

Full Length Research Paper

The effect of vanadium on rat hypercholesterolemia in the presence and absence of statins

Nasr L. R.^{1*}, Saber M. E.^{1,2}, Hussam B.⁵, Salma A. R.³ and Muzaffar I.⁴

¹Clinical Laboratory Sciences, College of Applied Medical Sciences, Taibah University, Madinah, Kingdom of Saudi Arabia.

²Biochemistry Department, Faculty of Science, Alexandria University, Alexandria, Egypt.

³Department of Pathology, School of Medicine, University of North Carolina, Chapel Hill, NC, USA.

⁴Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh Kingdom of Saudi Arabia.

⁵Biochemistry and molecular medicine department, college of medicine, Taibah University, Madinah, Kingdom of Saudi Arabia.

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Vanadium may or may not be a cholesterol-lowering agent. It could potentially be used as an additional therapy or alone in therapies for treatment of dyslipidemia. This study aimed to investigate the effect of vanadium on hypercholesterolemia in the presence and absence of statins. Sixty rats were divided into five groups. The first group was kept on a normal diet and the second group was kept on a high fat diet. The three remaining groups of rats were prepared for the treatment; one group received simvastatin, one was given vanadium, and the third group was tested with both. Blood samples from all groups were investigated. Body functions were considered a tool in expressing efficacy and toxicity for the three types of treatment and were compared with the control groups. Vanadium alone causes marked increases in cholesterol levels. When added to statins, all lipid values were negatively affected as compared to the statins-only treated group. Rats with high fat diets showed significant ($P \leq 0.05$) elevation in the levels of serum triglycerides (TG), total cholesterol (TC), low density lipoprotein concentration (LDL-C) and very low density lipoprotein concentration (VLDL-C) as compared to the control group. All generated data proved that vanadium is impracticable for treating dyslipidemia. Vanadium is not safe or efficient in therapies for lowering cholesterol and other lipid levels.

Key words: Vanadium, statin, body functions, cholesterol, toxicity, rats.

INTRODUCTION

Dyslipidemia is one of the key risk factors for cardiovascular diseases and several studies have shown that there is a strong causal relationship between

dyslipidemia and cardiac diseases (Subramanian et al., 2014). Hypercholesterolemia is the presence of high levels of cholesterol in the blood of living organisms.

*Corresponding E-mail: arezk@taibahu.edu.sa or naser21@gmail.com.

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Longstanding elevation of cholesterol can lead to several health complications such as atherosclerosis. The most efficient antihypercholesterolemia therapeutic agents are known as statins. Statins or hydroxy-methyl-gluteryl-coenzyme-A-CoA reductase inhibitors (HMG-CoA reductase inhibitors), are the highest selling drugs worldwide (IMS Health, 2012; Lecarpentier et al., 2012; Bhatnagar et al., 2008). These drugs revolutionized the treatment of hypercholesterolemia. Yet, a large number of statins in the market continue to cause a plethora of side effects graded from moderate to severe (Mancini et al., 2011).

The Food and Drug Administration issued safety warnings in 2012 about statins. The most popular drug causes several side effects including cognitive problems, such as memory lapses, dementia, and confusion. In addition to its serious neurological side effects, statins can cause liver damage, muscle pain and heart muscle damage, and is found to increase blood glucose (type II diabetes) (Shechter and Shisheva, 1993). For these reasons, searching for a drug agent to replace statins is necessary. Combining other therapeutic agents to statins may be able to reduce these side effects.

Vanadium is an ultra-trace element believed to be important for normal cell function and development (Verma et al., 1998; Badmaev et al., 1999; Meyerovitch et al., 1991). Vanadium deficiency (less than 1 mg/day) is associated with reproduction impairment, changes in red blood cell formation and iron metabolism (Mingxia et al., 2014). Katheriene (2004) have investigated several metal compounds such as vanadium, zinc, cobalt, chromium and molybdenum, to test their potential of being used as therapeutic agent to treat diabetes mellitus. Vanadium produced the highest effect in lowering glucose and lipids in Wistar rats. The metabolic effects of vanadium are known to be dose dependent and require more than 4 weeks for a complete response (Pugazhenti et al., 1991; Battell et al., 1992; Cam et al., 1995; Liu et al., 2012). The main food sources of vanadium are rice, oats, beans, radishes, barley, buckwheat, lettuce, peas, potatoes, dill, parsley, black pepper, shellfish, meat, mushrooms, soy, wheat and olives (Yuen et al., 1995). Vanadium has toxic effect, but believed to have therapeutic uses as well (Yuen et al., 1995). Diabetes, cancer, chlorosis, anemia and tuberculosis are the most known diseases directly or indirectly affected by vanadium. The mechanism by which vanadium restricts elevation of plasma cholesterol appears to involve both inhibition of cholesterol synthesis and accelerated catabolism of cholesterol (Deborah et al., 2008). The treatment for insulin resistance includes the use of vanadium compounds, which have been shown to enhance insulin responsiveness in animal models (Yuen et al., 1995).

