Studies on dietary hypocholesterolemic agent in Briolers

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ABSTRACT

In the present study, we evaluated and tested the hypocholesterolemic effect of statin (atorvastatin) in chickens. Thirty six (36) of one day old Cobb chicks were kept for 2 weeks for adaptation then divided into 3 equal groups each (12). Control (Gp.1) did not receive any treatment.GP-2 treated orally with 0.02% atorvastatin (0.2gm/kg ration). GP-3 treated orally with 0.05% atorvastatin (0.5gm/kg ration).Serum and whole blood sample were collected from all groups 2nd week and 4th week post treatment to evaluate some hematological and biochemical changes as well as immunomodulatory cytokines TNF-α, IL1β, and IL6.

Oral treatment of the atorvastatin caused significant benefit results in chickens appeared in the correction of some biochemical parameters also try to stimulate cytokine response represents in TNF-α, IL1β, and IL6.

From the present work, it could be concluded that statin (atorvastatin) has potent hypocholesterolemic effect, this appear in the correction with hematological, biochemical and immunological results.

INTRODUCTION

Poultry industry in recent years has occupied a leading role among agricultural industries in many parts of the world. The potential for further growth is obvious in view in the value of eggs and poultry meat as basic protective foods in the human diets. The increase of poultry meat production during the past decade in developing regions was in average 43%, while in the developed regions it was only 28.4% *(Daghir, 1995)* .The size of the poultry industry has been made possible by the introduction of intensive production systems utilizing new technologies. Successful disease control has been a necessary corollary to the efficiency of such systems. Thus recognition, treatment or prevention of disease is of crucial importance *(Jordan, 1990).*

Cholesterol is not a life-threatening toxin, it has many benefits, but cholesterol can also create problems. Cholesterol in the bile can crystallize to form gall stones that may block the bile ducts.

Cholesterol is also strongly implicated in the development of atherschelorosis: fatty deposits (plaques) that form on the inside of blood vessels and predispose to heart attacks. The major culprit seems to be levels of LDLs that are in excess of the body's needs *(Wainwright, 2005).*

Statins, inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, have revolutionized the treatment of hypercholesterolemia. They are the most efficient agents for reducing plasma cholesterol, being also appreciated for their good tolerance *(Wainwright, 2005).* Atorvastatin (AT) differs from other statins in that it has a longer action and presents active metabolites which are biotransformed mainly by cytochrome P3A4 in the liver *(Clarke and Mills, 2006).*

Chickens were chosen as our experimental model because of the similarity of their lipid metabolism to that of human. The relative contribution of various tissues to overall fatty acids and cholesterol synthesis differs among animal species. All fatty acids synthesis takes place in the liver of chicken and humans, whereas in rats and swine, adipose tissue is appetitively inhibits the conversion of HMG-COA to mevalonic acid, a substrate for cholesterol synthesis *(Parkre et al, 1993; Goldstein and Brown, 1990; Nakanishi et al, 1988; Brown and Goldstein, 1986 and Illingrowth, 1987).*

The objective of the present work to evaluate the hypocholesterolemic effect of statin (atorvastatin) in chickens.

MATERIAL AND METHODS:
1. Laboratory animals used:
Thirty six (36) one day old Cobb chicks were obtained from national hatchery in Mansoura Governorate. The chicks were housed in batteries and fed formulated balanced ration and water were supplied all the day.
2. Treatment drug:
Atorvastatin, Hunan Dinuo pharmaceutical Co., China.
3. Haematological test:
Erythrocytes (RBCs) and white blood cell count (WBC) ,calculation of blood indices according to *Feldman et al (2000)* packed cell volume (PCV) *(Barbra, 1988)* haemoglobin (Hb) levels *(Drabkin, 1949)* and differential leukocytic count *(Coles, 1986).*
4. Serum biochemical analysis:
Prepared frozen samples were used and analyzed for some serum analysis including AST, lactate dehydrogenase, creatinine, uric acid, total protein, albumin, globulin ,A/G ratio, total lipids, cholesterol, HDL cholesterol, LDL cholesterol, triglycerides , Catalase , Lipid peroxidase and Glutathione reduced were determined with semi-automatic spectrophotometer (BM-Germany 5010) using commercial test kits (Randox Co. UK.) according to enclosed pamphlet.
5. Immunological studies:

Tumor necrosis factor – α (TNF α) was assayed by Enzyme Amplified Sensitivity Immunoassay (EASIA) performed on microplate (Bio-Source, Co. Belgium). The assay uses monoclonal anti-bodies (MAbs) directed against distinct epitopes of TNF- α acc ording to *(Aukrust et al, 1994).*

ILIβ and IL6 were assayed by ready made Chicken Interleukin-1 beta and Interleukin-6 ELISA Kit provided by Themo scientific company according to *(Chan and Perlstin, 1987).*

6. Statistical analysis:

Our results were analyzed by (ANOVA) using SPSS software statistical program (SPSS for windows (ver.15.00, USA). Two groups were significantly different if P value was statistically lower than 0.05.

