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Effect of Silver Nanoparticles and Rosuvastatin on Endothelin and Obestatin in Rats Induced By High Fat- Diet.

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ABSTRACT

Hyperlipidemia is one of the major risk factors that precipitate coronary heart disease and atherosclerosis. In the present study, the effects of silver nanoparticles and rosuvastatin on serum endothelin and Obestatin in high cholesterol diet-induced hyperlipidemia in rats have been evaluated. This study was carried out on 55 male rats. They were randomly divided in to 11 groups. The first group served as a control group (negative group) fed on standard diet. The second group fed on high cholesterol diet referred to positive group. The other groups that fed on high cholesterol diet and treated with silver nanoparticles subdivided in to four groups according to concentration (5, 10) mg/kg and periods of treatment (8, 12) weeks. The remaining four groups fed on atherogenic diet and treated with rosuvastatin .This group also subdivided according to concentration (5, 10) mg/kg and periods of treatment (8, 12) weeks. The obtained results showed that, endothelin concentration was statistically significant decrease ($p < 0.05$) in groups treated with silver nanoparticles and rosuvastatin in compared with atherogenic group. While there was no important or statistically significant difference ($p < 0.05$) in Obestatin between a group of atherogenic and groups which treated with silver nanoparticles and rosuvastatin drug. The results have been shown significant positive correlation between Endothelin and TC, TG, LDL and VLDL and significant negative correlation with HDL. Also, the results have been shown significant positive correlation ($p<0.05$) between Obestatin and TC, TG, LDL, VLDL and significant negative correlation with HDL.

Keywords: silver nanoparticles, hyperlipidemia, diet.

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INTRODUCTION

Usually increase in the cholesterol levels and lipids in the blood define as hyperlipidemia, and is also referred to dyslipidemia that appear the different disorders of lipoprotein metabolism. The main characterization of dyslipidemia is elevated in cholesterol or triglycerides, or decline in high density lipoprotein cholesterol (HDL) level and also increase in (LDL) level, which is considered as an indicator of atherosclerosis [1].

Hyperlipidemias may be classified into two types: primary hyperlipidemia and secondary hyperlipidemia. Primary hyperlipidemia is commonly accrued due to genetic problems, but secondary hyperlipidemia will take place as a result of other underlining diseases such as diabetes. Hyperlipidemia furthermore divided according to the type of lipid elevated in to hypercholesterolemia, hypertriglyceridemia or both united called hyperlipidemia [2]. The high risk of cardiovascular disease is strongly related to LDL, and a lower risk is associated with HDL in adults. During childhood and adolescence the risk factors of cardiovascular disease are associated with severity of atherosclerosis in adults [3].

Rosuvastatin is an artificial statin that have a many number of binding interactions with HMG-CoA reductase [4]. Statins are (3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA reductase inhibitors) that are first drug of choice treating elevated LDL cholesterol levels [5]. Rosuvastatin possess the lengthiest terminal half-life among statins and it is relatively hydrophilic and selectively take-up and active in hepatocytes [6]. Oral administration of rosuvastatin had an action that longer on hepatocytes and cause a strong inhibitor on cholesterol synthesis in hepatic cell compared with other statins [7].

Nanotechnology is currently being applied in all manner of commercial industries to confer unique properties on products or improve those existing ones [8]. Nanoparticles (NPs) are widely used in biomedical application. They are used as deliver pharmaceutics, novel diagnostic and therapeutic approaches [9]. Silver NPs (SNPs) are one of the nanomaterials have unlike the shape (rods, spheres, triangles, wires,) and covering (peptide ,citrate, sugars, polymer). [10].The main Characterization of SNPs morphology is spherical and smooth surface. The nanoparticles diameter range from (< 50) nm. Trans electron microscopic image (TEM) expressions that the nanoparticles are more or less uniform in shape and size [11]. Aim of present study is show the effect of different concentration of silver nanoparticles and rosuvastatin on serum endothelin and obestatin in rats fed on high cholesterol diet.

