Effect of atorvastatin on the liver enzymes, electrolytes and histology of organs in male albino Wistar rats

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ABSTRACT

Statins (atorvastatin) are well known for their lipid lowering potentials and reduction of cardiovascular events in patients. The study evaluated the effect of atorvastatin on the electrolytes (Na+, K+, Cl− and Ca2+) liver enzymes (aspartate aminotransferase; AST, alanine aminotransferase; ALT, alkaline phosphatase; ALP) and the histology of some organs (heart, kidney and liver) in a dose dependent manner. Twenty-four male rats weighing between 150–180 g were divided into four groups of six (6) rats in each group. Group one which was the control was given distilled water as placebo. Groups two, three and four were given 40 mg/kg, 80 mg/kg and 120 mg/kg per body weight respectively of atorvastatin orally for 21 days. The animals were humanely sacrificed 24 h post administration. Blood samples were collected by cardiac puncture into plain sterile tubes. They were centrifuged to obtain the serum which was further used for liver enzymes analysis. The organs were collected and fixed in formalin for histological analysis. The results indicated that there was a significant increase (P < 0.01) for AST in groups II, III and IV, ALT also showed a significant increase (P < 0.01) in groups III and IV. There was also a significant increase (P < 0.05) for K+ in groups III and IV. Histological analysis also revealed that tissues of the liver showed vacuolation and vascular congestion as they were affected by the drug. In conclusion, the biochemical report was corroborated with results of histological evaluation. Higher dosage of atorvastatin is hepatotoxic as evidenced in the increase in the activities of ALT, AST, and the degeneration in the liver cells. Taking atorvastatin with caution is recommended and a lower dose at a longer time is preferable.


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Ethical approval: The authors have declared that principles of laboratory animal care were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

Competing Interests: The authors have declared that no competing interests exist.

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Keywords: atorvastatin; cardiovascular; electrolytes; hepatotoxic; histology; liver enzymes.

1. INTRODUCTION

Statins are also known as 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors. Reports have shown that they are the best-selling prescription drugs in the world – indeed in history [1]. These drugs are perceived to have a favorable safety profile and have well documented benefits to cardiovascular disease in many groups, including persons who are younger and older, male and female, at moderate and high cardiovascular risk. In addition, benefits have been objectively shown to exceed risks on average, specifically in clinical-trial equivalent middle-aged men who are at high cardiovascular risk [2]. 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, and are also appreciated for their

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good tolerance. Studies have demonstrated that these compounds reduce the progression and may induce the regression of atherosclerosis. These effects were translated in significant cardiovascular morbidity and mortality reductions in many clinical trials [3].

2. MATERIAL AND METHODS

2.1 Experimental animals

Twenty four (24) male albino Wistar rats weighing between 150 - 180 g were obtained from the animal house facility of Biochemistry Department, Faculty of Basic Medical Science, University of Uyo, Uyo, Nigeria and were used for the study. Prior to the experiment, the animals were acclimatized for three days and their weights taken. The animals were housed in wooden cages designed with wire gauge on top for ventilation and with provision for feed and water nozzle. They were kept in the animal house under adequate ventilation and room temperature of 26 ± 3 ºC and relative humidity with a 12 hour day light cycle. They were maintained in water and animal feed ad libitum.

2.2 Experimental design, drug dosage formulation and administration

The experimental design and the drug dose formulation is explained in table 1 below

<table>
<thead>
<tr>
<th>Group</th>
<th>No of rats</th>
<th>Treatments</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Control)</td>
<td>6</td>
<td>Distilled water</td>
<td>0 mg/kg b.w.</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>Atorvastatin</td>
<td>40 mg/kg b.w.</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>Atorvastatin</td>
<td>80 mg/kg b.w.</td>
</tr>
<tr>
<td>IV</td>
<td>6</td>
<td>Atorvastatin</td>
<td>120 mg/kg b.w.</td>
</tr>
</tbody>
</table>

2.3 Animal Sacrifice and Preparation of Sera for Analysis

At the end of drug administration (after 21 days), the animals were fasted overnight (12 h) and euthanized by dropping each in a transparent plastic jar saturated with chloroform vapour. Incision was made on the abdomen. Blood sample was collected through cardiac puncture using sterile syringes and needles into sterile plain tubes for sera preparation and anticoagulant (EDTA) bottles were used for whole blood. Serum samples were obtained from clotted blood into sterile plain tubes after centrifugation at 2000 rpm for 15 min using a bench top centrifuge. The serum was stored in the refrigerator until when used for analyses. Tissues of the animal was fixed in formalin for histopathological studies.

