High Fat Diet Induce Hyperlipidemia Incidences With Sever Changes in Liver Tissue of Male Albino Rats: A Histological and Biochemical Study.

Jasem Hannon Hashim Al-Awadi[1], Karem Hammed Rashid[2], Alaa Jawad Hassen[3].
1-College of Science, Al-Qadesiya University. 2-College of Agriculture, Karbala University.3-College of Science, Babylon University.

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Abstract:

This study was designed to investigate the effects of high fat diet on liver tissue as well as biochemical changes of lipid profiles of hyperlipidemic and normolipidemic rats which treated with atorvastatin before and after induction of hyperlipidemia by feeding the rats with high fat diet, the result showed:

There was high significant increase($p<0.0005$)in TC,TG,LDL,VLDL , and AI, but there was high significant decrease in HDL in rats fed on HFD for seven months if compared with negative control group, while atorvastatin treatment caused high significant decrease($p<0.0005$)in lipid profile parameters after three months of treatments if compared to positive control group. Atorvastatin treatment result in high significant decrease ($p<0.0005$,$p<0.005$)in TC,TG,LDL,VLDL, and AI in normolipidemic rats as compared with negative control group.

The histological sections of liver were revealed presence of severe histopathological changes which classified into grades between 0-4. The most severe changes were in liver sections of hyperlipidemic rats which consist: infiltration of lipids in micro, mid, and macro vascular steatosis, while some livers were observed to contain onset of fat sacs, damage of unique radial appearances of hepatocytes in hepatic lobule, lymphocytes infiltration, congestion also observed in some liver section of these animals, whereas the histopathological changes in livers of normolipidemic rats which treated with atorvastatin were less severity as compared with positive control rats these changes included: sever lymphocytes infiltration especially around central portal vein, pyknotic nuclei, severe congestion and loss radial appearances of hepatocytes also there was dilatation of central portal vein and some bile ducts, while atorvastatin treatment reduce the effects mentioned in some hyperlipidemic individuals.

الغذاء عالي الدهن يستحث حدوث فرط الدهن مع تغيرات حادة في نسيج الكبد لذكور الجرذان المهق:دراسة نسجية وكيموحيوية

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

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

مفتاح البحث:فرط الدهن,الغذاء مرتفع الدهن,الكبد,اتورفاستاتين,الكوليستيرول,الكليسيريدات الثلاثية,الدهون واطنة الكثافة,الدهون واطنة الكثافة جدا,دالة تصلب الشرايين.

الخلاصة:

صمم هذا العمل لغرض دراسة تاثير الغذاء ذات النسبة العالية من الدهون على نسيج الكبد والمتغيرات الكيموحيوية لدهون الدم وايضا تاثير العلاج بعقار الاتورفاستاتين على ذكور الجرذان المهق بعداستحثاث توليد حالة فرط الدهن في حيوانات التجربة وذلك بتغذيتها على غذاء عالي الدهون. وقد اوضحت نتائج الدراسة مايلي:

ارتفع كل من الكوليستيرول,الكليسيريدات الثلاثية,الدهون البروتينية واطئة الكثافة وواطئة الكثافة جدا وكذلك معامل تصلب الشرايين ارتفاعا معنويا عاليا جدا($P<0.0005$) فيما كان هناك انخفاض معنوي عالي جدا في الدهون البروتينية عالية الكثافة في الجرذان التي تناولت غذاء عالي الدهون لمدة سبعة اشهر اذا ما قورنت المتغيرات المذكوره اعلاه بمجموعة السيطرة السالبة,فيما تسبب عقار الاتورفاستاتين بحدوث انخفاض معنوي عالي جدا($P<0.0005$)في المتغيرات المذكورهTC,TG,LDL,vLDL, AI بعد ثلاثة اشهر من العلاج اذا ماقورنت بمجموعة السيطرة الموجبة. ادى العلاج بعقار الاتورفاستاتين الى حدوث انخفاض معنوي عالي($p<0.0005,p<0.005$)في كل مستويات الدهون TC,TG,HDL,LDL.vLDL في الجرذان اعتيادية الدهن اذا ماقورنت بقيم هذه المتغيرات بمجموعة السيطره السالبة .

