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# Assessment of the Diabetogenic Potential of Atorvastatin and the Effects of Atorvastatin on Body Weight, Haematological Indices, Serum Calcium Level in Female Albino Wistar Rats

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author EUM designed the study, wrote the protocol and supervised the work. Authors BUO and NGI carried out all laboratories work and performed the statistical analysis. Author EOA managed the analyses of the study. Author BUO wrote the first draft of the manuscript. Author BUO managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aim:** This study was designed to assess the effects of different doses of atorvastatin on fasting blood sugar, body weight, haematological indices, serum calcium and CRP levels in female albino wistar rats.
**Study Design:** Twenty-four female albino wistar rats weighing 125 to 150 g were used for this experiment. They were separated into four groups of 6 rats each and administered different

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concentrations (0, 40, 80 and 120 mg/ml) of atorvastatin daily for 21 days. Group 1 received placebo treatment, groups 2, 3 and 4 received 40, 80 and 120 mg of atorvastatin respectively. Fasting blood sugar was routinely monitored at 3 days interval while the body weight was monitored at 7 days (weekly) interval. At the expiration of the treatment, serum samples were analyzed for effects of the drug on the levels of serum calcium of the animals. Whole blood samples were also analyzed for its effect on some hematological parameters.

**Results:** The results obtained showed that in all the treated groups (groups 2, 3 and 4) there was an overall decrease in C-reactive protein and haematological indices (WBCs, RBCs, platelets and hemoglobin) compared to the control. The result of the fasting blood sugar showed a non-significant and non-consistent increase in fasting blood sugar between the control and treated groups. Treated groups II and III showed significant increase ($p<0.05$) in iCa levels compared to the control. The values obtained for body weight did not show overall significant difference ($p>0.05$) in body weight among the study groups.

**Conclusion:** Short term exposure to atorvastatin did not produce diabetic (hyperglycaemic) side effect. The body weight of the animals did not significantly change, the haematological indices decreased, serum C-reactive protein level decreased and there was increase in serum calcium level.

*Keywords: Fasting blood sugar; haematological indices; C-reactive protein; calcium; body weight.*

## 1. INTRODUCTION

Over the years and even in recent times, the number of deaths caused by cardiovascular disease (CVD) has been on the increase even in civilized nations like the USA, Germany, Canada, etc. According to World Health Organization statistics, more than 16 million people die of cardiovascular disease each year, and 7.2 million deaths in 2001 were caused by heart disease [1]. Cardiovascular disease, which includes hypertension, coronary heart disease (CHD), peripheral arterial disease, heart failure, stroke, etc. is the leading cause of death worldwide [2]. Statins have been described as the principal and most effective class of drugs to reduce serum cholesterol levels and cardiovascular events in patients with or without coronary artery disease [2]. Statins lower cholesterol levels by inhibiting the enzyme HMG-COA reductase, which plays a central role in the synthesis of cholesterol in the liver [3]. Cholesterol is important in building and maintaining cell membranes, and also as a precursor to bile salts and steroid hormones like progesterone and oestrogen [4]. Abnormally high level of cholesterol is the main cause of atherosclerosis [3]. The disease is associated with increased levels of LDL-cholesterol, reduced levels of HDL-cholesterol, impairment of cholesterol retrieval, cytotoxic effects on the endothelium, increased oxidation of lipoproteins, and stimulation of thrombogenesis. Diabetes, also is a major risk factor for coronary disease because it is a source of oxidative stress. Other factors that can contribute to atherosclerosis include lack of nitric oxide, insulin resistance, and inflammation. Cellular components of atherosclerotic plaques include foam cells, which are transformed macrophages, and smooth muscle cells filled with cholesteryl esters [5]. The atheroma grows with accumulation of foam cells, collagen, fibrin and frequently calcium, such lesions can slowly occlude blood vessels, symptoms are mainly produced by rupture of unstable atheromatous plaques leading to activation of platelets and formation of occlusive thrombi [5].

The relationship between elevated low-density lipoprotein cholesterol and increased risk for cardiovascular disease has been proven beyond reasonable doubts [6] and is the primary principle by which statins exert their effect. In the light of this, clinicians have recognized that much of a statins therapeutic effect is derived from its low-density lipoprotein (LDL)-lowering effects [7]. Therefore, the greater the LDL reduction, the greater the clinical benefit in terms of risk reduction for CVD events [8]. In addition, there is evidence that statins, beyond their LDL-lowering effects, reduce vascular inflammation, improve endothelial function and decrease thrombus formation [9-11].

