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# Effects of Simvastatin and/or Metformin Administration on Lipid Profile and Reproductive Function in Adults Male Rats

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## Abstract

**Background** : HMG-CoA reductase inhibitors, are large used lipid lowering medical that are effective in the functions of male reproductive. In addition, Metformin is affected on level of cholesterol that results in production of sperm. This study was designed to evaluate whether influence of simvastatin, metformin alone and combination in male rats. **Methods**: In this experiment 24 adult male rats were randomly divided into four groups(n=6) as following: control group (G1). 2nd group(G2)20 mg/kg simvastatin. 3rd group(G3)150mg/kg of metformin. 4th group(G4)150mg/kg metformin + 20m/kg simvastatin. The administration rout by oral gavage for 40 days. **Results**: There is a significant increase ( $P \leq 0.05$ ) in blood parameters, HDL cholesterol, blood glucose, total protein and sperm abnormality in treated groups. Histopathological study of testes reveals degeneration, vacuolation of germinal epithelium, destruction the wall of some seminiferous tubules. Liver tissue shows dilated, congested sinusoid, vacuolation of hepatocytes and central vein congestion, on the other hands, kidney reveals a vacuolation of some epithelial lining of glomeruli and renal tubules, atrophy of some glomeruli and dilation of bowman`s capsules. **Conclusion**: Simvastatin have a better effect on lipid profile. On the other hands, have more effects on testicular function.

**Key Words**: Metformin, Simvastatin, HMG-CoA reductase.

## Introduction

Statin affect cholesterol synthesis and thus may be contribute in inhibits steroidogenesis<sup>(1)</sup>. Due to their cholesterol-decreasing properties, this class of inhibitors might be expected to have adverse effects on reproduction by reducing the supply of circulating cholesterol which is required for steroidogenesis <sup>(2)</sup>. Nevertheless, HMG-CoA reductase inhibitors are considered teratogenic due to studies conducted with lovastatin <sup>(3)</sup>, and sporadic testicular effects have been observed in dogs <sup>(4,5)</sup>. Metformin controlling blood glucose level. Metformin works by helping to restore the body's response to insulin. It decreases the amount of blood sugar that the liver produces and that the intestines or stomach absorb <sup>(6)</sup>. Metformin have a positive impact on sperm quality,

this due to it is ability to reduce oxidative stress and lipid peroxidation, enhance 5'-AMP activated protein kinase activity, and restore the normal levels of pituitary-gonadal hormones <sup>(7)</sup>.

## Materials and Methods

This study was conducted in College of Pharmacy and it's approved by the ethical committee in the College. In this study,24 adults male rats (335 -365 g) were used. Rats housed in plastic cages (2 rats /cage) under standard laboratory conditions (12/12 light/dark cycle,  $22 \pm 2$  °C), they are allowed for pellet and tap water.

## Experimental design

Animals are divided randomly into 4 equal groups (n=6). The control group dosed with D.W. for 40days, simvastatin group 20mg/kg simvastatin, metformin group 150mg/kg metformin, simvastatin (20mg/kg) and simvastatin + metformin group dosed 20mg/kg simvastatin and 150 mg /kg metformin. Blood was

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collected from inferior vena cava, serum was stored in Eppendorf tubes at  $-20^{\circ}\text{C}$ . Blood glucose was measured according<sup>(8)</sup>, total cholesterol (TC)<sup>(9)</sup>, Triglyceride (TG)<sup>(10)</sup>, HDL cholesterol with total cholesterol<sup>(11)</sup>. VLDL according<sup>(12)</sup>. Sperm count according<sup>(13)</sup>. Individual sperm motility according<sup>(14)</sup>. The organs fixed in 10% formalin, stained with H&E and examined under light microscopy.

### Statistical Analysis

The data were expressed as mean  $\pm$ Standard deviation (SD), ANOVA analysis in our study. Least significant difference (LSD) was used to test the differences among means, indicated a significant ( $P<0.05$ ), using computerized SPSS v3.

## Results

### 1. Biochemical tests and Sperm analysis

Table(1) showed that was a significant increase ( $P\leq 0.05$ ) in HB, PCV, RBCs and WBCs count of simvastatin group compared with control and other

treated groups. While, it showed a significant decrease in PTL count of all treated groups compared with control group.

