 90g of powdered chow diet supplied by AL-Haffiz industries-karbala-Iraq. Simultaneously, this oil solution was added in to powdered mixture of normal chow diet to obtain homogeneous soft cake. The Iraqi animal oils -rich diet (HFD) preparation was modeled as a pellets of about 0.5g each according to the method**[9]**.

At the end of experiment which continuous for Six months, all animals in the control and hyperlipidemic groups were weighted and scarified after overnight fasting.

Blood Sampling:

A bout 0.5ml of Blood was collected by direct heart puncture after overnight fasting at end of six months, and after anesthetized of animal with ketamine hydrochloride injection, blood was placed in gel test tube and left to stand for 30 minutes at room temperature to allowing clotting. The sera samples were prepared by centrifugation at 3000 rpm for 10 minutes to estimate the levels of, TC, TG, HDL-C, LDL-C, an AI (biochemical assays).

Biochemical assays:

Measurement of serum total cholesterol and lipid profiles (TC, TG, HDL,LDL, VLDL, AI)was done as follow.

1- Measurement of serum total cholesterol(TC): The reagents were supplied by Randox, and serum total cholesterol (TC) was measured according to the**[10]**.

2-Estimation of serum Triglycerides (TG): The reagents were supplied by Randox,UK, and serum triglycerides (TG) was measured according to**[11]**.

3-Estimation of serum High Density lipoprotein-cholesterol. (HDL-C) : 1-Reagents composition: The used reagents were supplied by SPINREACT, and serum cholesterol HDL was measured according to**[12].**

4-Measurement of low density lipoprotein-Cholesterol (LDL-C), very low density lipoprotein (vLDL) and atherogenic index(AI):

The LDL-C, vLDL concentrations and AI were calculated from the Friedewald equation:

LDL -C = Total cholesterol (TC) – (HDL-C+ VLDL-C)

And vLDL-C= Triglycerides / 5, Atheroganic Index (AI) = TG/HDL-C According to the manufacturer's instructions**[13].**

Histopathology

According to**[14]** processing and staining technique as follow: Tissue (aorta) obtained from all experimental groups were washed immediately with saline and then fixed in 10% buffered neutral formalin solution for 48 hours. The tissue sectioned (5Mm thick sections) and stained with hematoxylin and eosin (H&E) and examined under high power microscope (Olympus) (100,200,400X) and photomicrographs were taken.

Statistical analysis:

Values are given as mean± standard deviation of the mean (S.D.). Differences were determined by the two tailed unpaired t-test; $p<0.05$ was accepted as significant; otherwise the results were deemed (NS).

Results:

Serum lipid profiles in study groups (TC, TG, HDL-C, LDL-C, VLDL, and AI).

Lipid profile levels of all experimental groups were showed in table(1) there were highly significant changes in TC, TG, LDL-C and AI ($P<0.0005$) and median significant changes ($p<0.005$) in,VLDL-C in hyperlipidemic group as compared to control mice., as well as there was significant change($p<0.05$) in HDL-C of hyperlipidemic mice when compared to control mice.

High fat diet (HFD) which contain high percentage of Iraqi Animal Oils (IAOD) caused highly significant increase ($p<0.0005$) in TC, TG, LDL-C, VLDL-C and AI of mice in all animals which fed on these diet as compared to control group, whereas same group have significant decrease ($P<0.05$) in its HDL-C levels as compare to control group of the values in the end of study period.

Table-1: effect of feeding mice on Iraqi animal oils on serum lipid profiles of control and hyperlipidemic mice.

Groups	T.C. (mg/dl)± S.D	T.G. (mg/dl)± S.D	HDL-C (mg/dl)± S.D	LDL-C (mg/dl)±S.D	VLDL (mg/dl) ±S.D	AI.±S.D
1-Control group	56.5±5.06^{a}	66.2±8.2^{a}	33.67±4.55^{a}	21.15±6.71^{a}	13.68±2.64^{a}	1.88±0.23^{a}
2-Hyperlipidemic group	255.27±13.79 ^{***b}	241.88±25.3 ^{***b}	21.45±3.11 ^{*b}	198.41±18.9 ^{***b}	50.39±4.70 ^{**b}	11.012±1.28 ^{***b}

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 at the end of study period.

Histopathological study of Aorta:

Microscopic examination of aortal sections stained with heamatoxyline- eosin stain showed different changes among the study group compared with control group which shown in figure(1). Control aortal sections illustrates tunica intima ,tunica media and tunica adventitia the most distinguish layers of the walls of the large arteries .these sections of normal mice appeared intact of all aortal layers in all animals without histopathological changes in aortal tissue.

Table (2)illustrate different changes in the aortal layers thickness among the study groups which represented by the increase of the thickness (89.7±21.3µm) of atherosclerotic plaque in intima layer significantly($p<0.0005$) in hyperlipidemic animals as compared to control animals, as well as there were significant increasing($p<0.05$) and abundant fibrosis(++) in media layer among aortal sections, these changes include mild fatty streak in the intimal layer of aorta represented by changes which includes: increase the thickness of atherosclerotic plaque of intima and fibrosis of media figures(2,3).