Statins act by competitively inhibiting HMG-COA reductase. This is the first committed enzyme of the pathway of cholesterol biosynthesis. The rate limiting step (and a major site of regulation) in the pathway to cholesterol synthesis is the conversion of HMG-CoA to mevalonate catalyzed by HMG-CoA reductase (4). Inhibition

of this enzyme (HMG-CoA reductase) is possible because of the molecular similarity between the HMG-CoA and statins which gives statins the chance of taking the place of HMG-CoA in the enzyme and reduce the rate by which it is able to produce mevalonate, the molecule produced by the action of HMG-CoA reductase which of cause eventually leads to the production of cholesterol, as well as a number of other compounds. The cardiovascular protective effect of statins is largely due to their low-density lipoprotein cholesterol (LDL-C)-lowering effect [12]. Although all statins act through the same mechanism, they are divided into two categories based on their origin: The fungus-derived lovastatin, simvastatin, and pravastatin; and the synthetic atorvastatin, fluvastatin, and rosuvastatin [13]. Apart from the reduction of LDL-cholesterol through inhibition of cholesterol synthesis, another way by which statins reduce blood LDL-cholesterol level is by stimulating increased synthesis of LDL-receptors to draw cholesterol out of the circulations thus increasing LDL-cholesterol uptake and lowering blood LDL-cholesterol level [14]. Statins also exhibit actions beyond lipid-lowering activity in the prevention of atherosclerosis such as:

a. Improvement of endothelial function
b. Modulation of inflammatory responses
c. Maintenance of plaque stability
d. Prevention of thrombus formation [11]

Statins have been central in the prevention of cardiovascular events associated with increased blood cholesterol and atherosclerotic lesions [14]. As such, statins have become some of the most widely prescribed drugs in the United States with many millions of patients taking them on a regular basis [8]. Statins are mostly taken up by the liver, but the remaining molecules bind with high affinity to plasma proteins. A meta-analysis conducted by Mills et al. in 2011 [8] on the effectiveness of statin in the reduction of cardiovascular events revealed that statin therapy provides consistent benefits derived from its lipid lowering effects and reduces major CVD events. The success of preventive measures depends in part on the accurate identification of individuals who are at risk for future cardiovascular events [15]. It is now recognized that subclinical cardiovascular disease can precede cardiovascular events such as myocardial infarction, for instance, subclinical atherosclerosis can be present for years before a myocardial infarction [15].

Among other side effects of statins such as myopathy, there has risen a concern that statins may slightly increase the risk of type 2 diabetes mellitus [16]. This observation stimulated various studies mostly involving the analysis of medical records of patients undergoing statin therapy and most of these studies observed this side effect in patients on statin treatment with higher doses appearing to have a larger effect [17].

Several trial data and meta-analysis also suggest that statins confer increased risk of development of diabetes [18]. In fact, recent overviews show that all statin agents are associated with a small increase in risk of incident type 2 diabetes and that intensive doses might be associated with higher risk than are lower doses [18].

Individual controlled trials dating back more than a decade have had conflicting results about new diabetes and poorer diabetic control in patients taking statins. On February 28, 2012, the US food and Drug administration (FDA) updated labeling requirement for statins. The JUPITER trial also confirmed existing findings of elevated glucose values in patients receiving high-dose statins [18]. It also confirms that the elevation is very mild, thus affecting individuals with glucose values close to the diagnostic threshold for diabetes at baseline [18]. Vigorous statin therapy can lead to the reduction in acute chronic events within 2-6 months. Intensive statin treatment produces greater reduction in both LDL-cholesterol and inflammatory markers such as C-reactive protein (CRP) and interleukin-6 (IL-6), which suggests a relationship between these markers and progressions of cardiovascular disease [19]. Whereas the mechanism by which statins may induce diabetes remains unknown, several meta-analyses have substantiated the relationship between statin use and elevation of blood glucose levels. As glucose levels rise, some will cross the arbitrary threshold for the diagnosis of diabetes [18].

Despite the diabetogenic side effect of statins, primary and secondary prevention trials have shown that the use of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (Statins) to lower an elevated low-density lipoprotein cholesterol level can substantially reduce coronary events and death from coronary heart disease [6]. Side-effect is a phenomenon that is associated with many drugs, its identification and management/prevention for a particular drug becomes a very important factor to be seriously

considered. The reduction of low-density-lipoprotein cholesterol (LDL-Cholesterol) by statin therapy successfully reduces atherosclerotic events in patients at risk, including individuals with diabetes. The benefits of preventing serious cardiovascular events seems to outweigh the high risks of diabetes and poorer glycaemic control, therefore it is advised that patients at moderate or high risk of cardiovascular events should continue to take statins including those with diabetes [20,18].

