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Effect of cholesterol biosynthesis inhibitor on some biochemical parameters in normal male rats

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Abstract

Endogenous cholesterol acts as a precursor of testosterone and other steroids hormones, this study was conducted to evaluate if there is a counterproductive effect associated with inhibition of cholesterol biosynthesis pathway specially in high doses and the degree of these effects in normal male rats. Forty eight adult Wistar rats divided into four groups, the first is control while the remaining three groups were treated with simvastatin (cholesterol biosynthesis inhibitor) in doses of 25, 50 and 100 $mg.kg^{-1}$ respectively. Serum samples were observed at the baseline then every fifteen days while tissue samples were observed at day 30 and 60. Results of statistic refered to a significant decrease ($p\leq 0.05$) in serum total cholesterol and triglycerides (by 24 and 49% ± 3) respectively, also serum testosterone was significantly decreased (by 71% ± 2) in all groups compared to control after thirty and sixty days. The activity of alanine aminotransferase was increased (57% ± 3) versus to aspartate aminotransferase. Liver cholesterol was significantly decreased (by 72% ± 2) while testicular cholesterol was decreased except the group of 100 $mg.kg^{-1}$ which in turns to elevate (61% ± 4), in addition also there was a decrease in body weight gain percentage neither the weights of liver nor testis was affected. In conclusion, the inhibition of denovo pathway of cholesterol biosynthesis negatively affects testosterone level in addition to cholesterol concentration in the tissues, body weight gain and alanine aminotransferase with no successful compensatory mechanism as related with testosterone level.

Keywords: Cholesterol; Simvastatin; Testosterone; Rats.
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تأثير مثبط التخليق الحيوي للكوليستيرول في بعض المعايير الكيميائية الحياتية لذكور الجرذان السليمة

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الخلاصة

يعمل الكوليستيرول المصنع داخل الجسم كمادة أولية لعملية تصنيع التيستيستيرون وباقي الهورمونات ذات التركيب الستيرويدي. صممت الدراسة الحالية لتقييم وجود التأثيرات الضارة المرافقة لتثبيط مسار تخليق الكوليستيرول وفي الجرع العالية على وجه الخصوص فضلا عن تقييم درجة الأذى الناتجة في مستويات هورمون التيستيستيرون لذكور الجرذان السليمة. استخدمت ثمانية وأربعين من ذكور الجرذان البالغة تم تقسيمها إلى أربعة مجاميع, عدت المجموعة الأولى مجموعة سيطرة في حين عوملت المجاميع الثلاثة المتبقية بدواء السمفاستاتين المثبط لتخليق الكوليستيرول وبجرع مضاعفة بلغت ٢٥، ٥٠ و ١٠٠ ملغم. كغم $^{-1}$ على التوالي، تم جمع عينات مصل الدم في اليوم الأول وبشكل نصف شهري بينما جمعت عينات الكبد والخصية بعد ثلاثين وستين يوما من زمن بدء المعاملة. بينت نتائج التحليل الإحصائي انخفاضا معنويا ($p\leq 0.05$) في تركيز الكوليستيرول والكليسيريدات الثلاثية بنسبة (٢٤ و ٤٩

± ٣%) على التوالي في مصل الدم فضلا عن انخفاض هورمون التيستيستيرون (٧١ ± ٢%) في المجاميع الثلاثة المعاملة مقارنة بمجموعة السيطرة بعد ثلاثين وستين يوما كذلك لوحظ ارتفاع فعالية إنزيم الألانين ناقل الأمين (٥٧ ± ٣%) على النقيض من إنزيم الأسبارتيت ناقل الأمين. كما لوحظ انخفاض الكوليستيرول في الكبد (٧٢ ± ٢%) شأنه في ذلك شأن الكوليستيرول في الخصية باستثناء المجموعة المعاملة بالجرعة ١٠٠ ملغم. كغم $^{-1}$ والتي أظهرت ارتفاعا لافتا للانتباه (٦١ ± ٤%) كما تبين حدوث انخفاض في النسبة المئوية للزيادة الوزنية للجسم دون أي أثر في أوزان الكبد والخصية. يستنتج من الدراسة الحالية أن عملية تثبيط المسار الداخلي للتخليق الحيوي للكوليستيرول من شأنها أن تؤثر سلبا في قيم هورمون التيستيستيرون فضلا عن خفض تركيز الكوليستيرول في الأنسجة، الزيادة الوزنية ورفع فعالية إنزيم الألانين ناقل الأمين دون آلية ناجحة من قبل الجسم لتعويض النقص في هورمون التيستيستيرون.

