

## The Effect of Statin Drug Abused on Body Weight and Liver Enzymes activity in Wistar Rats

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### Abstract

**Background:** Researchers proved the association between statins drugs and fatty liver. Recently in Sudan statin abused for losing weight. Herein we determined the effect of statin abused on liver enzymes and histological sections in Wistar rats. **Materials and Methods:** In experimental study, 18 male Wistar rats weighting (129.6±17.3g) were randomly divided into three groups, group one: received high dose statin (40mg/kg/day), group tow: received low dose (20 mg/kg/day) for twenty-one days and group three received placebo as control group. Body weight and weight gained were estimated at baseline, 7, 14 and 28 days. Serum Alaine Transaminase (ALT), Aspartate Transaminase (AST) and Alkaline Phosphatase (ALP) were measured using Mindray BC200 kinetic assay. Liver histological sections were examined. **Results:** The results showed that, statin significantly decreased body weight at day 7, while increased body weight at day 14 and 28. ALT and AST were significantly elevated after treatment. Histological section showed fatty liver following one-month statin abused. **Conclusion:** The data suggests that, abuse of statin drugs for losing weight had an adverse effect on liver functions.

**Keywords:** Statin, ALT, AST, Aminotransferases, Losing weight, Sudan

### Introduction

Statins are inhibitors of hydroxyl methyl glutaryl coenzyme A reductase (HMG-CoA R), the enzyme that catalyzes the rate-limiting step of the cholesterol biosynthetic pathway. Lovastatin was the first statin introduced, since then, simvastatin, pravastatin, fluvastatin, atorvastatin, cerivastatin, rosuvastatin, and pitavastatin (Livalo, Kowa) have been used clinically (Tobert, 2003). Researchers investigated that statin therapy associated with mild transient elevations of serum aminotransferases levels (Muthiah *et al.*, 2016). Moreover, atorvastatin is associated with hepatocellular injury, cholestatic injury, autoimmune-type reaction and fulminant liver failure (Bhardwaj and chalasani, 2007). Meanwhile, clinical trials have reported a 0.5 - 3.0% occurrence of elevations in aminotransferases among patients receiving rosuvastatins and few episodes of severe liver injury (lolwa Barakhat *et al.*, 2013). On the other hand, the frequency of liver-related adverse effects was low (1.1%) and did not differ from rates reported in patients (Athiros *et al.* (2010), Lochhead and Chan., 2013, Manish Thapar *et al.*, 2013). Other studies found that, statin associated with an improved response to interferon treatment for

chronic hepatitis C and a reduction in portal pressure in patients with portal hypertension and metabolic syndrome (Lochhead and Chan., 2013; Lai *et al.*, 2012). Hopefully, the Liver Panel could find “no direct evidence of death due to liver failure caused by statin therapy.” This does not mean that statins will not elevate the liver enzymes because this is a known side effect (Chiu *et al.*, 2011; Harrison *et al.*, 2010; Rao and pandya, 2011). Therefore, the present study carried out to investigate whether the abuse of statin by Sudaese women for losing weight potentially effects on body weight, liver enzymes activity and histological section.

### Materials and Methods

#### Drugs preparation

This study was conducted in the Neelain University at Khartoum state from April to May 2018.

The drug purchased from local pharmacy. Tablet was crashed (20mg) into powder then mixed with 1 ml of distilled water and used as stock drug. Finally, the stock suspension was

homogenized by using Sonicator and diluted to appropriatedose concentrations.

**Experimental animals**

Eighteen 2-month old female Wistar rats with average weight of(129.6 ±13.8 g)for group one, which treated with low dose (20mg/kg/day), (129.6 ±13.3 g) for group two treated with high dose (40mg/kg/day) and (130.4 ±24.9g) for control group which received placebo. The rats were clinically healthy and housed under standard husbandry condition (30 ± 2°C, 60 to 70% relative humidity 12 h: 12 h day night cycle) and fed on rats’ diet. Animal experiments were designed in accordance of institutional animal ethical committee. Average body weight and weight gained were measured at day 7<sup>th</sup>, 14<sup>th</sup> and 28<sup>th</sup>. After 30 days of statin administration blood samples were collected after rats scarified under mild chloroform anesthesia, then serum was obtained by centrifuged at 4000 rpm and stored at - 20°C till used, the rats were dissected and the livers were collected and preserved in formalin, finally livers were embedded in paraffin.

**Estimation of transaminases activity**

ALT and AST were estimated by using spectrophotometric fully automated Mindray BS-200 Systems, which automatically calculated the activity of enzymes.

**Estimation of alkaline phosphatase activity**

Alkaline phosphatase (ALP) were estimated by using spectrophotometric fully automated Mindray BS-200 Systems, which automatically calculated the activity of enzymes.