Sanchez and co-workers studied the bioavailability of vanadium and its hypoglycemic effect in magnesium-deficient rats (Sanchez et al., 2011). The group

generated data proving that vanadium plays an important role as a micronutrient and as an antidiabetic agent. In that study, vanadium was supplied in the form of bis(maltolato)oxovanadium (IV) in rats drinking water for a duration of five weeks. Recently, Soveid et al. (2013) studied long-term efficacy and safety of vanadium in the treatment of type one diabetes. This research team found that vanadium compounds can reduce blood glucose in experimentally-induced diabetic rats and type 2 diabetic patients. They also reported that cholesterol levels declined but the extent was not reported. Between therapy and toxicity, the research question is, "is vanadium a safe and efficient therapy in lowering cholesterol levels or is it solely a toxic agent"? The evaluation of vanadium as a toxic agent or treatment has not been investigated in the presence of communally used cholesterol lowering agents such as statins. It is believed that comparing vanadium and statins individually, and in combination, can guide in a clear description of the heavy metals value.

The purpose of this work was to examine the effect of vanadium on the level of cholesterol and other body functions in the presence and absence of statins. Effect of vanadium on healthy and high cholesterol rates was studied.

MATERIALS AND METHODS

Chemicals and diet

Vanadium (III) chloride (purity 97%) was purchased from Acros, Belgium whereas, cholesterol (purity $\geq 92.5\%$) powder was purchased from Sigma Aldrich, St. Louis, MO, USA. All other chemicals were of analytical grade unless specified. To prepare high-fat diet, cholesterol (1 %, w/w) powder was thoroughly mixed with crushed rat pellet diet. The pellets mixed with cholesterol were reconstituted with water and dried properly to avoid any fungal contamination.

Animal treatment

Thirty healthy male Wistar albino rats, weighing between 150 and 200 g, were obtained from the Experimental Animal Care Centre, College of Pharmacy, King Saud University, Riyadh, KSA. They were kept at constant temperature ($22 \pm 2^\circ\text{C}$), humidity (55%) and 12 h light/dark conditions during the experiment. Animals were randomly divided into five groups and each group comprised of 6 rats.

Group 1: rats fed with a normal pellet diet for 45 days in addition to normal saline for one week (control group); group 2: rats fed with a cholesterol mixed pellet diet for 45 days in addition to normal saline for one week (HFD group); group 3: rats fed with a cholesterol mixed pellet diet for 45 days plus simvastatin (30 mg/kg) orally by gavage for one week (HFD+ S group); group 4: rats fed with a cholesterol mixed pellet diet for 45 days plus vanadium chloride (15 mg/kg) orally by gavage for one week (HFD + V group); group 5: rats fed with a cholesterol mixed pellet diet for 45 days plus vanadium chloride (15 mg/kg) and simvastatin (30 mg/kg) orally by gavage for one week (HFD + SV group).

Table 1. Effect of vanadium chloride or simvastatin or both on lipid profile in serum of rats fed with high fat diet*.