Introduction

Cholesterol is present in the tissues and plasma either as free or cholesteryl esters in which a little more than half of body total cholesterol arises by an endogenous synthesis and the remainder supplied via receptor mediated uptake from circulating lipoproteins (1), virtually all tissues containing nucleated cells are capable of cholesterol synthesis from acetyl Co- A. The enzyme 3- Hydroxy 3-methyl glutaryl Coenzyme- A (HMG Co-A) reductase, is a principle regulatory (rate limiting) step and a site of action of most effective cholesterol lowering drugs (2, 3).

Androgens are a group of steroid hormones that include testosterone which is the main hormone responsible for the male reproductive activity (3), steroidogenesis is performed in gonads and adrenal from cholesterol precursor. Testicular androgens are synthesized in the interstitial tissue by the Leydig cells using cholesterol delivered by the transport protein StAR to the inner membrane of mitochondria where chain cleavage enzyme P450 scc. in turn to results in pregnenolone, this action is promoted by leutinizing hormone (LH) (4), testosterone formation from pregnenolone required five enzymes localized in microsomal portion of rat testis, although there was a traces of testosterone synthesized in the adrenal (3).

Several studies refered to that the inhibition of denovo cholesterol biosynthesis pathway leads to a significant reduction in the steroidogenesis in the WHHL rabbits females (5) and women's ovary (6). In males, it was demonstrated that testosterone biosynthesis requires a continuous cholesterol supply (7) so the inhibition of cholesterol biosynthesis pathway may results in a decline in plasma testosterone which may lead to a marked decrease in the fertility index and sperm cell count (8), also the supplementation of hen's diet with HMG Co-A inhibitor (0.03 or 0.05%) may lead to an increase in the incidence of unfertilized eggs and decreased hatchability (9).

Although studies have examined the effect of HMG Co-A reductase inhibitors on gonadal function, but there was no univocal results (10, 11) because some authors found that statins did not produce biological adverse effects on steroidogenesis in males (7, 12, 13) and in females (14).

Simvastatin is an efficient HMG Co- A reductase inhibitor which is widely used in most countries as a hypocholesterolemic drug (15).

The aim of this study was to investigate the dose related potential effects that is affecting the normal testicular secretion of testosterone arising from the inhibition of denovo cholesterol biosynthesis pathway in the normal male rats using simvastatin, also to inspect it's effect on some liver enzymes activity and testicular content of cholesterol.

Materials and methods

Animals and diet: Forty eight Wistar rats (3- months old) with weight range about 255 ± 15 g obtained from animal house, College of Veterinary Medicine were divided randomly into four groups (twelve rats each) and brought in room temperature with 12:12 light:dark cycle. Diet ingredients supplied in order to meets the rats nutritional and physiological requirements (16), diet was moistened with distilled water and formed in the shape of pellets which dried in an electric oven at 55 C°, prepared diet check up was done for insurance emptiness of cholesterol by qualitative test of Salkowski (17).

Simvastatin: SIMVOR 20 mg (RANBAXY laboratories limited, India) was used as a form of suspension in the treatment of rats by oral gavage.