Severe changes in aortal layers of some individual of hyperlipidemic group included: heavy thickness of atherosclerotic plaque in intima, as well as increase the thickness, and fibrosis of media with semi blocked of aortal blood stream in some cases figure.(4).

Table (2): thickness of aortal layers (µm) among two study groups:

Groups	Thickness of Atherosclerotic plaque (µm) ± S.D	Thickness of Intima (µm) ± S.D	Thickness of media(µm) ± S.D	Thickness of Adventitia(µm) ± S.D	Fibrosis Of media
1-control group	0±0 µm^{a}	5.4±1.6 µm ^{a}	78.4±6.8 µm^{a}	23.1±5.7 µm^{a}	-
2-Hyperlipidemic	89.7±21.3µm^{***b}	6.2±2.5 µm ^{a}	98.6±7.2 µm^{*b}	26.3±7.8 µm^{a}	++

All values represent mean ± S.D (n=10) , *Significant differences ($p<0.05$),***Significant differences ($p<0.0005$),a=no significant differences ,b=significant differences at the end of study period.

Discussion:

Induction of hyperlipidemia by feeding mice on HFD(10%iraqi animal oils) for six months resulted in several alterations in the serum TC,TG, LDL-c, VLDL-c and HDL-c levels associated with a dramatic increase in the atherogenic index. This effect resembles type IIa hyperlipidemia in humans **[15]** as shown in the present study. Our model diet consist of high percentage of Iraqi animal oils which expected to use in Iraqi meal prepared daily in our community, this material well known to aggravate induce hyperlipidemia by two ways: it contain high percentage of saturated fatty acids, as well as cholesterol. Therefore, this increase in cholesterol concentration and other lipoproteins (LDL,VLDL, with decrease HDL)and TG lead to imbalance in cholesterol homeostasis with increase its deposition in different organs particularly the aorta, thus we found different scores of lipid deposition as heavy thickness of atherosclerotic plaque in intima, as well as increase the thickness and fibrosis of medial layer of aorta in some individual of some sever cases . Our results were consistent with**[16]** who noticed that dietary cholesterol is known to cause a temporary increase in the plasma cholesterol level and a marked increase in the liver cholesterol level, biliary excretion of bile acids and fecal excretion of sterols and bile acids. The hypercholesterolemic effect induced by HFD may be due to the activity of the rate-determining enzyme in cholesterol biosynthesis, HMG-CoAreductase, stimulating the cholesterogenesis rate**[17].**On the other hand, development of hyperlipidemia may be also due to a decrease in catecholamine level which leads to low $ß_2$ - adrenergic receptor function**[18]**,and decrease lipolysis of fat cells**[19]**. Thus, decrease fat catabolism and increase the circulating lipid levels.

In the present study, HFD increased LDL levels, could be attributed to saturated fatty acids suppress hepatic receptor-dependent LDL uptake and increase levels of plasma LDL this result

harmony with **[20]**.Similarly, cholesterol alone also suppresses hepatic LDL uptake and increases plasma LDL cholesterol **[21]**. Increase the levels of LDL in our study was consistent with **[21]** that attribute this increase into amount of lipoproteins in serum were carriers of lipids and proteins all over the circulatory system, in excess amount low density lipoprotein cholesterol in blood cause oxidation and production of free radicals, leading to increased oxidative stress, which cause what called oxidized-LDL-C eventually development of atherosclerosis.

The reduction in HDL cholesterol level in animals fed HFD, in the present study, may be due to the decrease in lecithin-cholesterol acyltransferase (LCAT) activity, the enzyme involved in the transesterification of cholesterol, the maturation of HDL and the flux of cholesterol from cell membranes into HDL this result was harmony with **[22].**

In the present study, the histological observations were parallel to the obtained biochemical findings. Inductions of hyperlipidemia and Atherosclerosis in mice showed severe fatty streak and lipid accumulation in the intimal layer as heavy thickness of atherosclerotic plaque in intima, of the aorta as well as fibrosis and increase thickness in the walls of aorta particularly in medial layer . This effect was consistent with**[23]** who reported that the effect may be due to the accumulation and deposition of abundant fat droplets and foam cells and cholesterol droplets in the intimal layers of aorta. which occupied the entire cell cytoplasm. thus, results of the histopathology of aorta showed different degrees of histopathological changes which include increase of the thickness of atherosclerotic plaque in intima layer in hyperlipidemic animals as compared to control animals, as well as there were significant increasing in thickness and abundant fibrosis in media layer among aortal sections. The most sever changes were observed in some cases of hyperlipidemic mice. may be due to the individual variation between the animals in hyperlipidemic groups.our result also harmony with**[24]** who noticed the increased blood cholesterol up to 25%, seems to be the result of aortal lipid deposition, which provides acetyl coenzyme A to liver cells for cholesterol synthesis.