**Histological methods**

The livers tissue was fixed in 10% formaldehyde for sample preservative, then the tissue was dehydrated by passing it through increasing concentrations of ethyl alcohol (from 0 to

100%), after replacement occurs, the alcohol was replaced with xylene, which is miscible with alcohol. Then the tissue was embedded in paraffin-wax which becomes harden, after which sections of 5 µm diameter were obtained by using rotary microtome. The sections were rehydrated by passing through xylene. The strengths alcohol was decreased (100 to 0%). The section was stained with Hematoxylin and Eosin (H and E) and then dehydrated again using xylene ,finally mounted on the microscope slide then cover slip was placed on top to protect the sample and examined under microscope X40(Fischer et al., 2008).

**Statistical methods**

Analysis of variance (ANOVA) with *post hoc* analysis and student *t*-test were employed to test whether the effect of statin in the body weight and mean activity of AST, ALT and ALP were significantly difference in study groups. Also, the data obtains were analyzed with multiple comparison test (LSD) to compare between groups. All results are presented as Mean± SD, with the level of significance set at *P*-value < 0.05.

**Results**

**Effect of statin on body weight**

In order to investigate the effect of various dose of statin on body weight, the body weights were measured at day 0, 7, 14and 28, and weights gained were calculated. The results showed that, administration of statin for 30 days was significantly decreased body weight in treated groups (low and high doses) (142 ± 22.2 and 143 ± 22.0 g) than control group (159 ± 34.0 g) respectively, with *p*-value <0.01, presented in table 1, whereas body weight gained found in figure 1.

**Table 1. The effects of statin on body weight**

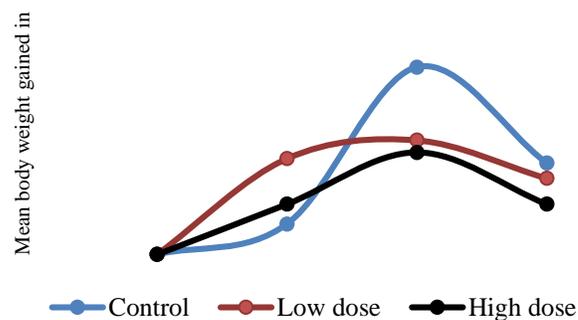
Groups	body weight			
	At Day 0	At Day 7	At Day 14	At Day 28
<b>1:High dose</b> (40mg/kg)	129.6 ±13.0	123 ± 17.3 (-6.60)*	136±20.9(13.4)**	143±22.0 (6.80)**
<b>2:Low dose</b> (20mg/kg)	129.6 ±13.8	117 ±16.9 (-12.6)**	132 ±20.3(15.0)**	142 ±22.2 (10.0)**
<b>3:Control (D.W)</b>	130.4 ±24.9	126 ±26.6 (-4.00)	147±36.0 (24.6)	159 ±34.0 (12.0)

Results are expressed as Mean±SD, values between brackets show gained weight/g, (\*) indicating *p*-valve ≤ 0.05 and (\*\*) indicating *P*-value ≤ 0.01

Comparison analysis shows that, following administration of high dose statin there were significant elevation in ALT and AST activity than control group, whereas unchanged was observed in ALP, presented in figure 2.

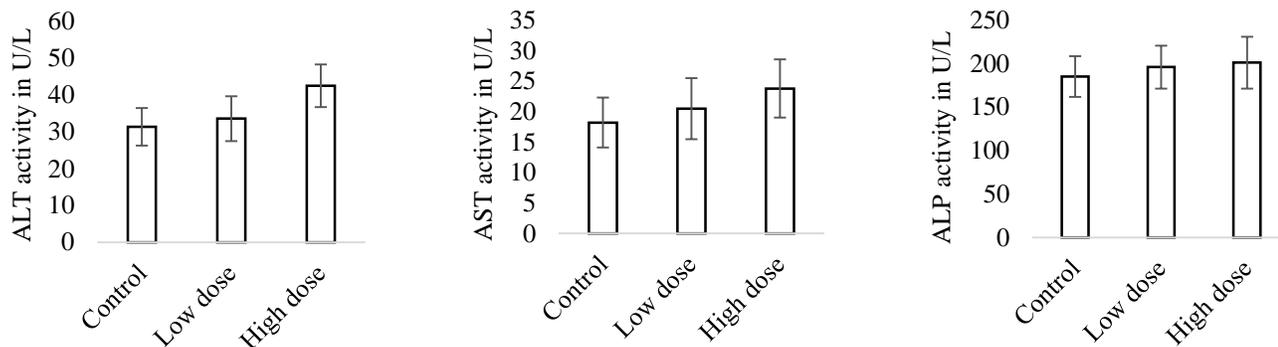
Histological section analysis shows that, high dose treated group had fatty liver than control group, presented in figure 3