Experimental design: The study includes the following four groups:

- Group one regarded as control which treated with methyl cellulose as a vehicle for sixty days.
- Groups two, three and four treated with simvastatin 25, 50 and 100 mg. kg^{-1} BW. respectively by oral gavage for sixty days.

At the day 30 of treatment, six rats of each group were sacrificed by separation of cervical spinal cord and specimens collected (serum and tissues) in addition to the detection of organ's weights (liver and testes) whereas the remaining rats were sacrificed in the same manner at the 60^{th} day.

Specimens collection: Blood specimens were collected every fifteen days from the baseline between 8:00 and 9:00

a.m. after about 12 hour of fasting from retroocular vein (18) by using capillary tubes. After coagulation blood samples were centrifuged (1000 ×g at 4 C°) for 15 min. then serum was collected and maintained in deep freezing. Liver and testes were dissected, immersed with ice cold saline (0.9% NaCl), dried on filter paper, weighed and frozen for subsequent analysis.

Weight gain calculation: Body weight gain was calculated every fifteen days throughout the period of the experiment, percentage of weight gain was calculated by subtracting the initial weight (g) from the recent weight then divided on the initial weight × 100.

Organ weight: Percentage of organ's weight was calculated at the end of experiment by dividing the weight of organ (g) on the ultimate weight of the animal (g) × 100.

Serum biochemical tests: Fasting total cholesterol TC and triglycerides TG were assayed enzymatically using specific kits manufactured from Fabricant Biolabo SA, France. Total testosterone was assayed by specific radioimmunoassay RIA (Immunotech, France). Serum transaminases activity was assayed using specific kits manufactured from BioMerieux sa, France.

Tissue total cholesterol: TC in the liver and testis were assayed by extracting the total lipids in the tissue by chloroform/ methanol (2:1 v/v) (19), the chloroform phase was collected and evaporated on water bath (45 C°) and lipids were redissolved in 200 µl ethanol (98%) then total cholesterol assayed spectrophotometrically (20).

Statistical analysis: Quantitative data were subjected to two way analysis of variance according to (21). Arcsine transformation were used for data expressed as percentages. Means differences were statistically compared according to Duncan multiple range test (22) using computer program, statistical package for social sciences (SPSS).

Results

There was significant decrease ($p \leq 0.05$) in serum cholesterol (mg. 100 ml^{-1}) among simvastatin- treated groups compared with baseline (Table 1). This decrease was more distinct in groups 50 and 100 mg. kg^{-1} since day 30 and since day 45 as considered with the group 25 mg. kg^{-1}.

Triglycerides (mg. 100 ml^{-1}) also decreased during the treatment in the groups received the three doses of simvastatin (Table 2), triglycerides concentration was nearly 1- fold lower in simvastatin- treated versus control at the days 30, 45 and 60 with no significant differences among treated groups except the value of the day 30 which illustrated significant difference ($p \leq 0.05$) between groups of 25 and 50 mg. kg^{-1} on one arm and group of 100 mg. kg^{-1} on the other arm which showed the least value.

Table 1: Effect of cholesterol biosynthesis inhibitor (simvastatin) on serum cholesterol level (mg/100 ml).

Groups	Period of treatment (day)				
	0	15	30	45	60
Control	74.06 ± 10.48 e	70.07 ± 1.14 de	72.83 ± 1.14 de	70.51 ± 2.04 de	69.65 ± 2.12 de
Simvastatin (25 mg/kg)	70.13 ± 1.13 de	70.71 ± 0.81 de	66.04 ± 1.82 c-e	60.24 ± 0.78 a-c	56.01 ± 1.21 ab
Simvastatin (50 mg/kg)	70.66 ± 0.99 de	71.42 ± 1.93 de	53.66 ± 1.84 a	53.97 ± 1.11 a	52.36 ± 2.11 a
Simvastatin (100 mg/kg)	71.48 ± 0.86 de	64.06 ± 1.71 b-d	53.64 ± 2.28 a	56.36 ± 1.74 ab	57.89 ± 1.77 a-c

- Different letters in the same column or in the same row refers to a significant difference ($p \leq 0.05$).
- Values are expressed as mean ± SE.