اوضحت المقاطع النسجية للكبد الى حدوث تغيرات مرضية-نسجية حادة وعديدة قسمت على اثرها الى درجات توزعت بين 0-4كان اكثرها حدة في المقاطع النسجية لاكباد الجرذان مفرطة الدهون حيث وجد ارتشاح وترسب للدهون وبهيئة فجوات دهنية صغيرة ومتوسطة وكبيره , فيما وجد في البعض منها بداية تكون للاكياس الدهنية,وتخريب للترتيب الشعاعي المميز للفصيصات الكبدية وارتشاح للخلايا المفية واحتقان وعائي.فيما وجدت تغيرات مرضية نسجية الا انها بدرجة اقل حدةفي الجرذان طبيعية الدهن والمعامله بعقار الاتورفاستاتين حيث وجد ارتشاح شديد للخلايا اللمفية حول الوريد المركزي تنكز وتفتت لانويه الخلايا الكبدية ,احتقان دموي شديد وكذلك تخريب للترتب الشعاعي المميز للخلايا الكبدية مكذلك توسع كل من الوريد المركزي وبعض القنوات الصفراوية.فيما خفف العلاج بعقار الاتورفا من الاثار المذكوره اعلاه في بعض الافراد المفرطة الدهون .

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 cells, and the condition can progress into more serious liver disease, such as non alcoholic steatohepatitis, liver fibrosis, cirrhosis, and more rarely, liver carcinoma **[7]**. Previous works have shown that feeding rats a high fat diet induces hepatic steatosis and liver 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 fats particularly cholesterol on the liver tissue and the role of atorvastatin treatment in both normal and hyperlipidemic rats.

Materials and Methods:

Animals and groups:

Eighty male albino rats (*Rattus norvegicus*) was purchased from animal care center collage 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 (40) 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 (table 1), for four months, then administered orally once daily for three months. The subgroups of treatment included:

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) listed in table (3-3) 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 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 cholesterol(TC): The reagents were supplied by Randox, and serum total cholesterol (TC) was measured according to the**[9]**.

2-Estimation of serum Triglycerides (TG): The reagents were supplied by Randox,UK, and serum triglycerides (TG) was measured according to**[10]**.

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**[11].**

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**[12].**

Histopathology

According to**[13]** processing and staining technique was as follow: Tissue (liver) 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:

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

Lipid profile levels of all experimental groups were showed in table(2) there were highly significant decreases in TC, LDL-C($P<0.0005$) and median significant decreases ($p<0.005$) in TG,VLDL-C in both groups(1-3,1-4) normolipidemic rats treated either with atorvastatin(5mg/kg/day) alone or supplemented with 2% soluble albumin s as compared to negative control group, but there was no significant change($p>0.05$) in both HDL-C and AI of above groups when compared to negative control rats.

High fat diet (HFD) caused highly significant increase ($p<0.0005$) in TC,TG,LDL-C,VLDL-C and AI of rats in both positive control group (2-2) and rats treated with 2%soluble albumin in normal saline compared to negative control group(1-1),whereas same groups have high significant decrease($P<0.0005$) in its HDL-C levels as compare to negative control group of the values in the end of study period. Treatment of hyperlipidemic rats by atorvastatin alone or supplemented with 2%soluble albumin resulted in improvement in the levels of TC,TG,LDL-C and VLDL-C which represented by significant decrease ($p<0.0005$) compared to positive control rats. This improvements also observed in AI in same above groups with highly significant decrease ($p<0.0005$) in athergenic index values(5.264±1.553,4.94±0.96)respectively compared to positive control AI values(11.112±1.29), as well as this improvement seen in HDL-C values which increased significantly ($p<0.005$) in both groups(2-3, 2-4)which reached(32.96±8.59, 34.66±3.31)respectively compared to positive control value(22.47±3.01).Although treatment of hyperlipidemic rats by atorvastatin alone or in combination with 2% soluble albumin result in an

improvement in the levels of TC,TG , LDL-C,and VLDL-C but their levels still at the high values if compared with negative control rats.

Table-2:effect of various treatments on serum lipid profiles of normolipidemic and hyperlipidemic rats.