RESULTS & DISCUSSION

Administration of atorvastatin in our work moderate doses did not lead to unfavorable hematological changes   (table1-2).These observations are suggestive of the protective effect of atorvastatin.

Liver transminase enzymes (AST) act as indicator on the statue of the liver and liver function, alteration in the enzyme level may be indicator on hepatic disease, but AST enzyme alone not specific hepatic enzyme, as shown in atorvastatin treated groups, transient elevation in AST level with rapidly return to normal (table-3), which is confirmed histopathologically by centrolobular

vacuolar degeneration, focal hepatic necrosis and dilated sinusoids besides some coagulative necrosis of some hepatocytes infiltrated with round cells (figure2-3), this may be agree with *Gehan and Manal (2010)* who observed an increase in the AST serum level at high dose of atorvastatin (80mg/kg), and confirmed histopathologically by severe hepatic changes were observed with high dose atorvastatin (80 mg/kg). In addition, cloudy swelling, hydropic degeneration and hepatic necrosis were the most predominant lesions. Such lesions were induced via the formation of free radicals, especially the reactive oxygen species. In consistent with the present findings, the hepatotoxic effect of high dose atorvastatin was ameliorated with partial disappearance of hepatic damage after treatment. Thus, the antioxidant effect of atorvastatin appears to play role in the attenuation of hepatic injury. The mechanism by which statins cause asymptomatic elevation in aminotransferases is unknown.

Most of the HMG-COA reductase inhibitors are metabolized by the liver. Damage to this organ is assessed by a persistent elevated level of its aminotransferases: ALT and AST *(Black, 2000).* Statins treatment commonly leads to an increase of ALT and AST which is dose dependent *(Parra et al, 2003).* The increase in aminotransferases levels more than three times the

upper normal limit indicates a potential liver toxicity *(Veillard and Marsh, 2002 and Waters, 2005).* Minor sporadic elevations in ALT, AST were observed for the atorvastatin-treated patients *(Rebecca et al, 1997).*

Uric acid is a major end product of nitrogen metabolism in birds. It constitutes approximately 60-80% of total excreted nitrogen in avian urine. Uric acid is synthesized in liver and 90% is excreted via tubular secretion largely independent of urine flow rate *(Kaneko et al, 1997)*.Small quantities of creatinine are ingested by animals consuming diets containing animal tissues, since creatinine is distributed throughout the body water. Muscle tissue contains phosphocreatinine, which undergoes spontaneous crystallization with loss of organic phosphate to form creatinine. Creatinine circulates in plasma and mainly excreted by kidney *(Kaneko et al, 1997).* Our results revealed insignificant change in creatinine and uric acid the chickens treated with 0.02% and 0.05% atorvastatin in the all groups along the whole experiment (table-3), so atorvastatin alone had no nephrotoxic effect, which is confirmed histopathologically by no changing in renal tissue of chickens after treatment with atorvastatin.

Dietary cholesterol is present in both free and esterified forms ,but only nonesterified cholesterol is absorbed and cholesterol ester are hydrolyzed within the lumen of the intestine by sterol esterase secreted by pancreas . The type of triglyceride present in diet significantly affects the absorption of cholesterol and its distribution in lymph lipids *(Kaneko et al, 1997).* Our results showed significant decrease in serum cholesterol and LDL cholesterol, HDL cholesterol, triglyceride and total lipid in the chickens treated with 0.02% and 0.05% atorvastatin in the 2nd week and 4th week (table-4), agreed with *Asaf and David (2000)* who observed significant decrease in cholesterol and LDL cholesterol (bad cholesterol) due to apart of lovastatin molecule resembles the structure of HMG-COA. For that reason, lovastatin and several compounds of this group act as competitive inhibitors of HMG-COA reductase enzyme.