Table 2: Effect of cholesterol biosynthesis inhibitor (simvastatin) on serum triglycerides (mg/100 ml)

Groups	Period of treatment (day)				
	0	15	30	45	60
Control	79.56 ± 1.78 d	81.21 ± 2.29 de	81.04 ± 1.02 de	87.14 ± 2.54 e	87.31 ± 2.54 e
Simvastatin (25 mg/kg)	82.32 ± 2.79 de	82.09 ± 1.66 de	47.50 ± 1.58 c	41.65 ± 1.51 a-c	43.39 ± 3.67 a-c
Simvastatin (50 mg/kg)	80.84 ± 2.53 de	78.15 ± 2.07 d	44.68 ± 1.82 bc	40.54 ± 1.16 ab	38.45 ± 1.40 ab
Simvastatin (100 mg/kg)	84.90 ± 1.93 de	81.07 ± 1.39 de	37.96 ± 1.94 a	40.42 ± 0.80 ab	42.88 ± 2.80 a-c

- Different letters in the same column or in the same row refers to a significant difference ($p \leq 0.05$).
- Values are expressed as mean ± SE.

Treated animals maintained good aspartate aminotransferase (AST) activity control (U. L^{-1}) throughout the study (Table 3) with no differences as compared with control group whereas the effect of simvastatin caused a persistent elevation in the activity of alanine aminotransferase (ALT) (U. L^{-1}) as table (4) refers to in a dose response manner from the baseline starting from day 30 in 25 and 50 mg. kg^{-1} groups and from day 15 in group of 100 mg. kg^{-1}. Evident that ALT activity was decreased just in group of 50 mg. kg^{-1} at day 45 however the value returned to rise at day 60.

Table 3: Effect of cholesterol biosynthesis inhibitor (simvastatin) on aspartate aminotransferase activity (U/L).

Groups	Period of treatment (day)				
	0	15	30	45	60
Control	57.84 ± 0.66 b-e	58.04 ± 0.89 b-e	57.80 ± 2.41 b-e	57.73 ± 1.30 b-e	58.27 ± 1.08 c-e
Simvastatin (25 mg/kg)	57.01 ± 1.62 b-e	58.56 ± 1.39 c-e	57.75 ± 1.14 b-e	56.95 ± 0.72 b-e	57.78 ± 0.95 b-e
Simvastatin (50 mg/kg)	53.22 ± 0.57 ab	55.42 ± 1.06 a-c	56.72 ± 2.09 b-d	61.98 ± 3.44 de	60.80 ± 0.34 de
Simvastatin (100 mg/kg)	51.65 ± 0.72 a	55.49 ± 1.59 a-c	57.07 ± 1.62 b-e	60.45 ± 0.52 de	61.31 ± 0.84 de

- Different letters in the same column or in the same row refers to a significant difference ($p \leq 0.05$).
- Values are expressed as mean ± SE.

As clearly evidenced from (Figure 1), treatment with an efficient 3- hydroxy 3- methyl glutaryl Co- A (HMG Co-A) reductase inhibitor significantly ($p \leq 0.05$) lowered serum total testosterone for all three treated groups compared with control in a dose related fashion for both 30 and 60 days of trials.

Table (5) summarizes the effect of cholesterol biosynthesis inhibition on liver and testicular of cholesterol. Liver, cholesterol declined after 30 days of treatment significantly ($p \leq 0.05$) in the three treated groups in a parallel mode as compared with control, the values obtained after 60 days of treatment were further declined with approximately 1- fold decrease compared with values of 30 days not related with dose, in contrast testicular cholesterol elevated in both groups of 50 and 100 mg. kg^{-1} at day 30, this elevation reversed to a drop below the values of 30^{th} day of treatment showing the least value in the group of 50 mg. kg^{-1} and the higher value in the group of 100 mg. kg^{-1}.