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
1-1-N.N.S.	65.5±5.06	63.9±8.2	32.77±4.55	20.15±7.71	12.78±2.64	1.98±0.23
1-2-N.N.S+S.alb	70.4±7.62[a]	73.3±15.98 [a]	34.4±4.32[a]	23.34±3.39[a]	14.66±3.2[a]	2.15±0.152[a]
1-3-N.ATO	44.2±9.64 [**c]	49.3±12.09 [*c]	29.14±5.61[a]	5.24±3.30 [***c]	9.86±2.42 [*c]	1.864±0.322[a]
1-4. N.ATO+S.alb	45.8±9.6[*c]	38.9±7.68[**c]	28.37±8.99[a]	12.85±8.55[a]	7.78±1.54[**c]	1.583±0.69[a]
2-1.H.N.S.	267.27±15.79 [***]	247.73±23.4[***]	22.47±3.01[***b]	195.35±17.96	49.46±4.68 [***b]	11.112±1.29 [**]
2-2. H.N.S.+S.alb	259.4±29.08[a]	205.1±27.55[**d]	20.66±1.913[a]	197.72±26.39	41.02±5.51[***d]	10.04±1.34[a]
2-3.H.ATO.	174.6±18.34 [***d]	164±24.106[***c]	32.96±8.59[***d]	108.84±18.09	32.8±4.821[***d]	5.264±1.6 [***d]
2-4.H.ATO+S.alb	165.9±25.98 [***d]	171.1±35.9 [***c]	34.66±3.31[***d]	97.02±18.53[*]	34.22±7.18 [***c]	4.94±0.96 [***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 between group(1-1)and group(2-1).,c=significant differences between group (1-1) and groups(1-2,1-3, 1-4) at the end of study period, d=significant differences between group(2-1)and groups(2-2,2-3,2-4) at the end of study period.

Histopathological study of liver:

Microscopic examination of liver sections stained with heamatoxyline- eosin stain showed different changes among the study groups compared with negative control group(normolipidemic rats treated with normal saline)which shown in figure(1). Negative control liver sections illustrates portal vein surrounded by cords of hepatocytes extended radialy from central portal vein and branches of hepatic artery around the lobular periphery .Among the nonparanchymal cells, the following types have been identified bile duct, endothelial, kupffer cells, kupffer cells are located preferentially in the per portal region figure(1) . There were flat endothelial cells around the central vein and sinusoids, and about 12.5%of all animals in this study with normal architecture of their liver sections.

Normolipidemic rats treated either with atorvastatin (5mg/kg/day)alone or supplemented with 2% soluble albumin about 6.25% and 7.5% with normal architecture respectively and about 3.75%,5% showed mild changes which includes: mild vascular congestion, some hepatocytes have large nuclei, and pre vascular lymphocytes infiltration figure(2). In ATO treated rats, about 2.5%was graded(2)with mild changes, but about 1.25% of ATO+S.alb treated rats graded(2), these changes includes, moderate congestion, necrosis, hepatocytes degeneration with enlargement of some hepatocytes(ballooning shape), and some of which have two nuclei and some of hepatocytes undergo hepatocytomegally with pyknotic nuclei figure(3,5).

Table (3)illustrate different changes among different study groups which graded from(0-4)and their percentages, grade(0)represent (no abnormality) normal architecture of liver section was observed in negative control as described previously figure(2) , grad(1)mild changes among liver sections which stained with hematoxylin-eosin stain, these changes include mild cytoplasmic fatty infiltration(little of micro vesicular steatosis),mild granular degeneration fig.(3). Grade(2)represented by moderate changes which includes: (large number of micro vesicular steatosis with little of macro vesicular steatosis),moderate granular degeneration,

blood congestion in portal vein figure(4). Grade(3) moderate to severe changes included: large number of micro vesicular steatosis, less than middle vesicular steatosis, little number of macro vesicular steatosis, leucocytes infiltration, necrosis, congestion, and lymphocytes infiltration fig.(6). Grade(4) severe changes among liver sections of positive control rats, these changes includes: large number of micro, middle and macro vesicular steatosis along with lymphocytes infiltration, necrosis, congestion, fibrosis, kupffer cells lining the sinusoids were prominent and increased in number, sinusoids and bile duct dilatation, pyknotic nuclei, loss the normal architecture(ballooning shape) of hypatocytes which extended radial from central vein fig.(7,8).