METHODS

Experimental animals

Male albino rats are (55) in number. Their weight ranged between (145-255) g and aged between (12-17) weeks were obtained from higher institutes of fertility and university of Babylon and the study begin from (1/7/2015) to (1/1/2016).Animals were housed in the animal house University of Al Kufa /Faculty of Science under control condition (light for12-h and dark for 12-h cycle) at room temperature (21-24) °C and give a standard and atherogenic diet.

Silver nanoparticle and Rosuvastatin

The silver nanoparticles were obtained from (Nanjing Nano Technology co, ltd) with average size 50 nm and purity 99.9 was examined by scanning electron microscope to confirm primary particle size image (3-1). 2mg of $AgNO_3$ were diluted in 10 ml of deionized water a stock solution for preparation of 5 and 10 mg/ml concentration of silver nanoparticles.

Rosuvastatin drug with 5 and 10 mg/kg concentration was obtained from (RANBAXY).

Blood samples

The blood was drawn by heart puncture by using a disposable syringe (same in volume) and then left at room temperature for coagulating, after that the clotted blood centrifuged for 15 minutes at 3000 rpm,

then the serum was isolated at storied at deep freeze in Al- Sadder Teaching City in Al-Najaf Al-Ashraf province until using for measurement of biomarkers and lipid profile.

Biomarker measurement

Measurements serum Endothelin

The assessment of endothelin rats ELSA kit provided by (Elabscience–China) sandwich immunoassay technique (enzyme linked immunosorbent assay-automated microtiter plate), ELISA reader (Bio kit EL×800 reader, EL×50, washer/USA). Appendix 1

Measurements serum Obestatin in rat ELSA kit

The assessment of obestatin rats ELSA kit provided by (Elascience–China) sandwich immunoassay technique (enzyme linked immunosorbent assay-automated microtiter plate), ELISA reader (Bio kit EL×800 reader, EL×50, washer/USA).

Measurements serum cholesterol rat estimation kit

This was done by a method based on enzymatic colorimetric test, executed with rats specific kit for test, supplied by BIOLABO.

Measurements serum triglyceride rat estimation kit

This was done by a method based on enzymatic colorimetric test, executed with rats specific kit for test, supplied by BIOLABO.

Measurements serum HDL-cholesterol rat estimation kit

This was done by a method based on phosphotungstic precipitation test, executed with rat specific kit for test, supplied by BIOLABO.

Calculation of LDL-cholesterol

LDL-cholesterol, (mg/dl) was calculated according to the following formula:

LDL Chol=Total Chol. – (Triglycerides/5) HDL Chol.

Calculation VLDL-cholesterol

VLDL-cholesterol, (mg/dl) was calculated according to the following formula:

VLDL Chol = (Triglycerides / 5)

Statistical analysis

Statistical analyses were performed using social sciences (SPSS). Data were expressed as mean ± SEM. The least difference (LSD) test was performed to determine the significant variances. $P< 0.05$ was used as statistically significant and for showing the associations between two continuous variables of study parameter was used Pearson correlation coefficient, which $P< 0.01$ was used as statistically significant.

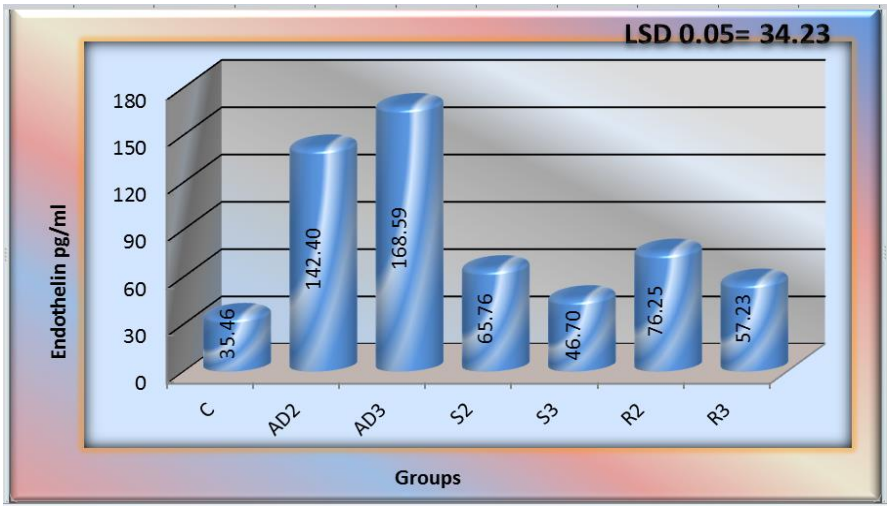
RESULTS

Effect on the Endothelin concentration in the male rat

The results in figure (1) showed that serum Endothelin was statistically significant decrease ($p < 0.05$)in control group in compared with group fed on atherogenic diet .There was statistically significant