Table 4: Effect of cholesterol biosynthesis inhibitor (simvastatin) on alanine aminotransferase activity (U/L).

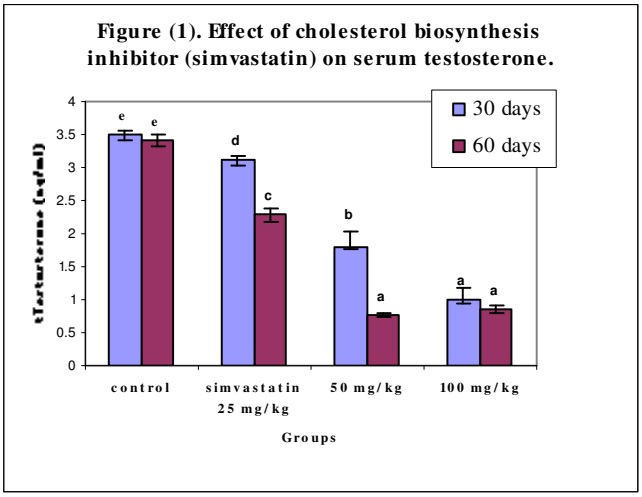
Groups	Period of treatment (day)				
	0	15	30	45	60
Control	41.33 ± 0.64 ab	40.83 ± 0.85 ab	43.50 ± 1.38 a-d	41.81 ± 0.61 a-c	42.65 ± 0.52 a-d
Simvastatin (25 mg/kg)	40.82 ± 1.10 ab	40.87 ± 0.50 ab	46.0 ± 1.93 cd	44.78 ± 1.35 b-d	53.54 ± 0.65 e
Simvastatin (50 mg/kg)	39.81 ± 1.35 a	41.52 ± 0.69 ab	51.11 ± 2.87 e	46.42 ± 1.12 d	65.35 ± 1.58 g
Simvastatin (100 mg/kg)	40.02 ± 0.35 a	51.30 ± 1.28 e	63.12 ± 3.28 fg	60.88 ± 0.81 f	66.31 ± 0.84 g

- Different letters in the same column or in the same row refers to a significant difference ($p \leq 0.05$).
- Values are expressed as mean ± SE.

Table 5: Effect of cholesterol biosynthesis inhibitor (simvastatin) on tissue cholesterol level (mg/g wet tissue)

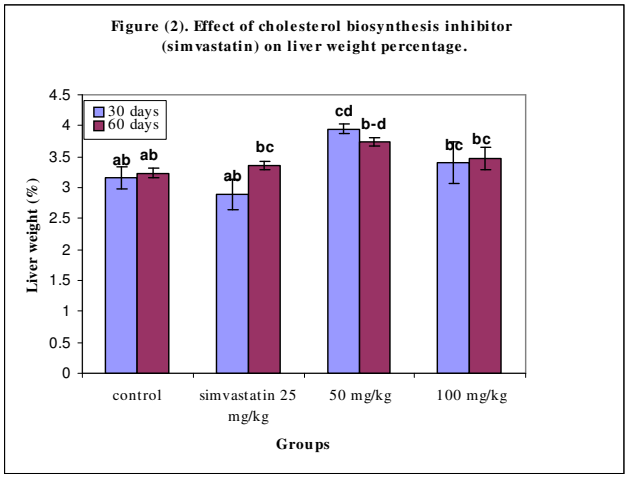
Groups	Period of treatment			
	day 30^{th}		day 60^{th}	
	Liver	Testis	Liver	Testis
Control	33.85 ± 2.43c	13.0 ± 1.29bc	33.06 ± 2.18c	13.37 ± 1.20bc
Simvastatin (25 mg/kg)	20.45 ± 0.32b	14.25 ± 0.92c	11.91 ± 0.71a	10.51 ± 0.61ab
Simvastatin (50 mg/kg)	22.17 ± 0.59b	21.47 ± 1.44e	9.12 ± 1.29a	8.57 ± 0.70a
Simvastatin (100 mg/kg)	19.29 ± 1.77b	24.49 ± 1.01f	12.06 ± 2.75a	18.14 ± 0.16d

- Different letters in the same column or in the same row refers to a significant difference ($p \leq 0.05$).
- Values are expressed as mean ± SE.