Hyperlipidemic rats treated with atorvastatin(5mg/kg/day) as well as atorvastatin supplemented with 2% soluble albumin there were observed improvement in their liver sections, there were no severe nor moderate-severe changes, but all animals distributed between grade(0) and grade(1) 30%for each grade, and 40% in grade(2). The changes includes mild microvascular steatosis with very little number of middle and macro vascular steatosis .

Table (3): grades of histopathological changes of liver among different study groups:

Groups	Grade(0)No	%	Grade(1) ts	%	Grade(2)No	%	Grade(3) of rats	%	Grade(4) ts	%
1-1.N.NS	10	12.5	-	-	-	-	-	-	-	-
1-2.N.NS+S.alb.	10	12.5	-	-	-	-	-	-	-	-
1-3.N.ATO	5	6.25	3	3.75	2	2.5	-	-	-	-
1-4.N.ATO+S.alb	6	7.5	4	5	1	1.25	-	-	-	-
2-1.H.NS	-	-	-	-	-	-	3	3.75	7	7.75
2-2.H.NS+S.alb.	-	-	-	-	-	-	4	5	6	7.5
2-3.H.ATO	3	3.75	3	3.75	4	5	-	-	-	-
2-4.H.ATO+S.alb	3	3.75	3	3.75	4	5	-	-	-	-

Grade(0)normal architecture of liver rats sections., grade(1)mild changes among liver section., grade(2)moderate changes among liver section., grade(3)moderate to severe changes among liver sections., grade(4)severe changes among liver sections. %=percentage.

Discussion:

Induction of hyperlipidemia by feeding rats on HFD(2%cholesterol) for seven 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**[14]**as shown in the present study. 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 **[15].**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**[16].**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**[17]**,and decrease lipolysis of fat cells**[18]**. 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**[19]**.Similarly, cholesterol alone also suppresses hepatic LDL uptake and increases plasma LDL cholesterol **[20]**.Lipoproteins in serum are 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.

Administration of Atorvastatin to rats fed HFD or normal diet caused a reduction in serum TC and LDL-c levels with a consequence reduction in atherogenic index, while serum HDL-c levels were elevated, a result which is in agreement with **[21]**and **[22].** These findings could be interpreted by the inhibitory effect of Ator on HMGCoA reductase enzyme which catalyzes the conversion of HMG-CoA to mevalonate, a rate-limiting step in the formation of endogenous cholesterol leading to the decrease in the intracellular stores of cholesterol.This in turn results in the up-regulation of LDL receptors on the cell membrane, thus increasing the clearance of LDL-c from plasma**[23]**. Another possible explanation of our results is that Ator may lower LDL-c level by inhibiting hepatic cholesterol synthesis in very low density lipoprotein (VLDL) which is the source of LDL-c. Thus may impair VLDL particle assembly and secretion from liver, decrease the VLDL levels in plasma, and further decrease the LDL level in plasma**[21].**

Other proposed mechanism for Ator in lowering serum cholesterol level is by inhibiting the absorption of dietary cholesterol. Statins may reduce the cholesterol content in the intestinal mucosal cells, with a subsequent reduction in acyl-CoA cholesteryl acyltransferase (ACAT) activity, which catalyzes the intracellular esterification of cholesterol and formation of cholesteryl esters, and thus decrease the absorption of cholesterol**[24].**

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**[25].** Atrvasatatin treatment could restore HDL-c levels may be attributed to the reduction of cholesteryl ester transfer protein (CETP) which plays an important role in HDL metabolism**[26]**.In plasma, CETP facilitates the transfer of TG and cholesteryl esters (CE) between apoB-containing lipoproteins and HDL, resulting in the net transfer of CE mass from HDL to apoB-containing lipoproteins**[27].**The increased level of HDL would facilitate and increase the clearance of free cholesterol which is produced by peripheral tissues from plasma, and thus help to further decrease the cholesterol level in plasma**[28].**

Atorvastatin appeared to reduce triglyceride levels by increasing LDLclearance and by inhibiting triglyceride synthesis. It also increases HDL cholesterol levels **[29].**

These results match with **[30]** who proved that 40 mg of atorvastatin decreased 35.12 mg/dl serum LDL-c, and HDL increased 7.11 %. Results of our study also consistent with results of research work conducted by **[31]** who observed 30.90 mg/dl decrease in serum LDL-c and 5% increase in HDL-cholesterol in animal models.