Table (2); the serum cholesterol, TG, and VLDL cholesterol level of sim, metf, and co-treated sim with metf groups were significantly lower than control group ( $P\leq 0.05$ ). While, markedly increase level of HDL cholesterol was observed in sim rats compared with control and other treated rats. Also, serum level of LDL cholesterol remained elevated in the control and metf rats compared with sim and co-treated rats.

Effects of metformin and simvastatin on serum blood glucose and plasma total proteins in adults male rats was shown in table (3). Compared with met, and co-treated sim-metf groups the serum level of blood glucose were significantly higher in group sim ( $p\leq 0.05$ ). On the other hand, the level of total proteins in co-treated group were significant higher than all groups are studies. However, simvastatin, metformin and co-treated rats showed a significant increase in abnormal sperm morphology compared with control.

**Table (1) Effect of metformin, simvastatin and co-administration of metf. and simv. on blood parameters. (M $\pm$ SD)(n=6)**

| Parameters groups | HB g/dl           | RBC $\times 10^6$ cell/mm <sup>3</sup> | WBCs $\times 10^3$ cell/mm <sup>3</sup> | PCV %             | PLT %            |
|-------------------|-------------------|--|---|-------------------|------------------|
| G1 cont.          | 13.25 $\pm$ 0.6 d | 6.06 $\pm$ 0.1 d                       | 11.51 $\pm$ 0.2 b                       | 37.53 $\pm$ 1.9 C | 443 $\pm$ 25.8 a |
| G2sim             | 15.61 $\pm$ 1.1 a | 6.39 $\pm$ 0.4 a                       | 14.63 $\pm$ 2.5 a                       | 39.44 $\pm$ 0.2 a | 269 $\pm$ 43.8 d |
| G3metf            | 14.26 $\pm$ 0.8 c | 6.10 $\pm$ 0.2 c                       | 9.96 $\pm$ 1.1 d                        | 36.46 $\pm$ 1.5 d | 410 $\pm$ 15.2 b |
| G4sim,metf        | 14.68 $\pm$ 0.6 b | 6.37 $\pm$ 0.2 b                       | 11.31 $\pm$ 0.6 c                       | 38.71 $\pm$ 3.5 b | 363 $\pm$ 25.8 c |
| LSD               | 0.41              | 0.02                                   | 0.20                                    | 0.72              | 24.81            |

*The different letters refer to significant differences among groups at level of ( $p\leq 0.05$ ).*

**Table (2) Effect of metformin, simvastatin and co-administration of metf. and sim. on lipid profile. (M±SD) (n=6)**

| Parameters groups | Chl. mg/dl    | Tg. mg/dl    | HDL mg/dl     | VLDL mg/d   | LDL mg/dl     |
|-------------------|---------------|--------------|---------------|-------------|---------------|
| G1 cont.          | 203.68±2.7 a  | 157.61±9.5 a | 71.36±3.7 d   | 31.52±1.9 a | 99.065±25.5 a |
| G2sim             | 176.06±7.4 d  | 78.38±8.0 d  | 185.01±5.5 a  | 15.73±1.6 d | 66.06±7.9 c   |
| G3metf            | 181.85±9.5 b  | 123.08±7.3 b | 84.23±4.9 c   | 24.61±1.4 b | 97.04±0.5 a   |
| G4sim,metf        | 181.11±11.8 c | 104.60±2.4 c | 145.65±10.5 b | 20.83±0.5 c | 85.13±12.9 b  |
| LSD               | 0.73          | 18.46        | 12.87         | 3.77        | 2.61          |

*The different letters refer to significant differences among groups at level of ( $p \leq 0.05$ )*

**Table (3) Effect of metformin, simvastatin and co-administration of metf. and sim. on blood glucose and total proteins. (M±SD)(n=6)**

| Parameter groups | Blood glucose g/dl | Total proteins g/dl |
|------------------|--------------------|---------------------|
| G1cont.          | 177.10±28.0 a      | 14.23±1.3 d         |
| G2 sim           | 184.06±8.2 a       | 17.61±1.9 b         |
| G3met            | 117.33±37.3 b      | 16.75±0.6 c         |
| G4sim,met        | 140.83±21.9 b      | 17.71±1.8 a         |
| LSD              | 36.33              | 0.10                |

The different letters refer to significant differences among groups at level of ( $p \leq 0.05$ )

## 2. Histopathological study

### 1. The Testes

Testes of control rat include normal seminiferous tubules. Testes of rat treated with simvastatin (20mg/kg B.W), shows degeneration with vacuolation of germinal epithelium and irregularity of basement membrane. Few numbers of spermatogonia as well as necrotic spermatocytes in some seminiferous tubules.