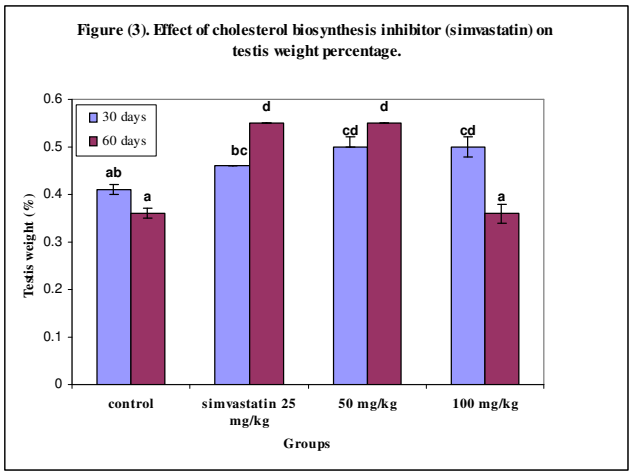
- Different letters refer to a significant difference ($p \leq 0.05$).
- Values are expressed as mean±SE.

Percentage of liver weight increased significantly ($p \leq 0.05$) in group received the dose 50 mg. kg^{-1} versus the groups of control, 25 and 100 mg. kg^{-1} after 30 days of treatment (figure 2), values obtained after 60 days of treatment demonstrated an approach in values with no significant differences among the three simvastatin- treated groups as well as the control one.



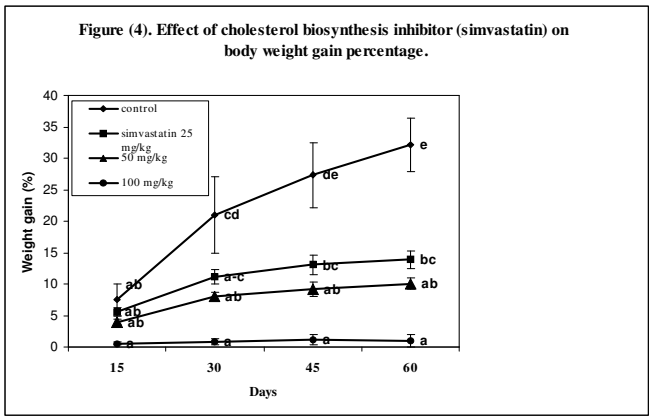
- Different letters refer to a significant difference ($p \leq 0.05$).
- Values are expressed as mean± SE.

Percentage of testis weight exhibited an increase in groups of 50 and 100 mg. kg^{1} compared with control with no differences among the three treated groups after 30 days of treatment. 60 Days of treatment indicated that there was more noticeable results that revealed an increase in percentage of testis weight of the group 25 mg. kg^{-1} and decrease in that of group 100 mg. kg^{-1} with respect to 30th day values. Both the values of the groups 25 and 50 mg. kg^{1} are significantly rise up regarding with both groups of control and 100 mg. kg^{-1} which there were interrelating values (Figure 3).



- Different letters refer to a significant difference ($p \leq 0.05$).
- Values are expressed as mean± SE.

As clearly evident (figure 4), mean body weight gain percentage began to diminish since the day 30 of treatment in an analogue mode for the three simvastatin- treated groups opposed to control, this finite weight gain continued in the same fashion throughout the period of experiment till the day 60 of the treatment. Also to which must be noticed that the falling in the weight gain percentages was inversely related to the dose.