Type 2 diabetes mellitus is a major risk factor for cardiovascular disease; in fact, many individuals with pre-diabetes already show diabetes-related complications and cardiovascular disease [21]. Collaborative Atorvastatin Diabetes Study (CARDS) in diabetic patients carried out by Calhoun et al. [22] showed that atorvastatin 10 mg daily is safe and efficacious in reducing the risk of first cardiovascular disease events, including stroke, in patients with type 2 diabetes without high LDL-cholesterol. The role of statins in primary and secondary prevention of cardiovascular disease (CVD), including among patients with type 2 diabetes is well established [9]. However, the relationship of statin therapy to incident type 2 diabetes is controversial [9]. Atorvastatin exerts beneficial effects in diabetes, but the underlying mechanisms are yet to be elucidated [23].

Previous reports have shown that patients with diabetes have elevated serum calcium levels compared with non-diabetic individuals [24], and this has mainly been linked to impaired insulin sensitivity rather than defective insulin secretion [25]. In diseases with markedly elevated serum calcium, such as primary hyperparathyroidism, there is a two- to four-fold higher prevalence of type 2 diabetes and glucose intolerance compared with the general population [26]. Increased parathyroid hormone (PTH) could stimulate calcium channels to increase calcium influx and the levels of intracellular calcium, which would influence insulin sensitivity and hypertension [27]. Increasing intracellular calcium levels have been shown to decrease the effect of insulin in adipocytes due to reduced number of glucose transporters (GLUT4) and decreased insulin receptor activity [26,28]. The dietary intake of calcium did not seem to influence insulin sensitivity [25]. Hypertension, dyslipidemia, and diabetes also significantly increases in a linear trend with increasing calcium [29].

## 2. MATERIALS AND METHODS

### 2.1 Materials

#### 2.1.1 Reagents

Assay kit for the estimation of C-reactive protein were obtained from Agappe Diagnostics, Switzerland.

#### 2.1.2 Acquisition of drugs

A widely prescribed statin drug, atorvastatin (brand name- Lipitor) was a purchased from a renowned pharmacy [Rufus Obi Pharmacy] in the city of Aba, Abia State, Nigeria.

### 2.2 Methods

#### 2.2.1 Dose determination and preparation

The different doses (or concentrations) of atorvastatin that were administered on the experimental animals were deduced from the normal licensed doses, according to drugs.com [30] that are prescribed to adults either for primary or secondary prevention of cardiovascular events. Adults who have not developed cardiovascular disease but have high risk of developing one receive prophylactic dose of 10 mg, 20 mg or 40 mg of atorvastatin daily in order to keep the cholesterol level substantially low so as to prevent the development of cardiovascular disease. Individuals, who have been diagnosed of one cardiovascular disease or the other, are often placed on therapeutic dosage of 80 mg of atorvastatin daily in order to prevent the progression of the disease and the occurrence of cardiovascular events such as stroke or heart attack. These doses and conditions as well as the body weight of the animals were taken into consideration while designing and preparing the doses that the animals were treated with. Therefore, four different doses of atorvastatin were designed for the administration; placebo, 40 mg/kg b.w. and 80 mg/kg b.w. A very high dose (experimental dose) of 120 mg/kg b.w. was incorporated into the work. The drug sample solutions were prepared from 40 mg atorvastatin tablet. A stock solution of the drug containing 1 mg/ml was prepared by dissolving one tablet (40 mg) in 40 ml distilled water. 1 ml of the stock solution was drawn and diluted using appropriate dilution factor which was extrapolated according to the weights of the animals; 1 ml from the different dilutions was equivalent to the different doses,

prophylactic (40 mg/kg b.w.), therapeutic (80 mg/kg b.w.) and experimental (120 mg/kg b.w.).