decrease ($p < 0.05$) in Endothelin level in group treated with silver nanoparticles for tow period (8 and 12 weeks) and rosuvastatin for (8 and12 weeks) in compared with atherogenic group.There was no important or statistically significant difference in mean serum Endothelin between group treated with silver nanoparticles and group which treated with rosuvastatin drug for tow period respectively .While, group treated with silver nanoparticles for 12 weeks was highly significant decrease ($p < 0.05$) in mean serum Endothelin in compared with other study groups (AD, S2, R2, R3).

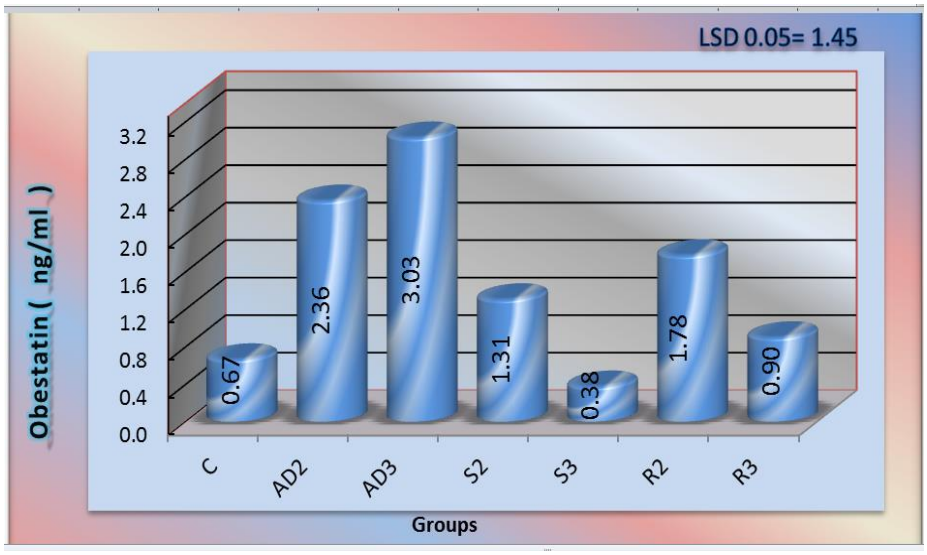
Figure 1: the difference in mean Serum Endothelin between study groups.



C: control, AD2: atherogenic diet for tow month, AD3: atherogenic diet for three month, S2: silver nanoparticles for tow month, S3: silver nanoparticles three month, R2: rosuvastatin drug for tow month, R3: rosuvastatin drug three month

Effect on the obestatin concentration in the male rat

Figure (2): the difference in mean Serum Obestatin between study groups.



C: control, AD2: atherogenic diet for tow month, AD3: atherogenic diet for three month, S2: silver nanoparticles for tow month, S3: silver nanoparticles three month, R2: rosuvastatin drug for tow month, R3: rosuvastatin drug three month

The results in (4-8) showed that serum Obestatin was significantly statistically significant decrease ($p <$

0.05) in control group in compared with group fed on atherogenic diet. There was statistically significant decline ($p < 0.05$)in Obestatin levels in group treated with silver nanoparticles and rosuvastatin for (12 weeks) in compared with atherogenic group. There was no important or statistically significant difference in mean serum Obestatin between group treated with silver nanoparticles and group which treated with rosuvastatin drug for tow period respectively .While, group treated with silver nanoparticles for 12 weeks was highly significant decrease ($p < 0.05$) in mean serum Obestatin in compared with other study groups (AD, S2, R2, R3).