2.4 Biochemical Analysis.

The Estimation of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) was done using the spectrum assay kit methods based on the principles of Reitman and Frankel [4]. Glucose analysis was done using a glucometer. Electrolytes were analyzed using the IVD Automatic Electrolyte Analyser.

2.5 Histopathological Studies

The fixed tissues of the heart, kidney and liver were cut (5- micron thickness) and sectioned. The sections of the tissue were stained with the dyes of Hematoxylin and Eosin (H and E) according to Conn [5].

2.6 Statistical Analysis

This was carried out using windows SPSS version 20. One way analysis of variance was adopted for comparison. The data were expressed as mean ± standard deviation and values of p<0.05 were considered significant.

3. RESULTS AND DISCUSSION

3.1 Effect of Statin on enzyme activities of male albino Wistar rat

The result of the effect of statin on enzyme activities of male albino wistar rat is presented in Fig 1.

3.1.2 Effect of Statin on the electrolyte of male albino Wistar rat

The effect of Statin on the Electrolyte in test groups and control shows a significant increase (p<0.05) only for K⁺ in group III and IV. It is presented in Table 2.

3.1.2 Effect of Statin on the histology of organs on male albino Wistar rat

The result of the histology of organs using the H and E technique is represented in plates I, II and III.
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**Fig. 1.** Enzyme activities in male albino wistar rat treated with statin

**Table 2: Effect of Statin on the Electrolyte of Male Albino Wistar Rat**

<table>
<thead>
<tr>
<th>Group</th>
<th>K (mmol/L)</th>
<th>Na (mmol/L)</th>
<th>Cl (mmol/L)</th>
<th>i Ca (mmol/L)</th>
<th>t Ca (mmol/L)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (0 mg)</td>
<td>3.48±1.32</td>
<td>134.00±1.41</td>
<td>94.90±1.84</td>
<td>0.72±0.04</td>
<td>1.4±0.08</td>
<td>7.94±0.07</td>
</tr>
<tr>
<td>II (40 mg)</td>
<td>6.63±6.06</td>
<td>137.77±2.81</td>
<td>95.47±3.01</td>
<td>0.75±0.03</td>
<td>1.42±0.02</td>
<td>8.02±0.09</td>
</tr>
<tr>
<td>III (80 mg)</td>
<td>14.09±7.26</td>
<td>137.50±6.58</td>
<td>96.47±1.67</td>
<td>0.77±0.09</td>
<td>1.50±0.17</td>
<td>8.14±0.27</td>
</tr>
<tr>
<td>IV (120 mg)</td>
<td>14.57±3.26</td>
<td>138.33±4.23</td>
<td>97.30±4.92</td>
<td>0.76±0.06</td>
<td>1.62±0.06</td>
<td>7.95±0.04</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± S.D, n=6; a=p<0.05 (compared with control), b=p<0.01 (compared with control).

The result of the biochemical assessment of statin-treated male albino Wistar rats from the study shows that the liver enzymes ALT and AST were significantly different from control. ALT for group III and group IV was significant at (P<0.01). AST for group II, III and IV also showed a significant increase at (P<0.01). The enzyme aspartate aminotransferase (AST) is widely distributed in erythrocytes and tissues principally in the heart, liver, muscles and kidney. Elevation of the serum level of AST is found in disease conditions such as myocardial infarction, viral hepatitis and muscular dystrophy [6]. A compromise to the tissue results in an increase of the enzyme in circulation thereby showing an increase in the serum level of AST [7]. The significant increase (P<0.01) in groups II, III and IV in the experimental is an indication that there was an increase in the amount of the enzymes in the serum. This may have resulted in the damage to the tissue [8].

Electrolytes are substances that become ions in solution and have the ability to conduct electricity. Its presence in the body is essential for normal function of our cells and organs. The result of electrolytes shows a significant increase in K⁺. The significant increase in K⁺ shows that statin dose at 120mg/kg had predisposed the kidney of the animals to damage thereby leading to their increase in the blood resulting in a condition called hyperkalemia [9].

The result of the histology of organs shows that the liver was affected at higher doses of the drugs. This shows that a higher dose of statin is harmful to the liver.
Plate I(a) - Histologic photomicrograph of heart tissues of normal male albino wistar rats without treatment with statin at Magnification A1 (X100) and A2(X400) stained with H and E technique. Heart tissue revealed normal area cellular pattern of cardiac muscle with myocardium area of purkinje fiber, interstitium, cardiac muscle cell and intercalated disc within normal limit (Not affected).