#### 2.2.2 Animal treatment

Twenty-four healthy female albino wistar rats weighing 125 g to 150 g were used in this work. The animals were obtained from the animal house of the Faculty of Basic Medical Sciences, University of Uyo, Uyo, Akwa Ibom State, Nigeria. The animals were acclimatized for a period of seven days in well ventilated cages maintained under controlled environmental conditions and 12 hours light/dark cycle. The animals were maintained on water and animal feed. After acclimatization period, the rats were reweighed and separated into four groups of close range average weights. Each of the groups contained a total of six animals. Thereafter the animals were subjected to different dosages of atorvastatin (Lipitor). Animals in group I (control) received an equivalent volume (1ml) of distilled water as placebo. Animals in group II were given prophylactic dose (40 mg/kg b.w), group III animals received the therapeutic dose (80 mg/kg b.w.) and group IV animals were given experimental dose (120 mg/kg b.w.). The animals received 1 ml of the statin solution orally at 4.00 pm on daily basis for a period of 21 days. All animal treatments were carried out in line with the guidelines of institutional Animal Ethical Committee as approved by the Graduate School, University of Uyo, Akwa Ibom State, Nigeria. Determination of the fasting blood sugar of the animals was carried out at three days interval, while the body weight was measured at one week interval until the end of administration.

#### 2.2.3 Collection of blood samples and preparation of sera for analysis

At the end of the administration (21 days), the animals were fasted overnight (12 hours) and the fasting blood sugar measured. Thereafter, the animals were anaesthetized and sacrificed and blood samples for sera preparation collected by cardiac puncture into sterile plain tubes, but the blood samples for haematological indices were collected in sterile EDTA tubes. Serum samples were extracted from the clotted blood into sterile plain tubes after centrifugation at 2000 rpm for 10 minutes using a bench top centrifuge (MSE Minor, England). The sera were assayed for serum calcium levels, while the whole blood samples (in EDTA tubes) were used in determining haematological indices.

#### 2.2.4 Measurement of fasting blood sugar

The fasting blood sugar of both control and treated animals was monitored using a glucometer. The tail of each rat was punctured with a new and sterile pin and the blood placed immediately in a strip and read directly in a glucometer to obtain the glucose value.

#### 2.2.5 Measurement of body weight

The body weight of the control and treated animals was monitored weekly using analytical weighing balance.

#### 2.2.6 Estimation of haematological indices

Estimation of Hematological indices (FBC) was determined using systex® automated haematology analyzer, kx-21n (non – cyanide haemoglobin analysis method), sysmex corporation kobe-Japan.

#### 2.2.7 Estimation of serum calcium

The total calcium (tcalcium) and ionised calcium (icalcium) was determined using automated electrolytes analyser.

#### 2.2.8 Estimation CRP

Assay principle- It is a latex enhanced turbidimetric immuno assay CRP samples binds to specific anti – CRP anti – CRP atitbodies which have been adsorbed to latex particles and agglutinate. The agglutination is proportional to the quantity of CRP in the sample.

## 3. RESULTS

The result of the study on the assessment of effects of statins (atorvastatin) on the glycaemia, body weight, haematological indices and serum calcium of female albino wistar rats are presented as follows:

### 3.1 Effect on Fasting Blood Sugar

The glycaemic effects of different statin (atorvatstatin) doses on female albino wistar rats are shown in Fig. 1. At the end of the study, it was observed that the animals did not develop hyperglycemia as there were no significant over all changes in the fasting blood sugar in both the control and test groups (following statin administration). However, some fluctuations were observed as the fasting blood sugar level

oscillates following drug administration in all the groups. Overall, there was non-significant (p>0.05) increase in the fasting blood sugar between the control and treatment groups.

### 3.2 Effects on Body Weight (g)

The effects of different doses of atorvastatin on the body weight of female albino wistar rats are shown in Table 1. The weights of the animals were taken before the statin treatment commenced and changes in weight were monitored on a weekly basis. Change in weight was deduced by comparing the current weight of the animals with their immediate previous weights. In the light of this, no significant (p>0.05) overall change in body weight of the animals in both control and test groups was observed. The treatment group III (80 mg/kg) had initial weight gains which was significantly (p<0.05) more than the control.

### 3.3 Effect on Serum Calcium and CRP

The effects of statin (atorvastatin) on serum ionised calcium and total calcium levels of rats in test and control groups are shown in Table 2. A significant (P<0.05) rise in the levels of serum ionised calcium (iCa) was observed in groups II and III when compared with the control group but group IV showed a non-significant (P>0.05) increase in iCa compared with control. There was also a non-significant increase in the serum tCa level in all the test groups when compared with the control group.

### 3.4 Effect on Haematological Indices of Female Albino Wistar Rats

The effects of different doses of atorvastatin on haematological parameters of female albino wistar rats are shown in Table 3. There was a significant (p<0.05) decrease in White Blood Cells (WBC) of test group III compared to the control but the decrease in WBC shown by test groups 1 and 4 were not significant (p>0.05) compared to the control. There were no significant (p>0.05) changes in Red Blood Cells (RBCs) and haemoglobin of study groups 2 and 3 when compared with the control. The platelets decreased non-significantly in all the treated groups compared to the control (p>0.05).