Two mechanisms of action attributed to reductase inhibitors are the indirect result of decreased cholesterol synthesis in the hepatocyte. The first and most commonly cited mechanism is an increase in LDL receptor expression *(Ma et al, 1986).* The second mechanism, although not widely discussed, is a decrease in the production and secretion of VLDL *(Naoumova et al, 1996; Kasim et al, 1992).* Atorvastatin has a liver-selectivity ratio in animals that is intermediated between pravastatin, lovastatin ,fluvastatin, and atorvastatin is more effective in inhibiting liver cholesterol synthesis

than either pravastatin or lovastatin *(shaw et al, 1990).*

Also, we agreed with **Istvan and Deisenhofer (2001)** who revealed that statins also have the ability to bind HMG-CoA reductase more potently than HMG-CoA leading to the inhibition of HMG-CoA reductase, and subsequently reduction in cholesterol synthesis in the liver. That inhibition induces hepatocytes to increase their surface expression of LDL-receptors, so as to increase uptake of LDL and reduce plasma cholesterol, also **Robert et al (2006)** reported decrease in plasma total cholesterol. The regulation of HMG-COA expression can be controlled at the level of transcription, as well as by changes in mRNA translation and stability and phosphorylation **(Edward and Davis, 1996).** In agreement with HMG-COA gene expression being down regulated in the presence of high circulating cholesterol levels **(Goldstein and Brown, 1990)**. Also, **Uma et al (2008)** observed that atorvastatin (10 and 80mg/day) in subjects with metabolic syndrome dose dependently decreased total, LDL, and oxidized LDL cholesterol. In addition **Kim et al (2004)** studied the effects of pravastatin were compared with lovastatin and simvastatin in laying hens. Pravastatin is a potent HMGR-inhibiting drug, known to cause liver specific inhibition of cholesterol synthesis.

Exposure of lipids in cell membrane to free radicals stimulates the process of lipid peroxidatin. The products of lipid peroxidation are themselves reactive species and lead to extensive membrane, organelles and cellular damage. The free radical activity and the extent tissue damage are related quantitatively to the amount of lipid peroxide level in the blood **(Davidraj et al, 2008).** Lipid peroxidation is a general mechanism, whereby free radicals induce tissue damages, and implicated under several diverse pathological conditions **(Saeed et al, 2009).** Catalase and Malondialdehyde (MDA) are considered as biomarker for the assessment of lipid peroxidation. Another class of actions for statins and a potential mechanism by which statins may improve endothelial function is through their antioxidant effects through our results which showed significant decrease in Catalase and Malondialdehyde (MDA) in the chickens treated with 0.05% and 0.02% atorvastatin (table-5). We agreed with **Martha et al (2008)** who measured serum MDA levels as a parameter for lipid peroxidation and observed a significant reduction in the lipid peroxidation with both the drugs atorvastatin and clopidogrel before and after the treatment period in various experimental groups. The degree of reduction was more or less same with both the drugs, but with the

combined treatment, there was less reduction in the lipid peroxidation. Oxidative stress may cause vascular damage and dysfunction and initiate a cascade of events that ultimately lead to atherosclerosis. Also agreed with *Karatzis et al (2005)* who reported that pravastatin therapy caused an early significant decrease in serum malondialdehyde concentration. In addition partially agreed with *Gehan and Manal (2010)* who reported that atorvastatin alone decreased malondialdehyde, but increased catalase and superoxide dismutase (SOD) levels.

SOD catalyzes thedismutation of $_{O2}$ in to oxygen and $_{H2O2}$, and while it is an important antioxidant defense mechanism in aerobic organisms, excessive SOD activity may be deleterious *(Saeed et al, 2009).* Our results revealed that Superoxide dismutase (SOD) serum level in the chickens treated with 0.02% and 0.05% atorvastatin showed significant decrease (table-5). We agreed with *Uma et al (2008)* who examined other markers of oxidative stress, such as urinary nitrotyrosine, F2-isoprostanes, and monocyte Superoxide release and showed significant decrease effect on these measures. Also, *Ebru et al (2011)* reported the significant decrease in superoxide dismutase activity in erythrocytes after 8 weeks of atorvastatin treatment; indicate the antioxidant effect of the drug.