The excessive aortal lipid deposition leads to atherosclerosis, which represents an imbalance between triglyceride synthesis in the liver and its secretion **[25],** the result of this study was consistent with this explanation. Our study of serum lipid profiles levels and histopathological changes in the aortal layers was consistent with**[26]** who reported that the main effect of fat–cholesterol enriched diet was accumulation of cholesterol and triglycerides in the serum and tissues, mainly in the aorta Thus, we can concluded, diet, which contain high percentage of Iraqi animal oil, has atherogenic effect on the levels of blood serum and tissues particularly the aorta.

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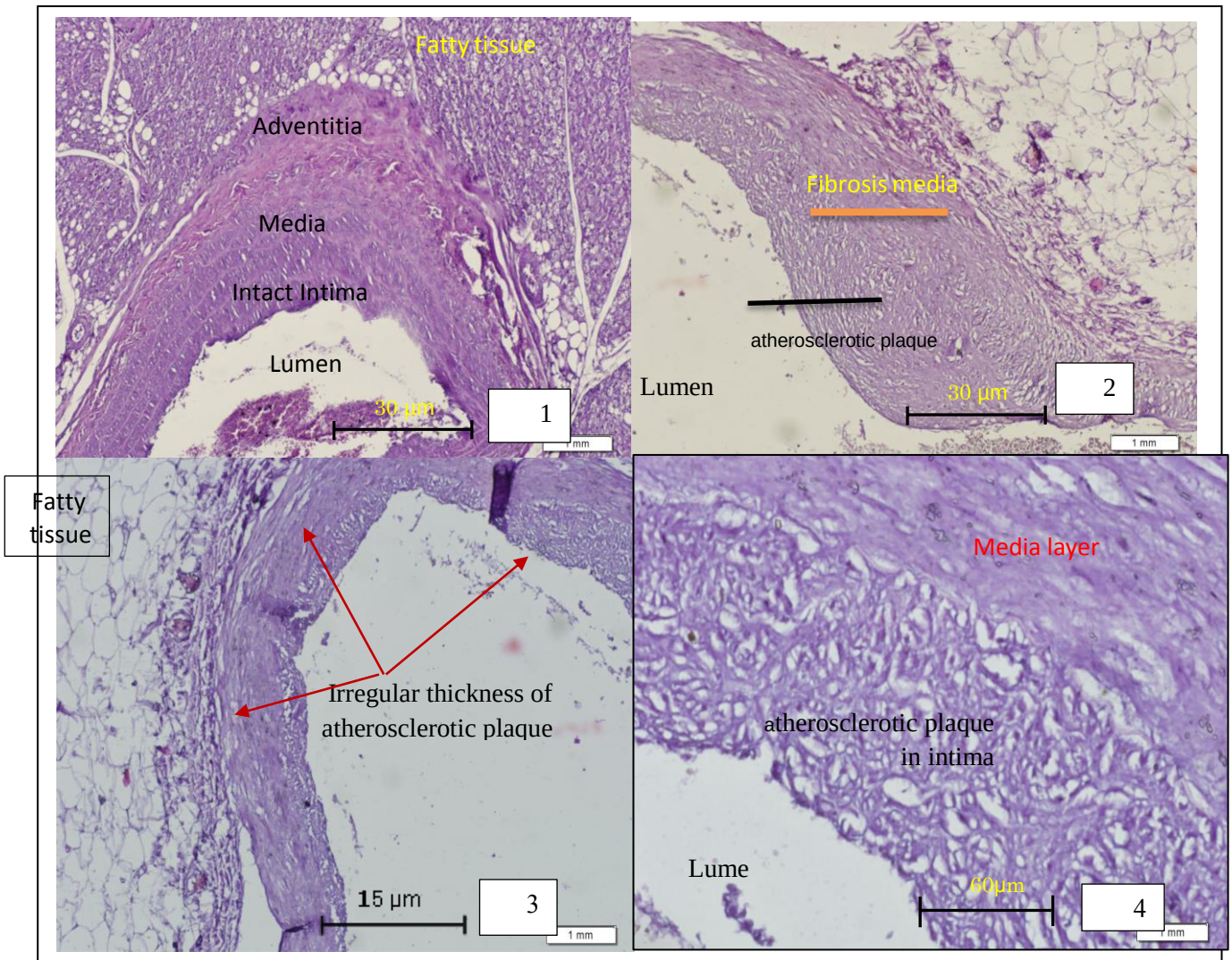

Figure(1)cross-section of aorta stained by heamatoxylin-eosin of control group 200x.showed normal histology of aortal layers. Figure(2)cross-section of aorta of hyperlipidemic group, 200x.,Figure(3)cross-section of aorta of hyperlipidemic mice,100x Figure(4)cross-section of aorta of hyperlipidemic mice feed on Iraqi animal oils, high thickness atherosclerotic plaque in intima layer 400x.