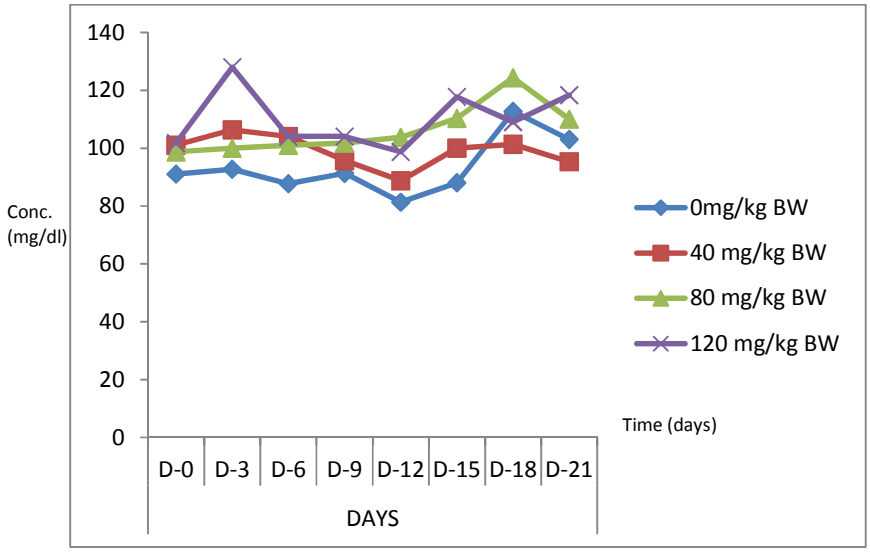


**Fig. 1. Blood glucose concentration (mg/dl) following administration of different concentrations of atorvastatin**

**Table 1. Effect of statin on body weight (g) of female albino wistar rats**

| Group | Day 0 | Day 7 | Day 14 | Day 21 |
|---|---|---|---|---|
| 1 (0 mg/kg) | 132±4.83 | 143.5±15.02 | 162.8±6.7 | 167.3±4.79 |
| 2 (40 mg/kg) | 131.5±4.65 | 142.3±6.55 | 154.3±12.69 | 163.5±11.9 |
| 3 (80 mg/kg) | 140.8±4.32 | 166.8±6.1* | 181.8±5.76* | 180±7.84 |
| 4 (120 mg/kg) | 133±4.12 | 142.5±8.08 | 176.8±6.61* | 168.5±6.02 |

*Values are expressed as Mean±S.D., n=6, * p<0.05 (test groups compared with control group)*

**Table 2. Effect of statin (atorvastatin) on serum calcium and CRP of female albino wistar rats**

| Group | iCa (mmol/L) | tCa (mmol/L) |
|---|---|---|
| 1 | 0.84±0.03 | 1.70±0.07 |
| 2 | 0.92±0.04* | 1.78±0.07 |
| 3 | 0.94±0.03* | 1.83±0.06 |
| 4 | 0.89±0.04 | 1.75±0.08 |

*Values are expressed as Mean±S.D., n=6, * =p<0.05 (test groups compared with control group)*

## 4. DISCUSSION

"Statins" is a class of drugs that lowers the level of cholesterol in the blood by reducing the production of cholesterol in the liver. Statins inhibit the enzyme in the liver that plays a key role in the synthesis of cholesterol. This enzyme is called hydroxyl-methyl-glutaryl-coenzyme A reductase (HMG-CoA reductase). Scientifically, statins are referred to as HMG-CoA reductase inhibitors. Cholesterol is critical to the normal function of every cell in the body. However, it also contributes to the development of atherosclerosis, a condition in which cholesterol containing plaques deposits form within arteries. These plaques block the arteries and reduce blood flow to tissues supplied by the arteries. When plaques rupture, a blood clot forms on the plaque thereby further blocking the artery and reducing the flow of blood. When blood flow is reduced sufficiently in the arteries that supply blood to the heart, the result is angina (chest pain) or a heart attack (myocardial infarction) [29]. If reduced flow is caused by plaques in arteries of the brain, the result is stroke [29]. Increased levels of cholesterol are associated with coronary heart disease (CHD), hyperprothrombinemia, diabetes, cirrhosis and various liver diseases [31,32].