**Comparison of the Body weight gained at day 0, 7, 14 and 28**



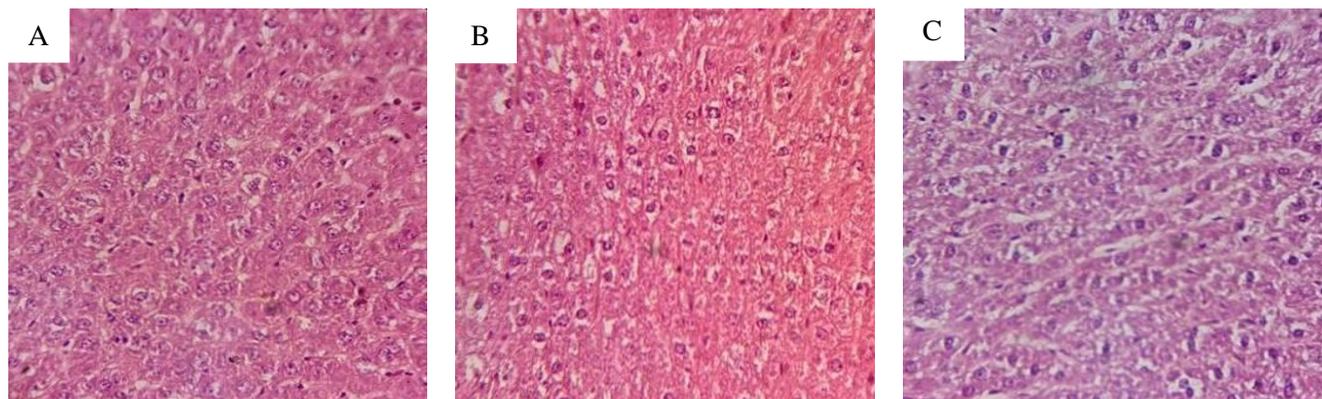
**Figure 1:** Plot of comparison the weight gaining/gram at day 0, 7, 14 and 28. The results shows a significant decreased in body weight gained of group treated with low and high statin doses than control at day 14 and 28.

**Comparison of mean liver enzymes of group treated using low and high doses with control group**



**Figure 2:** Comparison analysis of ALT, AST and ALP activity. Results expressed as Mean ±SD, significant differences considered as *p*-value ≤0.05.

### Histological section results



**Figure 3:** Liver histological sections of A: High dose, B: low dose and C: control groups. These results show unremarkable changes when compare groups received high and low doses with control group. The sections were examined under microscope X40.

### Discussion

In spite of abusing statins drug for losing weight in Sudan, the side effects of perversions remain unknown. Unfortunately, no study carried out to investigate the effect of statin abused on liver enzymes and functions. Therefore, this study hypothesis that, abuse of statin for losing weight by normal subject has an adverse effect on liver functions.

The present study demonstrated that, 7 days following the administration of low and high doses atorvastatin were significantly increased rats body weights. Whereas at day 14 and 28 body weight was significantly decreased. Controversial findings were previously report that, the weight gained following the statin used is not well explained, few studies shown that, the administration of statins decreased body weight, whereas others reported increased in body weight and liver fat accumulation (Abralde *et al.*,2009; Russo *et al.*, 2009; Ramage-morin, 2009). Therefore, we suggesting that, the contradictory findings have been occurred as the results in the duration and doses of treatment.

Concurrent with previous studies, ALT and AST activity were significantly higher, whereas ALP was unchanged after treatment with statin for month. In fact, atorvastatin therapy is associated with mild transient elevations of serum aminotransferases activity (Muthiah *et al.*, 2016; Manish Thapar

*et al.*, 2013; Lochhead and Chan, 2013). Therefore, suggesting hepatocellular damage, after statin abused by normal subjects for losing weight. These findings further were reinforced by histological sections analyses that, statin drugs abused was significantly increased fatty liver in group treated with high dose. Thus, confirming previous findings that, statin is associated with many adverse effects such as hepatocellular injury, fatty liver, cholestatic injury, consequently liver failure (Muthiah *et al.*, 2016). In addition to that past reports also found that, there were many adverse events after using statins including ALT and AST elevations, and sever liver necrosis (Lochhead and Chan.,2013 ; Lai *et al.*,2012; Ramage-morin, 2009;Newman *et al.*,2006). There for, further study is needed to investigate the mechanisms of statins in relation to body weight and adverse effect of abused.

### Conclusion

The data of present study concludes that, administration of statin drugs for one month increases ALT, AST and fatty liver. Moreover, 7 days after treatment increase body weight, whereas 14 and 28 days decrease body weight. Therefore, abuse of statin drugs for losing weight had an adverse effect on liver functions and body weight. Farther investigation must be done with more study groups and long duration time to study the effect of the statin clearly, the drug must be study at the human subject level to see wether have the same effect at human or not, in this study

we use the atorvastatin drug I recommended other studies to do on the other statin types for more reveal of the drug's effects.

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