Testes of rats treated with metformin (150 mg/kg b.w) shows vacuolation of germinal epithelium. There few numbers of spermatogonia. Testes of rats treated with simvastatin (20mg/kg b.w) and metformin (150mg/kg b.w) shows disarrangement, sever sloughing of epithelium and destruction the wall of some seminiferous tubule. There is a lot of number of spermatids in seminiferous tubules.



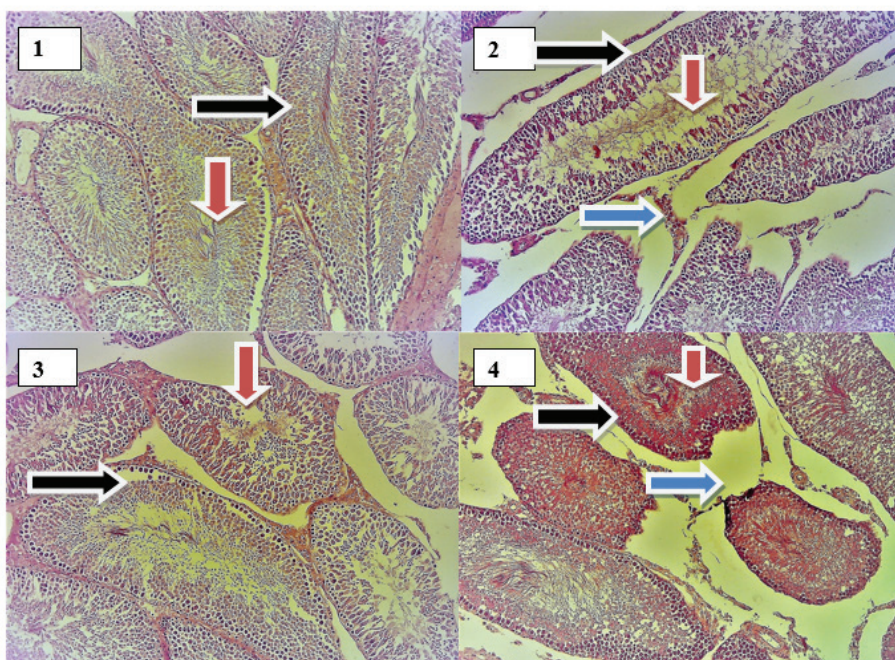


Figure (1): tests of control, normal structure (black arrow) full with sperm(red arrow). (2): tests of rat treated 20mg/kg simvastatin, degeneration in addition to vacuolation (red arrow) (3): tests of rat treated 150mg/kg metformin, vacuolation & degeneration (red arrow). (4): tests of rat treated simvastatin and metformin, thickens within seminiferous tubules and vacuolization (red arrow). H&E, 200x.

## 2. The Liver

The liver tissue of control group has shown normal liver tissue. The cellular cords separated by sinusoids. The plate hepatic cells were separated by narrow, congested blood sinusoids with vacuolation of hepatocytes (figure

2). Also there is vacuolization of hepatocytes, congested central vein, enlarged sinusoid (figure 2). Hepatic nuclei are enlarged pyknotic and light chromatinic stained, disappearance of the radiated hepatic architecture, massive vacuolation in hepatocyte, the central vein thick and narrow (figure 2).