Glutathione reduced (GSH) is the major intracellular non-protein thiol compound, and plays a major role in the protection of cells and tissue structures from oxidative injury. Erythrocytes play an important role in the delivery of GSH to tissues with high rate of GSH utilization *(Anderson and Meister, 1989).* On the other hand, protects protein thiol groups from oxidation by serving as a biological redox agent *(Stadtman, 2004 and Meister and Anderson, 1983).* Our results showed significant decrease in GSH level in the 2nd week in the chickens treated with 0.02% and 0.05% atorvastatin (table-5). However, in the 4th week GSH was decreased significantly for the chickens treated with 0.02% atorvastatin. We agreed with *Aydin et al (2009)* who reported that atorvastatin therapy caused significant decreases in MDA and GSH levels.

The inhibition of lipid peroxidation observed with atorvastatin might be secondary to lipid lowering effect as well as its antioxidant effects *(Yogendrasinh and Rajendra, 2010).*

No significance changed in biochemical parameters in atorvastatin treated groups. atorvastatin is without having any serious toxicity or side effects known and thus can be safely used (table-3).

TNF- α is amplify, and coordinate proinflammatory sign also, resulting in the synchronized expression of effectors molecules that mediate

diverse aspects of innate immunity. TNF is capable of eliciting expression of chemokines and adhesion molecules and thus may be critical to the recruitment of neutrophils from the blood and has severe damage to the target organs *(Isogai et al, 1998 and Joseph et al, 2001).* The transcription factor NFkB regulates the expression of cytokines, chemokines, adhesion factors, and inducible pro inflammatory receptors .The abnormal activation of NFkB has been established for a series of inflammatory diseases and cancer.

IL1β Produced by activated macrophages, IL-1 stimulates thymocyte proliferation by inducing IL-2 release, B-cell maturation and proliferation, and fibroblast growth factor activity. IL-1 proteins are involved in the inflammatory response, being identified as endogenous pyrogens, and are reported to stimulate the release of prostaglandin and collagenase from synovial cells.

IL-6 is one of the most important mediators of fever and of the acute phase response. It is capable of crossing the blood brain barrier *(Bastard et al, 1999)* and initiating synthesis of Prostaglandin E2 (PGE2) in the hypothalamus, thereby changing the body's temperature setpinnate immune system, called pattern recognition receptors (PRRs), including Toll-like receptors (TLRs).IL-6 is responsible for stimulating acute phase protein synthesis, as well as the production of neutrophils in the bone marrow. It supports the growth of B cells and is antagonistic to regulatory T cells. Our esults showed that IL1β, IL6, TNFα serum level in chickens treated with atorvastatin (AT0.02& AT0.05) significant increase in comparison with control group (table-6), which agreed with *Michel et al (2005)* who studied the anti-inflammatory and analgesic effects of atorvastatin in a rat model of adjuvant- induced arthritis received 1-10 mg/kg atorvastatin for 10-15 days. They reported that atorvastatin caused inhibition of the increase of paw volume, increased of amyeloperoxidase activity in arthritic animals, and had stimulatory effect on the local expression of all cytokines and inhibition of IL-1β, TNF-α, IL-6.

Histopathological examination did not show any change in lung, spleen, bursa, heart, intestine and kidney except changes in hepatic tissue at 2nd week post treatment with atorvastatin (figure 2-3) , but at 4th week post treatment did not show any change which agreed with *Gehan and Manal (2010).*

Table (1): *Erythrogram Parameters (Mean ± S.E.) in Chickens experimentally treated with atorvastatin.*

Time/ Week	Group	RBC ×10⁶/µL	Hb g/dl	PCV %	MCV fl	MCH Pg	MCHC %
2nd week	Cont.	2.56±0.13a	8.24±0.5a	30±1.27a	117.8±5.59a	33.4±1.92a	29.6±2.3a
	AT 0.02	2.8±0.12a	9.27±0.24a	31±0.71a	118.2±4.12a	33.2±1. 25a	28.1±0.24a
	AT 0.05	2.52±0.09a	8.66±0.41a	30.4±0.98a	128.5±1.83a	34.4±1.16a	25.9±1a
4th week	Cont.	2.33±0.05a	7.83±0.27a	36.4±0.98a	156.52±6.2a	33.7±1.8a	21.55±0.87a
	AT 0.02	2.56±0.12a	7.63±0.36a	36.8±1.36a	144.96±8.9a	30.1± 2a	33.7±0.79a
	AT 0.05	2.62±0. 21a	7.73±0.43a	35.2±1.86a	135.62±6.6a	29.7±0.96a	34.9±0.79a

Table (2): *Leukogram Picture (Mean ± S.E.) in Chickens experimentally treated with atorvastatin.*