In the present study, the histological observations were parallel to the obtained biochemical findings. Inductions of hyperlipidemia in rats showed severe fatty changes in liver hepatocytes. This effect may be due to the accumulation and deposition of abundant fat droplets in hepatocytes which occupied the entire cell cytoplasm **[32].** thus, results of the histology of liver showed different degrees of histological changes, due to large number of histological changes in different study groups, therefore, our classification of these changes in the liver tissue consist of five scores. the most sever changes were observed in hyperlipidemic positive control rats these changes included: large numbers of micro, middle and macro-vesicular steatosis in the liver tissue. Our diet model consist of cholic acid this material well known to aggravate induce hyperlipidemia by two mechanism of actions: an increase in cholesterol absorption in mucosal

intestine and a concomitant suppression of cholesterol 7α-hydroxylase activity a key enzyme in the synthesis of bile acids from cholesterol that result in decreased cholesterol excretion , cholic acid improve cholesterol absorption by its emulsifying property**[33].**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 liver , thus we found different scores of lipid deposition in the hypatocytes in form of micro, middle, and macro vesicular steatosis may be due to the individual variation between the animals in hyperlipidemic groups. The increased blood cholesterol up to 25%, seems to be the result of liver lipid deposition, which provides acetyl coenzyme A to liver cells for cholesterol synthesis**[34].**

The excessive liver lipid deposition leads to steatosis , which represents an imbalance between triglyceride synthesis in the liver and its secretion **[35].**The main effect of fat–cholesterol enriched diet was accumulation of cholesterol and triglycerides in the serum and tissues, mainly in the liver**[34].**

The accumulation in the liver of triacylglycerols, defined as hepatic steatosis, is proposed to be the first stage of more severe liver diseases such as nonalcoholic steatohepatitis, which shows histological signs of fibrosis and necroinflammation, through cirrhosis **[36].**

liver injury in HF diet-fed rats results from increased production of peroxides and/or increased sensitivity to peroxides due to cross-talk between the pathways of cholesterol and selenoprotein biosynthesis **[37].** Injury to liver tissues due to hyperlipidemia alters their transport function and membrane permeability, leading to leakage of enzymes from the cells. Therefore, the marked release of ASAT, ALAT and GGT into the circulation indicates severe damage to hepatic tissue membranes **[38].**

Liver sections in most severe cases revealed different degrees of hepatocytes degeneration, apoptotic cells and pyknotic nuclei all of which refer to cell death during induction of hyperlipidema, these changes probably due to increase of oxygen free radicals which are reported to be generated during hypercholestrolemic atherogenesis, thus, Oxidative modification of lipid, i.e. oxidized low-density lipoprotein (Ox-LDL) has been suggested to play a critical role in the pathogenesis of atherosclerosis , LDL oxidation plays an important role in plaque growth and degenerative changes leading to cell disruption and death**[39].**

hyperlipidemic rats showed well improvement in liver histology after three months of Atorvastatin treatment, there were no severity or moderate changes, only mild changes or normal liver sections were observed, these results could attributed to effects of atorvastatin which reduced most lipid profiles(TG,TC,VLDL,LDL) particularly oxidized-LDL, as well as lipid peroxidation. Atorvastatin exert its effects by different mechanisms of actions: an increase HDL-c levels by reduction of cholesteryl ester transfer protein (CETP),the increased level of HDL would facilitate and increase the clearance of free cholesterol, increase expression of LDL receptors on the surface of hepatocytes which lead to further clearance in both native and oxidized LDL-C and finally reduced lipid peroxidation all of these actions will lead to decreased lipid deposition in hepatocytes **[28].**