The correlation between Endothelin and lipid profile levels

The result of correlation and liner regression between Endothelin and lipid profile are indicated:

- The presented of significant positive correlation ($p<0.05$) between Endothelin and cholesterol levels (mg/ml)of the male rat ($R^2= 0.802$), (figure:3)
- The presented of significant positive correlation ($p<0.05$) between Endothelin and triglyceride levels (mg/ml)of the male rat ($R^2= 0.894$), (figure:4)
- The presented of significant negative correlation ($p<0.05$) between Endothelin and HDL-C levels (mg/ml)of the male rat ($R^2= 0885.$), (figure:5)

The correlation between Obestatin and lipid profile levels

The result of correlation between Obestatin and lipid profile are indicated:

- The presented of significant positive correlation ($p<0.05$) between Obestatin and cholesterol levels (mg/ml)of the male rat ($R^2= 0.810$), (figure:6)
- The presented of significant positive correlation ($p<0.05$) between Obestatin and triglyceride levels (mg/ml)of the male rat ($R^2= 0.896$), (figure:7)
- The presented of significant negative correlation ($p<0.05$) between Obestatin and HDL-C levels (mg/ml)of the male rat ($R^2= 0. 888$), (figure:8)

Figure (3): correlation between Endothelin and TC

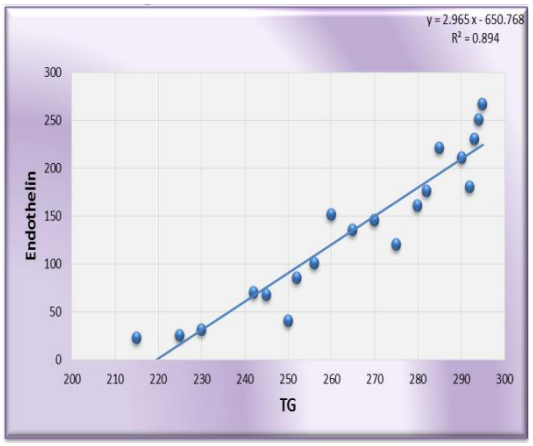


Figure (4): correlation between Endothelin and TG

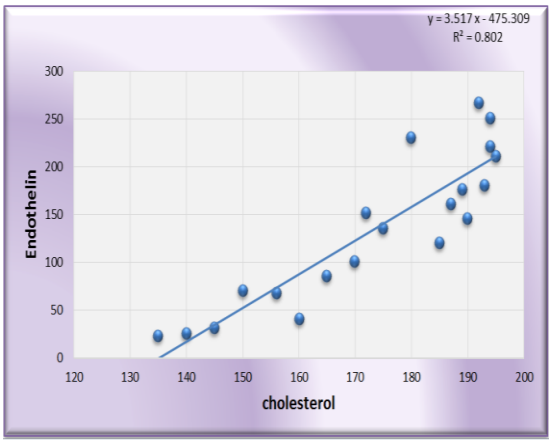


Figure (5): correlation between Endothelin and HDL-C

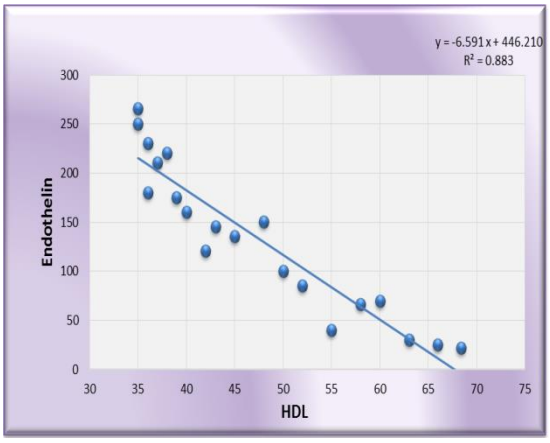


Figure (6): correlation between Obestatin and TC

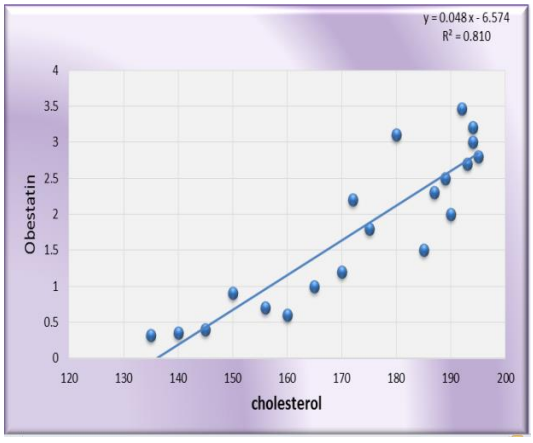


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Figure (7): correlation between Obestatin and TG