### 4.1 Effect on Fasting Blood Sugar

The effect of statin on blood sugar level is shown in Fig. 1. There was inconsistent rise/fall in fasting blood sugar. This result shows that the short term exposure of the female albino wistar rats to statins did not produce any hyperglycaemic/diabetic effect despite the dosage. This is consistent with the previous analogy that statins produce diabetogenic side effects mainly on chronic exposure and mostly affect individuals with already existing high risk of developing diabetes [18,9]. Dormuth and colleagues [33] studied over 136 936 patients hospitalised for a major cardiovascular event or procedure, a group in which statin treatment is strongly indicated for secondary prevention, and observed a moderately increased risk of new onset diabetes in patients prescribed higher potency statins compared with lower potency statins. Sato et al. (2012) also reported that statin intake is associated with decreased insulin sensitivity during cardiac surgery in non diabetic, dyslipidemic patients. In their study, hypercholesterolemia patients that were receiving statins experienced large oscillation in blood glucose compared to the groups that were not receiving statin. Yan et al. [34] reported that for non-diabetes mellitus patients, chronic statin use play a key role in the risk of developing stress-induced hyperglycemia. The finding of Rautio et al. [35] showed that the incidence of type 2 diabetes did not differ between statin users and non statin users. But fasting glucose increased in statin users compared to non users suggesting that the use of statins might have unfavorable effects on glucose metabolism and that statins might hamper beneficial effects of life style intervention in people at high risk of type 2 diabetes [35]. Mansi and colleagues [36] in their study, reported that statin use was associated with a significantly higher risk of new-onset diabetes, even in a very healthy population, they also reported that statin use was also associated with a very high risk of diabetes complications. An increase in fasting glucose in statin users suggests deterioration in insulin secretion capacity in statin users and also suggests that statins may also interfere with insulin sensitivity [35]. However, the relationship of statin therapy to incident type 2 diabetes is controversial (9).

### 4.2 Effect on Haematological Indices

Haematological parameters are important in diagnosing toxicity in animals exposed to toxicants [37]. The blood indices of the treated animals decreased compared to the control but were still within normal range. RBCs play a vital role in transporting oxygen from the lungs to the rest of the body. These oval-shaped cells contain haemoglobin, the protein that binds oxygen while it is being carried to all the stationary cells in the body (cells in the skin, muscle, bone and organs). Decrease in RBCs either indicates excessive damage to erythrocytes or inhibition of the formation of erythrocytes [29]. Since haemoglobin is contained in RBCs, a low number of RBCs usually leads to a low number of haemoglobin. The reduction in Haemoglobin could have been due to interference with the

**Table 3. Effect of statin (atorvastatin) on haematological parameters of female albino wistar rats**

| GRP | WBC X10$^3$/µL | RBCX 10$^6$/µL | HB g/Dl | Platelets X10$^3$/µL |
|---|---|---|---|---|
| 1 | 19.23±3.03 | 7.23±0.11 | 13.5±0.95 | 953±27.15 |
| 2 | 20.53±4.83 | 6.97±1.05 | 11.8±0.76 | 875±68.53 |
| 3 | 16.17±3.12* | 7.23±0.41 | 12.1±0.32 | 914±14.57 |
| 4 | 17.90±0.60 | 7.22±0.20 | 11.7±1.16 | 890±35.37 |

*Values are expressed as Mean ± S.D., n=6, * =p<0.05 (test groups compared with control group*

enzymatic machinery necessary for haem synthesis through binding to proteins by statins. They provide important clinical data relevant in monitoring of infections, malignancies and immune derangements, hence, they have come to occupy critical roles in the diagnosis, prognosis and management of various diseases and disorders [38].

Haemoglobin is an iron-containing compound found in the red blood cells. Measuring the concentration of haemoglobin in the blood can diagnose anaemia, which is caused by a deficiency of haemoglobin. Red cell count is an estimation of the number of red blood cells (RBCs) per liter of blood. Abnormally low numbers of red blood cells may indicate anaemia as a result of blood loss, bone marrow failure, malnutrition, iron deficiency, over-hydration, or mechanical damaged to red blood cells [38]. White blood cells are blood components that protect the body from infectious agents. They also play an important role in the immune system of the body by identifying, destroying and removing pathogens and foreign materials from the body. The most fundamental clinical measure of inflammation is the white blood cell count. Even within the reference interval, higher total leukocyte counts precede and predict the incident risk of type 2 diabetes and coronary heart disease, primarily because of the granulocyte subpopulation, rather than the lymphocyte and monocyte subpopulations [32]. A high WBC is an indication of an infection or tissue damage and a low WBC is an indication of a disease state. The decrease in WBC in the test groups compared to the control showed that statin can reduce/control inflammation of walls of blood vessels which can cause plaque rupture, blood coagulation and blockage of arterial blood supply, the reason is because inflammation is mediated by certain types of WBC [29,39]. Furthermore, the decrease in platelet observed in all the treated groups compared to the control, though not significantly different from the control but is a pointer to the ability of statin to discourage or reduce intravascular blood coagulation which can lead to the formation of a thromboembolus that is capable of blocking an artery and causing cardiovascular disease [11]. The decrease in red blood cells and other blood indices that were measured showed that statin might cause increased fragility of the cells and destruction by the reticuloendothelial system which can predispose patients that are on statin to anaemia of some sort.