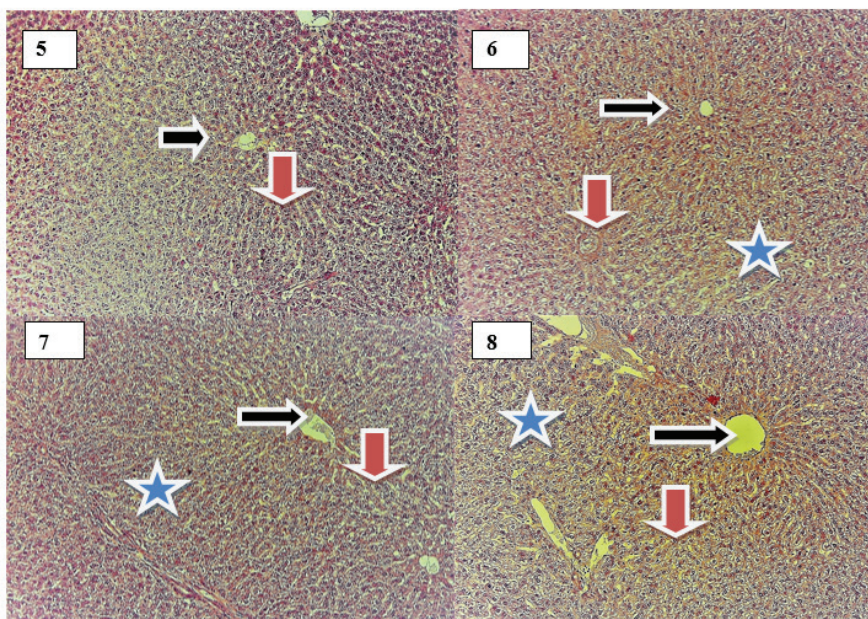


Figure (2): liver control rat (black arrow) and hepatocytes (red arrow). (6): liver of male rat exposed 20mg/kg B.W. of simvastatin, vacuolization (blue star), congested of sinusoid (red arrow). (7): liver of male rat exposed 150mg/kg B.W. of simvastatin, vacuolization (blue star), congested of sinusoid (red arrow). (8): liver of male rat exposed 150mg/kg B.W. of simvastatin, vacuolization (blue star), congested of sinusoid (red arrow).

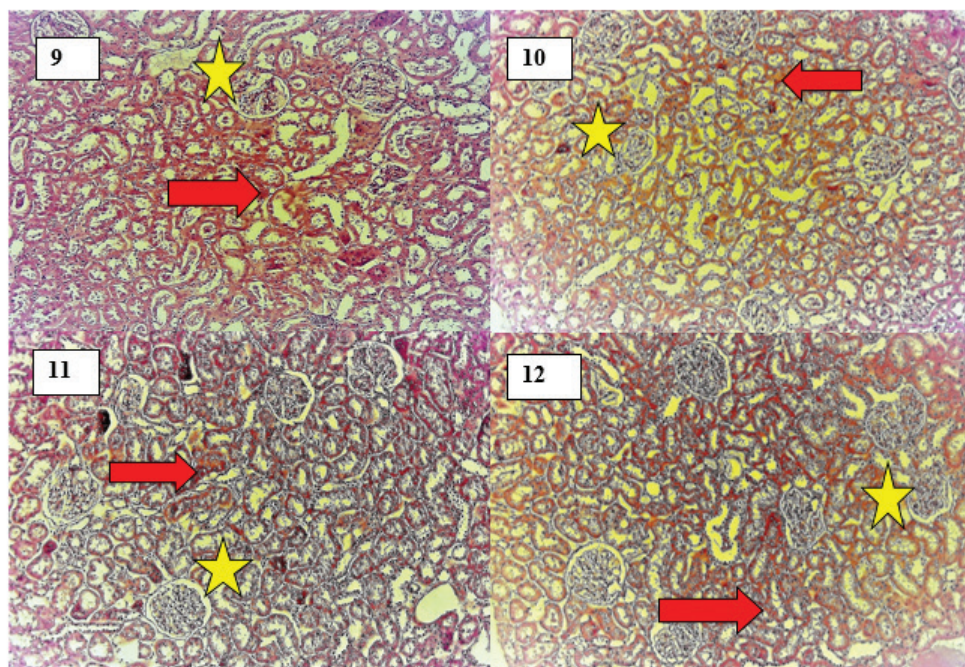


**metformin, vacuolization, congested central vein (black arrow), enlarged sinusoid (blue star). (8): liver of male rat exposed 20mg/kg B.W. of simvastatin and 150 mg/kg B.W of metformin, vacuolated hepatocyte. H&E, 200x.**

### 3. The Kidney

The kidney of control shown normal renal tissue (figure 3). Kidney of rats exposed to 20mg/kg B.W simvastatin, shows necrosis of the epithelial

lining of glomeruli and tubules and glomerular atrophy (figure 3). Kidney of rat exposed to 150mg/kg B.W. of metformin, shows enlargement of glomeruli with dilatation of bowman's capsule and hemorrhage between tubules (figure 3). Kidney of rat exposed to 20mg/kg B.W of simvastatin and co-treated with 150mg/kg B.W. of metformin, shows atrophy of glomeruli with narrow of bowman's capsule and hemorrhage between tubules (figure 3).