Time / Week	Group	TLC ×10³/µL	Heterophils ×10³/µL	Lymphocytes ×10³/µL	Monocytes ×10³/µL	Eosinophils ×10³/µL	Basophils ×10³/µL
2nd week	Cont.	17.6±1.166a	7.4±0.51a	9.7±1.2a	0.47±0.1a	o.46±0.21a	0.0±0.0
	AT 0.02	11.88±2.332a	9.4±1.7a	8.5±1.2a	0.64±0.2a	o.09± 0.09a	0.0±0.0
	AT 0.05	16.4±1.166a	7.2±1.3a	8.4±0.47a	0.54±0.2a	0.2± o.13a	0.13±0.13
4th week	Cont.	20 ±2.449a	12.1±1.96a	7.31±1.37a	0.11 ±0.11a	o.5±0.23a	0.0±0.0
	AT 0.02	18.4±2.135a	11.9±1.42a	5.78±0.8a	0.09±0.09a	o.5±0.22a	0.0±0.0
	AT 0.05	15.2±1.854a	8.34±1.33a	6.29±1.75a	0.08±0.08a	o.38±0.17a	0.0±0.0

Means in the same column not followed by the same letter differ significantly (P<0.05).

Table (3): *Some Biochemical Parameters   Mean (±S.E.) in Chickens experimentally treated with atorvastatin.*

Time/ Week	Group	Uri A mg/dl	Creatinine mg/dl	T.Protein g/dl	Albumin g/dl	Globulin g/dl	A/G Ratio	AST U/L	LDH U/L
2nd week	Cont.	5.58± 0.24a	0.33± 0.025a	3.12± 0.22a	0.91± 0.48a	2.21± 0.21a	0.43±0. 52a	20.3± 1.3a	2227.5± 61.11a
	AT 0.02	6± 0.31a	0.39± 0.047a	2.76± 0.33a	0.90± 0.46a	1.86± 0.30a	0.53± 0.75a	56.90±4 .7c	2211.75±90 .74a
	AT 0.05	5.84± 0.47a	0.45± 0.057a	2.41± 0.21a	0.93± 0.52a	1.48± 0.22a	0.67± 0.80a	45.1± 3.3b	2233.4± 97.56a
4th week	Cont.	5.18± 0.47a	0.37± 0.019a	2.42± 0.10a	0.94± 0.29a	1.49± 0.11a	0.65± 0.60a	43.2± 1.5a	1969.7± 172.75a
	AT 0.02	5.95± 0.41a	0.38± 0.019a	2.27± 0.12a	0.96± 0.89a	1.31± 0.13a	0.78± 0.12a	44.25±2 .8a	2377.71± 182.95a
	AT 0.05	5.6±0. 17a	0.38± 0.026a	2.31± 0.7a	0.91± 0.15a	1.41± 0.64a	0.65± 0.28a	41.99±1 .9a	2225.1± 35.59a

Means in the same column not followed by the same letter differ significantly (P<0.05).

Table (4): *Serum Lipid Profile parameters (Mean ± S.E.) in Chickens experimentally treated with atorvastatin.*

Time / Week	Group	Cholesterol mg/dl	Total lipid mg/dl	TG mg/dl	HDL mg/dl	LDL mg/dl
2nd week	Cont.	202.2±1.96c	1380.4±14.1c	156±3.3c	132.6 ±1.3c	38.4±2.6c
	AT 0.02	109.2±1.02b	1114±16.96b	93±2.7b	105.5±1.4b	14.9±0.48b
	AT 0.05	104.6±1.29a	981.6±29.1a	62.4±4.6a	100.9±1.5a	8.8±0.33a
4th week	Cont.	193 ±3.44b	1381±20.93b	179±3. 9c	101.3±1.94c	55.9±2.7c
	AT 0.02	81 ±2.19a	913±17.34a	100.6±0.51b	75.2±2.13b	14.3±0.31b
	AT 0.05	87±1.38a	923.4±28.14a	62.2±3.57a	66.2±1.91a	8.36±0.41a

Means in the same column not followed by the same letter differ significantly (P<0.05).