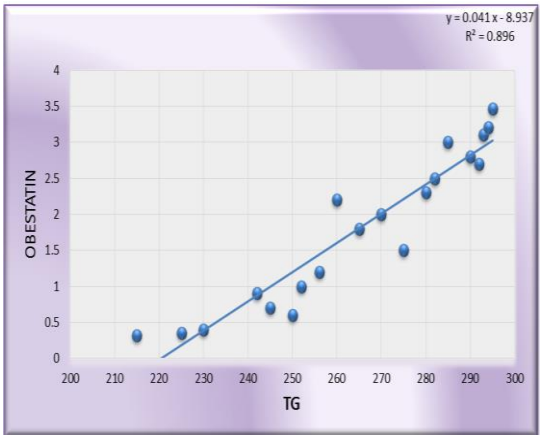
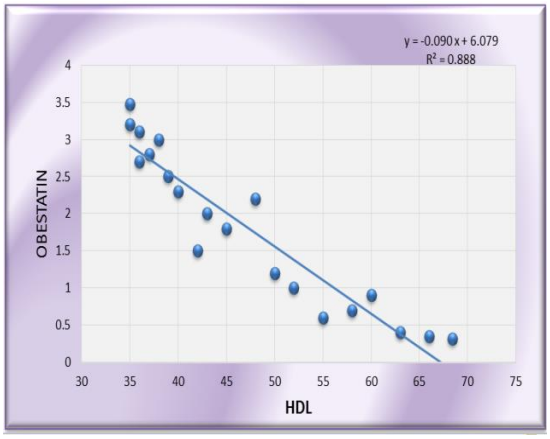


Figure (8): correlation between Obestatin and HDL-C



DISCUSSION

The current study revealed a significant elevated ($p < 0.05$) in the mean serum Endothelin concentration in the group fed on atherogenic diet in comparison with control group. These findings were in agreement with the study of [12].

Previous studies suggested that plasma ET-1 is also increased in hypercholesterolaemia before the progress of atherosclerosis [13, 14]. Hypercholesterolemia causes an increase in the formation of reactive oxygen species (ROS) and in companied with elevation LDL and decline in HDL, thus lead to reduce in the functions of endothelial and increase in the endothelin levels [15].

Many studies have revealed that oxidized low-density lipoprotein (oxLDL) and HDL numerous are contributed in the synthesis of prepro-ET-1 [16, 17]. ET-1 was functioning as a potent chemoattractant for monocytes, stimulates macrophage cells [18] and enhance the vascular smooth muscle hypertrophy [19].

The result of present study indicated a significant decline ($p < 0.05$) in Endothelin concentration in groups treated with rosuvastatin drug in comparison with an atherogenic group. These findings were in agreement with the study [20].

Many studies have demonstrated that statin drugs were displayed as a pleiotropic effect to reduce in the plasma LDL, like lowering the endothelins production and act beneficial action on endothelial function [21]. A previous study has shown that the statins have antioxidant action to reduce the ROS formation, so that, the statin used for improvement of endothelial dysfunction, dyslipidemia and cardiovascular disease therapy [22].

Another study showed that statin increase the expressing of endothelial nitric oxide synthase (eNOS) [23, 24]. NO prevents the secretion of ET-1 from the endothelium [25, 26].