## 4.3 Effect on Serum Calcium

The effects of statin (atorvastatin) on serum ionised calcium and total calcium levels of rats in test and control groups are shown in Table 2. Electrolytes are charged molecules also called ions, distributed in tissues and body fluids and have the ability to conduct electricity. Their presence in the body is essential for normal function of our cells and organs. Some important electrolytes include $Na^+$, $K^+$, $Cl^-$ and $Ca^{2+}$. The concentrations of these ions in the blood plasma or serum remain fairly constant throughout the day in a healthy person. Changes in the concentration of one or more of these ions in plasma can occur during various acute and chronic disease states and can lead to serious consequences [40]. Tests that measure the concentration of electrolytes are useful in obtaining clues for the diagnosis of specific diseases. Changes in electrolyte occur in dietary deficiencies, excess loss of nutrients due to urination, vomiting, and diarrhea, or abnormal shifts in the location of an electrolyte within the body. Electrolyte disturbances can occur with malfunctioning of the kidney (renal failure), infections that produce severe and continual diarrhea or vomiting, drugs that cause loss of electrolytes in the urine (diuretics), poisoning or diseases involving hormones that regulate electrolyte concentrations [41]. High levels of calcium ions (hypercalcemia) occur at free calcium ion concentrations over 5.2 mg/dl or total serum calcium above 10.4 mg/dl. Hypercalcemia usually occurs when the body dissolves bone at an abnormally fast rate, increasing both serum calcium and serum phosphate. Sudden

hypercalcemia can cause vomiting and coma, while prolonged and moderate hypercalcemia results in the deposit of calcium phosphate crystals in the kidneys and eye. Hypocalcemia occurs when serum free calcium ions fall below 4.4 mg/dl, or when total serum calcium falls below 8.8 mg/dl. Hypocalcemia can result from hypoparathyroidism (low parathyroid hormone), from failure to produce 1, 25-dihydroxyvitamin D, from low levels of plasma magnesium, and from phosphate poisoning (the phosphate enters the bloodstream and forms a complex with the free serum calcium). Hypocalcemia can cause depression and muscle spasms [42]. Serum calcium has been discovered to be directly linked to type 2 diabetes mellitus [24,28]. The rise in serum calcium observed in this study therefore shows that the animals were predisposed to impending loss of insulin sensitivity/type 2 diabetes mellitus as a result of exposure to higher doses of atorvastatin. This therefore suggests that higher doses of atorvastatin and its prolonged usage could be associated with diabetogenic side effect in experimental animals (albino wistar rats).

### 4.4 Effect on Body Weight

Accumulation of triglycerides in the fat cells results in weight gain [4]. One will expect a progressive decline in weight following statin administration since it reduces the triglyceride level, but the result of this research reveals progressive rise in the body weight of the animals in both control and test groups up to the end of second week of statin administration. Triglycerides are gotten from fats eaten in the diet. They are formed in the intestinal mucosa by the esterification of glycerol and fatty acids. They can also be synthesized from carbohydrates. The triglycerides are transported in the blood stream bound to cholesterol and proteins in complexes called lipoprotein particles. The calories that are not used immediately by the body are converted to triglycerides and stored in fat cells (adipocytes) until required between meals [4]. Ingestion of excess quantities of fat or carbohydrate beyond the body's needs results in a buildup of fats in adipocytes and hence weight gain [4]. This result shows that short-term exposure to statin does not significantly alter/affect body weight. There are many risk factors for type 2 diabetes such as age, race, pregnancy, stress, certain medications, genetics or family history, high cholesterol and obesity. Most patients with type 2 diabetes are usually overweight or have obesity [43]. People who are overweight or have obesity have added pressure on their body's ability to use insulin to properly control blood sugar levels, and are therefore more likely to develop diabetes.