Plate I(b) - Histologic photomicrograph of the Heart tissues treated with 40mg/kg of statin at Magnification B1 (X100) and B2 (X400) stained with H and E technique. Tissue revealed normal cellular pattern with no abnormality as compared to control group. (Not affected).

Plate I(c) - Histologic photomicrograph of the Heart tissues treated with 80mg/kg of statin at Magnification C1 (X100) and C2 (X400) stained with H and E technique. Tissue revealed normal cellular pattern with no abnormality as compared to control group. (Not affected).
Group 4: Heart

Plate I(d) - Histologic photomicrograph of the Heart tissues treated with 120mg/kg of statin at Magnification C1 (X100) and C2 (X400) stained with H and E technique. Tissue revealed normal cellular pattern with no abnormality as compared to control group. (Not affected).

Group 1: Kidney

Plate II(a) - Histologic photomicrograph of kidney tissues of normal male albino wistar rat without treatment with statin at Magnification A1 (X100) and A2(X400) stained with H and E technique. Tissue revealed normal area of renal corpuscle with squamous lining epithelium, distal and proximal convoluted tubules, collecting ducts and loop of henles, all within normal cellular profile. (Not affected).

Group 2: Kidney

Plate II(b) - Histologic photomicrograph of Kidney tissues treated with 40mg/kg of statin at Magnification B1 (X100) and B2 (X400) stained with H and E technique. Tissue revealed normal cellular pattern with no abnormality as compared to control group. (Not affected).
Group 3: Kidney

Plate II(c) - Histologic photomicrograph of Kidney tissues treated with 80mg/kg of statin at Magnification C1 (X100) and C2 (X400) stained with H and E technique stained with H and E technique. Tissue revealed normal cellular pattern with no abnormality as compared to control group. (Not affected).

Group 4: Kidney

Plate II (d) - Histologic photomicrograph of the kidney tissues treated with 120mg/kg of statin at Magnification D1 (X100) and D2 (X400) stained with H and E technique. Tissue revealed normal cellular pattern with mild area of inflammation as compared to control group (Not affected).

Group 1: Liver

Plate III(a) - Histologic photomicrograph of liver tissues of normal male albino wistar rat without treatment with statin at Magnification A1 (X100) and A2 (X400) stained with H and E technique. Tissue revealed normal cellular pattern of central vein, hepatocytes, radiating from the sinusoidal tracks and portal triad. (Not affected).
Group 2: Liver

Plate III(b) - Histologic photomicrograph of liver tissues treated with 40mg/kg of statin at Magnification C1 (X100) and C2 (X400) stained with H and E technique. Tissue revealed area of pyknotic nucleus, vacuolation and vascular congestion as compared to control group. (Not Affected).

Group 3: Liver

Plate III(c) - Histologic photomicrograph of liver tissues treated with 80mg/kg of statin at Magnification C1 (X100) and C2 (X400) stained with H and E technique stained with H and E technique. Tissue revealed area of pyknotic nucleus, vacuolation and vascular congestion as compared to control group. (Moderately Affected).

Group 4: Liver

Plate III (d) - Histologic photomicrograph of the liver tissues treated with 120mg/kg of statin at Magnification D1 (X100) and D2 (X400) stained with H and E technique. Tissue revealed drug pyknotic nucleus, vacuolation and vascular congestion as compared to control group. (Moderately affected).
4. CONCLUSION

The result obtained for the enzymes show that statin has an hepatotoxic effect. This may be attributed to the generation of free radicals by the drug which compromises with the organ. Increase in $K^+$ shows that was a disruption of the renal cell which lead to the increase in $K^+$. A degeneration of the liver tissue is seen in the histological studies.

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AUTHOR CONTRIBUTIONS

NGI designed the study, performed the experiments and statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. BUO managed the literature searches, EOA and UME supervised and monitored the activities and progress of the research as well as read and corrected the final manuscript. SAO managed the histopathology of the work. All authors read and approved the final manuscript.

REFERENCES


DEFINITIONS, ACRONYMS, ABBREVIATIONS

ALP alkaline phosphatase
ALT alanine amino transferase
AST aspartate amino transferase
BM - Basement Membrane
CT - Collecting Duct
DCT - Distal Convulated Tubules
H - Hepatocytes
HA - Hepatic Artery
HV - Hepatic Vein
ICS - Interstitial Cellular Spaces
LH Loop of Henle
MF - Myocardial Fibres
N - Nucleus
PCT - Proximal Convoluted Tubules
PT - Portal Triad
RBC - Red Blood Cells
RC - Renal Corpuscles
RF - Reticular Fibers
SEL - Squamous Epithelial Lining
SL - Sinusoidal Lining