- Different letters refer to a significant difference ($p \leq 0.05$).
Values are expressed as mean± SE.

Discussion

It is not surprising that the inhibition of cholesterol biosynthesis process results significant decrease in the serum total cholesterol as the data suggests (table 1), these observations are fully agree with the previously reported observations of Adah and Ormiston and their teams who detected a significant reduction in serum total cholesterol after three weeks of treatment with simvastatin (23, 24), also Friberg and co- investigators conformed these results through an in vitro study on rat granulosa cells (25), despite some papers disagree with these findings which did not found significant changes in serum total cholesterol of rats (26, 27). The hypocholesterolemic potency of statins has been elucidated by inhibiting activity of the rate limiting enzyme, 3- hydroxy 3- methyl glutaryl Co- A reductase in the cholesterol biosynthesis pathway leading to decrease in both free and cholesteryl esters (28).

Statins related compounds increases and stabilizes the low density lipoproteins cholesterol (LDL-c) receptors gene expression through triggering the up regulation of the LDL-c receptors mRNA in rat's hepatocytes (29), through the effect of serum cholesterol level on the sterol regulating element- binding protein which controls the specific gene expression participating in the cholesterol cellular uptake mechanism and metabolism leading to a drop in it's plasma level (3), all the former results are corresponding and explaining the observations of the present study relating to serum total cholesterol level.

In view of the decreased triglycerides concentration caused by simvastatin oral doses (table 2), our findings somewhat resemble those of a previous studies in human (30), in normal female rats (31) and in alcohol- treated rats (32).

The reduction in serum triglycerides level may be a consequence of the action of HMG Co-A reductase inhibitors which inhibits lipase activity probably by decreasing lipase mRNA (31), another theory elucidates the reason of decreased serum triglycerides level was because of the inhibitory action of simvastatin on the cytoplasmic form of the enzyme diacylglycerol acyltransferase which catalyzes the final reaction in the synthesis of triglycerides in the rat liver microsome (33) furthermore the effect of treatment with high doses of statins markedly decrease plasma very low density lipoproteins cholesterol (VLDL-c) and the related triglycerides level (34) but in comparison with results of the present study, which shown that the high dose (100 mg. kg^{-1})- triglycerides responsive level was statistically similar with other two doses (25 and 50 mg. kg^{-1}) since the day 45 of treatment.

Data scorer as considered with aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are partially fit with previous studies related with ALT activity (table 4) which shown an increment in normal human serum (35), normal rabbits and rats ALT at a high dose for two weeks of treatment (36, 37) Despite our data disapprove last studies as related with raised AST activity in them which continue insignificant in the present study (table 3).

Some experiments explained several possible mechanisms for statin- associated high ALT activity with the occurrence of skeletal myopathy which have been proposed recently, depletion of secondary metabolic intermediates and induction of apoptosis and alteration of chloride channel conductance within myocytes (38).

The significant decrease in serum total testosterone shown in figure (1) are some kind identical with previously reported data by Azzarito and co- investigators who found that the treatment of hypercholesterolemic men with simvastatin for twelve months resulted a moderate reduction in serum testosterone (7) also statin treatment inhibited the testosterone production by 25 % in rats and in women with polycystic ovary syndrome (39), on the other aspect studies refer to that serum testosterone was unchanged by statin treatment in human males (10, 13) and in normal rats (23).

Some studies found a reverse results as related to testosterone level which in turns to increase during the treatment with statins (40), these results disagree with the observations of the present study.

The cholesterol biosynthesis inhibitor was found to suppress gonadal steroidogenesis which includes androstenediol and testosterone through it's known inhibitory effect on HMG Co-A reductase activity but an additional mechanism was demonstrated by affecting the later steps of testicular steroidogenesis by selectively inhibition of 17- ketosteroidoxidoreductase which catalyze the conversion of dehydroepiandrosterone and androstenedione to androstenediol and testosterone respectively (41), another study found that simvastatin caused a significant decrease in the testosterone 6- beta hydroxylation in the cytochrom P450 of human hepatocytes (42) as well as the evidence of that statins to reduce receptor mediated uptake of cholesterol in the gonads for steroidogenesis (43) and suppress the lutenizing hormone (LH) level which controls on a part of steroidogenesis (23).