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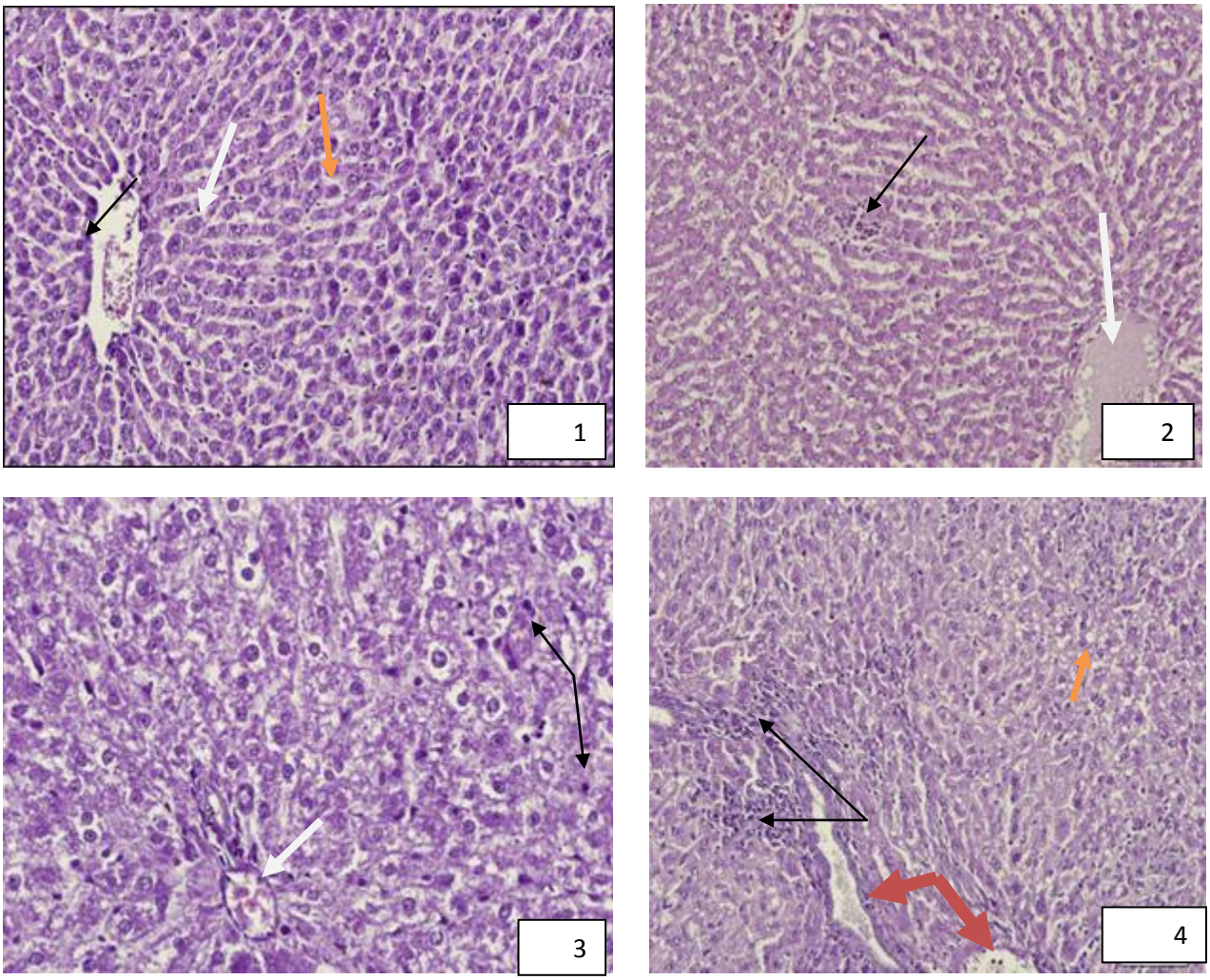
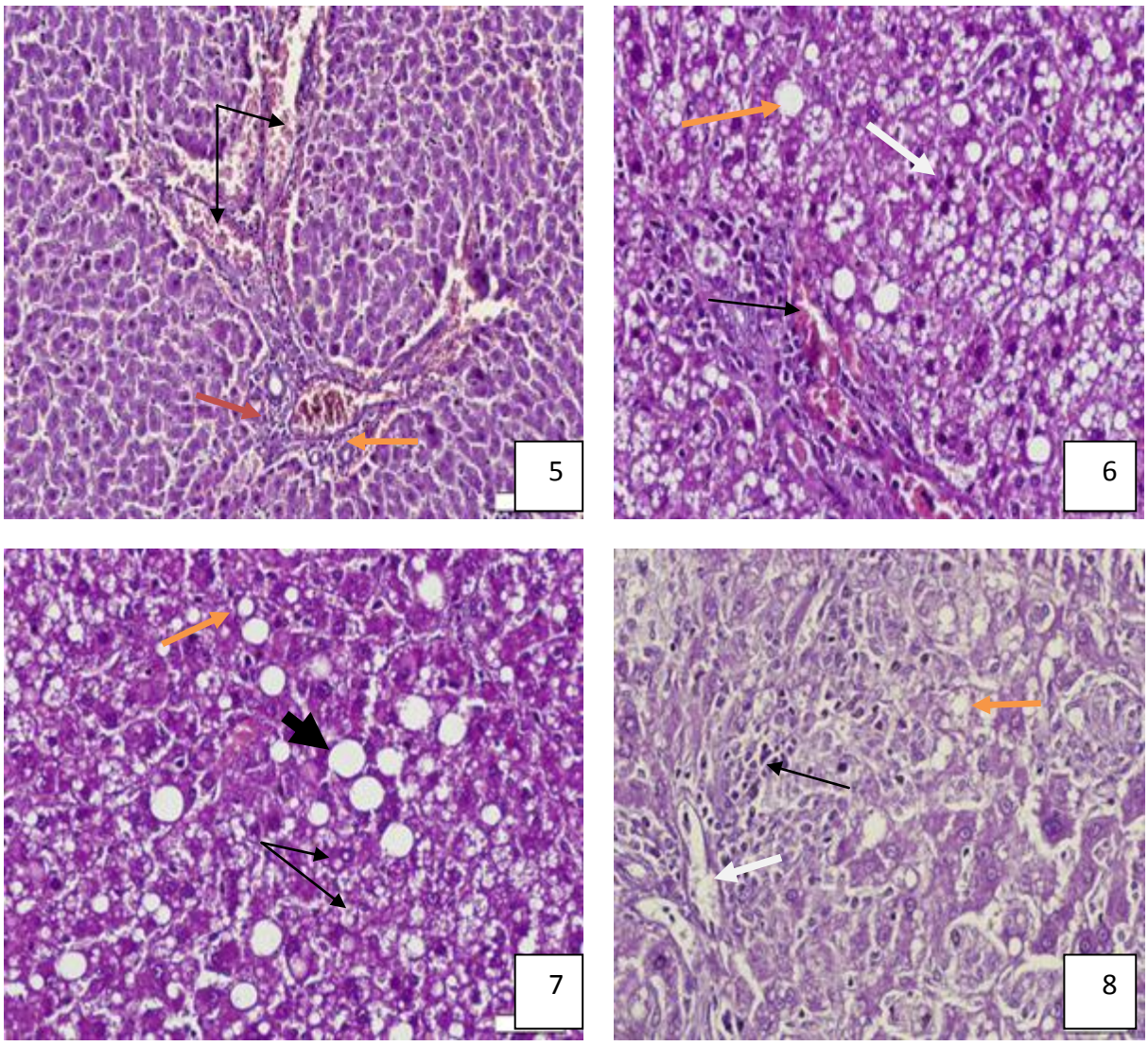