Table (5): *Some Oxidative Stress parameters (Mean ± S.E.) in Chickens experimentally treated with atorvastatin.*

Time / Week	Group	GSH mg/dl	Catalase u/ml	MDA nmol/ml	SOD mg/dl
2nd week	Cont.	0.42± 0.18a	1271± 9.92c	14± 1.05b	209.51± 4.8b
	AT 0.02	0.35± 0.41a	1148± 13.2b	11± 0.71a	173.8± 1.1a
	AT 0.05	0.48± 0.61a	584± 15.7a	10.4± 0.23a	172.35± 4.92a
4th week	Cont.	1.12± 0.88b	1520.4± 11.14c	9.4± 0.23b	202.88± 2.04b
	AT 0.02	0.49± 0.55a	1141± 4.29b	6.2± 0.37a	261.94± 1.77a
	AT 0.05	0.38± 0.13a	530± 44.47a	5.6± 0.4a	273.3± 1.13a

Means in the same column not followed by the same letter differ significantly (P<0.05).

Table (6): *Some Immune Response parameters (Mean ± S.E.) in Chickens experimentally treated with atorvastatin.*

Time / Week	Group	TNFα	IL1β	IL6
2nd week	Cont.	14.93±1.63a	32.43±2.66a	21.57±1.44a
	AT 0.02	35.57±5.30b	43.53±1.43b	33.7±1.24b
	AT 0.05	53.07±4.29c	45.67±2.71b	35.77±2.43b
4th week	Cont.	14.93±1.63a	32.43±2.66a	21.57±1.44a
	AT 0.02	60.47±1.85b	47±4.88b	55.10±5.76b
	AT 0.05	65.17±1.99b	46.93±4.23b	44.83±2.83b

Means in the same column not followed by the same letter differ significantly (P<0.05).
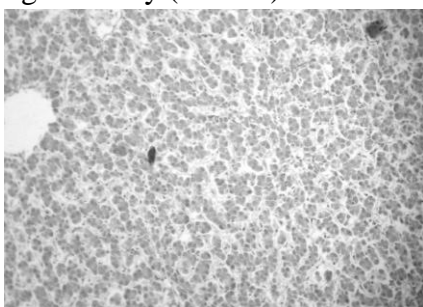

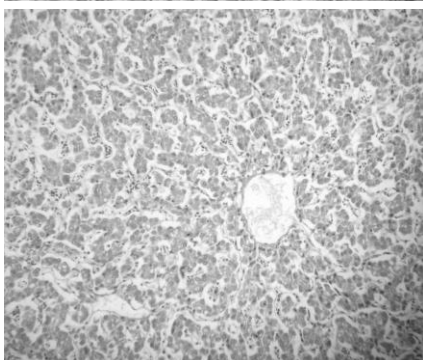**Fig, (1):** liver of control groups chickens showing apparently healthy tissue.

Fig, (2): liver of chickens experimentally treated with atorvastatin 0.02% showing centrolobular vacuolar degeneration, focal hepatic necrosis and dilated sinusoids.
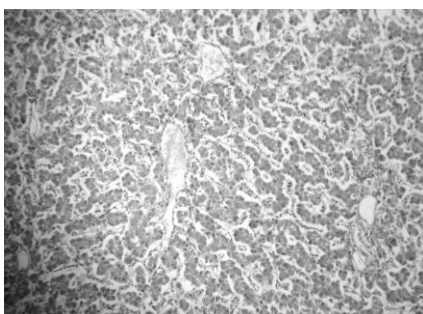

Fig, (3): liver of chickens experimentally treated with atorvastatin 0.05% showing hepatic degeneration besides some coagulative necrosis of some hepatocytes infiltrated with round cells.

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الملخص العربى

تم دراسة تأثير دواء أتورفاستاتين بعمل تجربه على ٣٦ كتكوت قمنا بتقسيمهم بعد عمر أسبوعين الى ٣ مجموعات، المجموعه الأولى لم تأخذ أى أدوية والثانية أخذت أتورفاستاتين بتركيز ٠,٠٢% والثالثة أخذت أتورفاستاتين بتركيز ٠,٠٥%، وتم بأخذ عينات بعد أسبوعين من العلاج ثم بعد ٤ أسابيع من العلاج.وقد أظهرت النتائج عدوم حدوث أى تغير فى كرات الدم الحمراء أوالبيضاء.وشملت بعض التغيرات الكيميائية كارتفاع انزيم الكبد بعد اول اسبوعين من العلاج ثم اختفاء هذا الاتفاع بعد ٤ أسابيع.أيضا أظهرت عدم تأثير أتورفاستاتين على وظائف الكلى.
إلى جانب حدوث انخفاض ملحوظ في صورة الدهون في الدم.كما اظهرت النتائج انخفاض فى الانزيمات المؤكسدة فى الدم وتحسن المناعة.