The result of current study indicated a significant decrease ($p < 0.05$) in Endothelin concentration in groups treated with silver nanoparticles in compared with atherogenic group. The study of Sheikpranbabu *et al.* (2009) indicate that Ag-NP have a curative property in vascular permeability. Ag-NP had an important action on decreasing the permeability of endothelial. This is showing that silver obestatine can be used for treating of several disorders associated with high permeability [27]. Previous studies have shown that the larger particle (50 nm) easily enters into the endothelial cell with absence of toxicity [28, 29]. Kalimuthu *et al.* (2008) suggested that the nanoparticles with size (40-50 nm) prevent the proliferation and migration in BRECs [30] and to enhance apoptosis [31].

Previous studies suggested that ET-1 is affected by cholesterol, which is important for the development of atherosclerosis. [32, 33].

Bergdahl *et al.,* (2003) shown that the endothelin increased by cholesterol [32]. This indicated that the endothelin has positive correlated with cholesterol. Study of Yu *et al.,* 2015 suggested that endothelin negatively correlated with high density lipoprotein [34]. In contrast, Chai *et al.,* 2010 found that Plasma levels of ET-1 did not correlate with cholesterol features (TC, TGs, HDL, LDL) level in patients with coronary heart disease [35].

The results in present study showed that there was no important or statistically significant difference ($p < 0.05$) in Obestatin among all study groups (AD, S5, R5, and R10). These findings were in agreement with previous study Zou *et al.*, (2009) which have shown that obestatin levels were not different between obese and control groups [36]. It has been reported that the reason for the increase in obestatin level is a negative feedback effect of obestatin on appetite.

Zhang *et al.,* (2005) demonstrated that there was not significant difference in obestatin level in the fasted or fed state animals [37]. In addition Yildiz *et al.*, (2014) observed no statistically significant differences in obestatin levels before and after the diet in overweight or obese patients [38]. In contrast, the results of other studies showed that obestatin level was significantly higher in obese compared with controls [39, 40].

Other study contrary expectation, peripheral blood obestatin levels significantly decrease in obesity [41. 42, 43].

Study of Guo *et al.,* (2007) confirmed that lower level of pre-prandial Obestatin in obese patients may be linked to the troubled satiety perception in obesity and anorexigenic effect of obestatin [44]. Bdeer *et al.,* (2015) suggested that after the feeding of a high cholestrole diet, the combined rise in glucose and insulin that is responsible for the reduction of obestatin in blood [45].

As obestatin suppresses the contraction of jejunal and inhibits emptying activity of gastric. It is important to notice that the anorexigenic impact depends on the external sites of action. Furthermore, suppression in contraction of jejunal might create an afferent vagus signal to stimulate satiety in the CNS [46].

Obestatin has a potential role in the pathophysiology of obesity [47]. Catalán et al. (2007) estimated the actual existing of obestatin receptor in adipose tissue and whether the expression levels are modified in obesity [48]. Sever study demonstrated that Obestatin decrease of body weight [49, 50 51] and others suggesting no effect [52, 53]. Nakahara *et al.* (2008) have shown that obestatin level was significantly decreased and negatively correlated with BMI obese subjects [54].

Furnes *et al.,* (2008) reported that stomach contributed to a certain amount of circulating obestatin [55].In addition, previous studies suggest that obestatin is involved in lipid metabolism. The treatment with Obestatin led to decrease in cholesterol and triglyceride levels [56, 57, 58]reported that.

Due to the impacts of obestatin on energy balance, levels of this peptide may be affected by physical activity and causes changes in appetite and weight [59].The current result revealed a significant positive correlation between obestatin and TC, TG, LDL and a significant negative between obestatin and HDL, that result was agreement with another study [39, 47] have also reported that obestatin levels were positively correlated with TC and TG levels.

Study of Gutierre-Grobe *et al.* (2010) shown that lower levels of obestatin are associated with overweight [60], TG and TC levels and Yildiz et al., (2014) confirmed that the correlation between obestatin and HDL was a significant negative [38].In contrast, Abou Fard *et al.*, (2014) revealed that plasma obestatin was negatively correlated with TC and TG in both diabetic and obese rats [43] and study of Bdeer et al., (2015) showed non- significant correlation between the changes in obestatin and the changes in HDL [45].These findings denote that obestatin homeostasis is more obviously related to the disturbances in insulin and glucose metabolism rather than the changes in the lipid profile.

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