### 4.5 Effect on Serum CRP Level

Cardiovascular disease remains the leading cause of death in the United States, a fact that underscores the importance of primary prevention [15]. Inflammation appears to participate in the pathogenesis of both type 2 diabetes and cardiovascular disease [32]. The use of biomarkers to augment traditional cardiovascular risk prediction has attracted considerable attention in the past decade. This interest has been fuelled by the realization that traditional risk factors do not identify everyone who will eventually develop cardiovascular disease [15]. This has been accompanied by the emergence of potential screening tests such as high-sensitivity C-reactive protein (CRP) [15]. Circulating biomarkers may be informative either early or late in the disease process, with some biomarkers reflecting activity in biological pathways that precede disease and other biomarkers triggered by the presence of subclinical cardiovascular disease. The success of preventive measures depends in part on the accurate identification of individuals who are at risk for future cardiovascular events (risk prediction). Traditionally, risk prediction has relied on assessment of risk factors such as hypertension, diabetes mellitus, hyperlipidemia, and smoking [15]. High concentrations of circulating cytokines, particularly C-reactive protein (CRP) and interleukin-6 (IL-6), have been associated with the development of type 2 diabetes and cardiovascular disease. Indeed, a variety of circulating proinflammatory cytokines and acute-phase reactants are increased in obesity, metabolic syndrome, hypertension, non-alcoholic steatosis, polycystic ovarian syndrome, type 2 diabetes and cardiovascular disease [32,44], Inflammation is an indicator of an acquired cause of both insulin resistance and impaired insulin secretion [32]. In preclinical rodent studies done by Hotamisligil, Shargil and Speigelman (1993), blockade of tumor necrosis factor (TNF) was shown to improve insulin resistance, a result that raised hopes that TNF blockade would also work in humans. Atherosclerosis is a complex process that involves more than just cholesterol. In addition to lowering cholesterol levels, statins also reduce inflammation, which could be another mechanism by which statins beneficially affects

atherosclerosis. Statins are also known to reduce C-reactive protein (CRP) levels and a variety of experimental observations suggest a direct role for CRP in the pathogenesis of atherosclerosis. Specifically, CRP renders oxidized LDL more susceptible to uptake by macrophages, induces the expression of vascular-cell adhesion molecules, stimulates the production of tissue factor, and impairs the production of nitric oxide [45-47]. Ridker et al. [48] concluded that patients with a low CRP level, after statin treatment, had better clinical outcomes than those with higher levels, regardless of the resultant level of LDL-cholesterol. In this study, a decreas in the levels of C-reactive protein was observed in a dose-independent manner compared to placebo though the decrease is not statistically significant compared to the control. However, this confirmed that statin can prevent cardiovascular diseases and events by modulating inflammation in the blood vessels which contributes to the formation of atheroma. The mechanism underlying CRP lowering remains incompletely understood; however, statins effectively lower CRP concentrations by approximately 25% to 30% [49]. All statins reduce CRP concentrations, and the effect does not appear to be dose dependent [32]. CRP lowering is not correlated directly with lipid lowering. Lowering LDL and CRP concentrations has been shown to be of clinical benefit in settings of acute coronary syndrome [50]. Multiple basic-science laboratories are investigating the underlying mechanisms that initiate inflammation and link it to insulin resistance and vascular impairment. These new findings may provide new opportunities for treating patients with type 2 diabetes and/or cardiovascular disease and may provide prevention strategies as well [51].

## 5. CONCLUSION

The values obtained in the glucose monitoring test shows that within 21 days of administration, atorvastatin was not able to induce hyperglycemia, that a longer period of exposure might be needed to precipitate such a side effect. The values obtained in body weight shows the tendency of gaining weight while on statin therapy so long as the person is eating high calorie rich meal and doing little or no exercise and thus calls for combination of statin and dietary restrictions as well as moderate exercise to maintain the weight of the patient on stain therapy. Decreased values of haematological parameters shows that the drug has the likelihood of increasing their fragility and destruction by reticuloendothelial cells or inhibit their formation in the bone marrow. Furthermore, the decrease observed in WBC and serum CRP level in the test groups confirms that statin has the potency to reduce inflammation in the blood vessels. The observed decrease in platelets shows that statin has the likelihood of discouraging thrombogenesis. The serum icalcium increased significantly in the treated groups compared to the control which indicates diabetogenic potential of the drug.

## ETHICAL APPROVAL

The experimental procedures and protocols used in this study were approved by the Institutional Health, Research and Ethical Committee. All efforts were made to minimize animal suffering and reduce the number of animals used. All authors hereby declare that the principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. The animals were used in accordance with NIH guide for the care and use of laboratory animals.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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