All the observations in text above assures and explains the results of the present study as related with testosterone level and the dose related testosterone reduction is fully associated with reduced serum total cholesterol as a normal result of testosterone precursor depletion.

The decline in liver cholesterol caused by inhibition of cholesterol biosynthesis pathway (table 6), agree with Hou and his team who recorded 50% reduction in tissue's total cholesterol after cholesterol biosynthesis inhibition in normal mice (44). Simvastatin- treated female rats shown a sharp increase in cholesteryl esters in the liver microsome as a result of the inhibitory action of simvastatin on hepatic

lipase mRNA expression (31) versus to the data of the present study which demonstrated a decline in liver total cholesterol.

It has been reported that the relatively selective inhibition of liver cholesterol synthesis by statin compounds might be due to the existence of a specific uptake mechanism mediated by the Na^+- independent multispecific anion transporter systems (38) whereas the surprising increase levels of total cholesterol in testis of group received 100 mg. kg^{-1} after sixty days of treatment are elucidated as a result of reduction of high density lipoproteins cholesterol (HDL-c) binding proteins (HB1 and HB2) with no effect on serum cholesterol concentration (26), now it is known that HMG Co-A reductase synthesis and activity increase following their competitive inhibition by statins leading to increase in rate of cholesterol biosynthesis in tissues (45) in addition to the high dose statin treatment of rats rises enzyme acyl Co- A: cholesterol acyl transferase mRNA level (34).

A recent in vitro study found that the apolipoprotein E incorporated in rat HDL-c has a high affinity for LDL-c receptors so suggested that rat Leydig cells can use HDL-c as a substitute to regulate testicular steroidogenesis via an apolipoprotein pathway (42), also this elevation in testicular cholesterol content may be due to the notable ability of rat's Leydig cells for autorecovary in a progressive manner, this event was associated with a striking proliferation of smooth endoplasmic reticulum and peroxysomes, this hypothesis discussed that these morphologic alteration are the counterpart of enhanced newly synthesis of HMG Co-A reductase that is a mode of compensatory response of Leydig cells aimed to maintaining an adequate production of cholesterol despite of the competitive inhibition of HMG Co-A reductase by statins (46). This compensation of cholesterol level in the testis did not resulted in up regulation of testosterone production probably due to the relative short- term duration of treatment.

The relative stable values in liver weight percentage relative to whole body weight among the three simvastatin-treated groups compared with control (figure 2) throughout the duration of treatment is in agreement status with results published by Adah and co- investigators in different species of male animals which demonstrated there were no influence of cholesterol biosynthesis inhibition on the weights of liver and reproductive organs (12, 23) however testis weight percentages are significantly increased in groups of 25 and 50 mg. kg^{-1} after sixty days of treatment (figure 3) without satisfying interpreting.

The dose related drop in body weight gain percentage relative to initial body weight described in figure (4) is against the observations of Adah and his co- investigators in animals treated with simvastatin for sixty-five days (12, 23), in the same time, normal mice received statin family shown a reduction in body weight possibly due to decreased food consumption (34). Another theory tried to explain the reduced weight gain as a result of less pronounced bile acids biosynthesis produced by the action of HMG Co-A reductase inhibitors progressively with dose doubling (47).

In conclusion, there were a direct proportion between serum total cholesterol and testicular concentration of cholesterol in normal rats, negatively reflected on serum testosterone and triglycerides, in addition to decreased body weight gain and increased serum alanine aminotransferase activity. Furthermore there was a compensatory mechanism for decreased level of cholesterol in the testis without affecting on dependant testosterone.

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