**Figure(3): kidney of control rat show normal glomeruli (star) and tubules (red arrow). (10): kidney of rat 20mg/kg B.W. of simvastatin, necrosis of the epithelial glomeruli, tubules (star), atrophy of glomeruli (red arrow). (11): kidney of rat 150mg/kg B.W. of metformin, enlargement of glomeruli, dilatation of bowman's capsule (star), hemorrhage between tubules (red arrow). (12): kidney of rat 20mg/kg B.W. of simvastatin and 150mg/kg B.W. of metformin, glomerular atrophy, narrow of bowman's capsule (star), hemorrhage (red arrow). H&E, 200x.**

### Discussion

The continues increasing of glycosylated hemoglobin as product of diabetic related with glucose over all in blood is give form to changes of Hb structure, this factors could have contribute to increase in all blood cells. Therefore, hyperglycemic increases the blood cells count<sup>(15)</sup>. Hence, the altered in blood picture (RBCs, WBCs, PCV, and PLT) may presented in table(1)<sup>(16)</sup>. Another interesting finding of our study is that the effect of simvastatin, metformin & combination on lipid profile in which there is a significant decrease in lipid profile of all treated groups in comparison to control group while there is a significant increase in HDL in simvastatin

group when compared with untreated rats as shown in table (2). Our result is in line with<sup>(17)</sup> who mentioned that there is a decrease in all lipid profile in mice treated with atorvastatin.

Several studies showed that rats treated with metformin cause a decrease in lipid profile, while the addition of natural honey to metformin significantly decrease all type of cholesterol and has a better effect in comparison to metformin alone in diabetic rats<sup>(18,19)</sup>. Our results are also in agreement with Wang et al<sup>(20)</sup>, who observed that the level of TC, TG significantly decrease & the HDL cholesterol increase in diabetics rat treated with simvastatin drug. Bellia et al, mentioned

that random clinical studies of patient treated with simvastatin drug at dose 20mg/day has no effect on glucose level control for 4weeks of treatment, also found that glycemic control and HbA1c may worsen with increase duration of treatment till 1year with no change in insulin sensitivity<sup>(21,22)</sup>.

Result in table(3)reveals the increment in glucose level in rats treated with statin drug significantly in comparison to those treated with metformin &the combination of these two drugs, this result may be related to the effect of statin drug on islet of Langerhans which may effect on their ability to secret insulin or may be due to decrease the sensitivity of insulin which lead to increase insulin resistance and increase glucose level. This result is in agreement with<sup>(23,20)</sup> they both reported that simvastatin associated with an increase in serum glucose level and impaired glucose homeostasis in patients and rats respectively.

The present study showed an effect of simvastatin, metformin and their combination on sperm count, motility and normal morphology presented as a decrease in sperm characteristics, on the other hands, these drugs also increase the abnormality of sperm morphology in comparison to control group , this result is in agreement with<sup>(24)</sup> who mentioned that a decrease in testes weight, low sperm count, motility and abnormal morphology of semen in rat administered high dose atorvastatin.

Adaramoye and Lawal,<sup>(25)</sup>; Banihani,<sup>(26)</sup> they mentioned that administering of metformin in rat may have a positive effect on sperm characteristics especially in diabetic rat and at dose 30mg/kg per day for 6weeks. While administering of metformin for 3 weeks at the same above dose in rat associated with a decrease in semen count and motility by 33%<sup>(27)</sup>. Our results corresponding with histopathological study of testes tissue associated with degeneration, vacuolation of epithelium, sloughing in germinal epithelium and spermatocytes necrosis in some of seminiferous tubules, this results is in line with<sup>(28,29)</sup> they reported that rat treated with rousovastatin and atorvastatin respectively cause changes in seminiferous tubules, death of some germinal epithelium and edema in interstitium.

Drugs exposed to metabolism by liver and kidney tissue, therefore histopathological study of these organs associated changes including dilation of liver sinusoid,

vacuolation of hepatocytes and congestion of hepatic sinusoids. Kidney associated with excretion of drugs metabolites therefore in some field showed atrophy of some glomeruli, dilation of bowman`s capsules and degeneration of some glomerular cells. These changes occur as an adaptation of these organ to accommodate the continuous administration of these drug, therefore considered as reversible changes and the organs can back to normal state if the cause annotate<sup>(30)</sup>.

### Ethical Clearance

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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