Figure(1)liver section stained by heamatoxylin-eosin of group (1-1) portal vein(black arrow) hepatic cord(white arrow),hepatic sinusoids (yellow arrow) 20x.,Figure(2)grade1liver section of group(1-3), mild congestion(black arrow), mild lymphocytes infiltration(white arrow),20x., Figure(3)grade1 liver section of group(1-3), pyknotic nuclei(black arrow), congestion(white arrow) 40x., Figure(4)grade2liver section of hyperlipidemic rat treated by ATO. high inflammatory cells(black arrow), little lipid deposition(yellow arrow),dilated bile duct(red arrow) 20X.



Figure(5)grade2normal rat treated with ATO. severe congestion(black arrow), lymphocytes infiltration(yellow arrow), necrosis(white arrow), dilated hepatic artery and bile duct(red arrow).20x., Figure(6)grade4,liver section of group(H.NS),pyknotic nuclei(white arrow), congestion(black arrow), fat infiltration(yellow arrow), 40x., Figure(7)grad3 liver section of group(2-1),macro vesicular steatosis (black arrow), mid vesicular steatosis(yellow arrow), micro vesicular steatosis(thick black arrow), 40x., Figure(8)liver section in hyperlipidemic rat treated with ATO,focal area of lymphocytes infiltration(black arrow)dilatation CPV(white arrow), fat infiltration(yellow arrow), 40x.,