Lipid	Control diet group	HFD group	HFD + S group	HFD + V group	HFA + SV group
Cholesterol (mg/dl)	105.51 ± 4.23	221.00 ± 10.61 ^a	135.82 ± 3.80 ^b	264.66 ± 5.27 ^b	159.83 ± 7.08 ^b
Triglycerides (mg/dl)	89.91 ± 3.9	214.66 ± 5.28 ^a	122.50 ± 2.33 ^b	249.16 ± 4.88 ^b	136.66 ± 3.21 ^b
LDL (mg/dl)	27.11 ± 3.67	154.08 ± 10.78 ^a	62.16 ± 5.11 ^b	194.66 ± 4.73 ^b	93.04 ± 6.40 ^b
VLDL (mg/dl)	17.98 ± 0.79	42.93 ± 1.05 ^a	24.50 ± 0.46 ^b	49.83 ± 0.97 ^b	27.33 ± 0.64 ^b

*The values are expressed as mean ± SE; ^amean values of HFD group are significantly different in comparison with the control ($P \leq 0.05$). ^bMean values are significantly different in comparison with HFD group ($P \leq 0.05$).

After one week of treatment, blood was collected from retro-orbital plexus under light ether anaesthesia in tubes. Serum was separated by centrifugation at 2500 \times g for 10 min and transferred to prelabelled Eppendorf tubes for various biochemical parameters. The experiment was carried out according to the guidelines of animal care and use committee of King Saud University, Riyadh, Saudi Arabia.

Lipid profile

Total cholesterol (Demacker et al., 1980), triglycerides (Lowell et al., 1973) and high-density lipoprotein (HDL) (Burstein et al., 1970) levels were estimated in serum using Roche diagnostic kits (Roche Diagnostics GmbH, Mannheim, Germany).

Liver function enzymes and bilirubin

Serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) (Reitman and Frankel, 1957), alkaline phosphatase (ALP) (Kind and King, 1954), gamma-glutamyl transferase (GGT) (Fiala et al., 1972) and bilirubin (Raedsch and Stiehl, 1983) were determined using a Reflotron Plus Analyzer as well as Roche kits (Roche Diagnostics GmbH, Mannheim, Germany).

Statistical analysis

Results are presented as mean ± SE (standard error). All analyses were carried out using Statistical Package (SPSS, Version 16.0). Data were analyzed using one-way ANOVA to assess differences between groups. Means were statistically compared using Dennett's multiple comparison tests at 0.05 significance level. Probability values $p < 0.05$ were considered to be statistically significant.

RESULTS

The effect of vanadium chloride alone and with simvastatin on serum TG, TC, LDL-C and VLDL-C of rats fed high fat diet

Rats with high fat diets showed significant ($P \leq 0.05$) elevation in levels of serum TG, TC, LDL-C and VLDL-C as compared to the control group. Adding simvastatin alone, or with vanadium chloride, to the high fat

diet groups 3 and 5, respectively, resulted in a significant ($P \leq 0.05$) decrease in the levels of TG, TC, LDL-C and VLDL-C as compared to the HFD-group. On the other hand, HFD-rats with vanadium chloride in group 4 showed further significant increase ($P \leq 0.05$) in the plasma levels of TG, TC, LDL-C and VLDL-C when compared with the HFD-group (Table 1).

The effect of vanadium chloride alone and with simvastatin on serum HDL-C of HFD-rats

Significant ($P \leq 0.05$) decreases in HDL-C levels were noticed in HFD-rats as compared to the control group. Further significant ($P \leq 0.05$) reduction in plasma levels in HDL-C was observed with the vanadium chloride treatment in group 4, as compared to the HFD-group. Oral ingestion of simvastatin alone, and with vanadium chloride in groups 3 and 5 caused significant ($P \leq 0.05$) elevation in plasma HDL-C concentration as compared to the HFD-group (Figure 1).

The effect of vanadium chloride alone and with simvastatin on integrity and secretory function of HFD-rats

Alanine aminotransferase (ALT) and γ -glutamyl transferase (GGT) activities, the specific biomarkers of hepatic damage, showed significant ($P \leq 0.05$) increase in HFD-group as compared to the control. The activities of aspartate aminotransferase (AST) and alkaline phosphatase (ALP) and the concentration of total bilirubin were also significantly ($P \leq 0.05$) elevated in HFD-group as compared to the control. The administration of vanadium chloride with the HFD-rats in group 4, exhibited further significant ($P \leq 0.05$) increase in all the above hepatic parameters as compared to the HFD-group, while simvastatin alone in group 3 or with vanadium chloride in group 5 exhibited significant improvement in hepatic function. This was indicated by the significant ($P \leq 0.05$) decrease in the activities of plasma ALT, AST, GGT and ALP and in the concentration of total bilirubin in both groups 3 and 5 as compared to the HFD-group (Table 2).

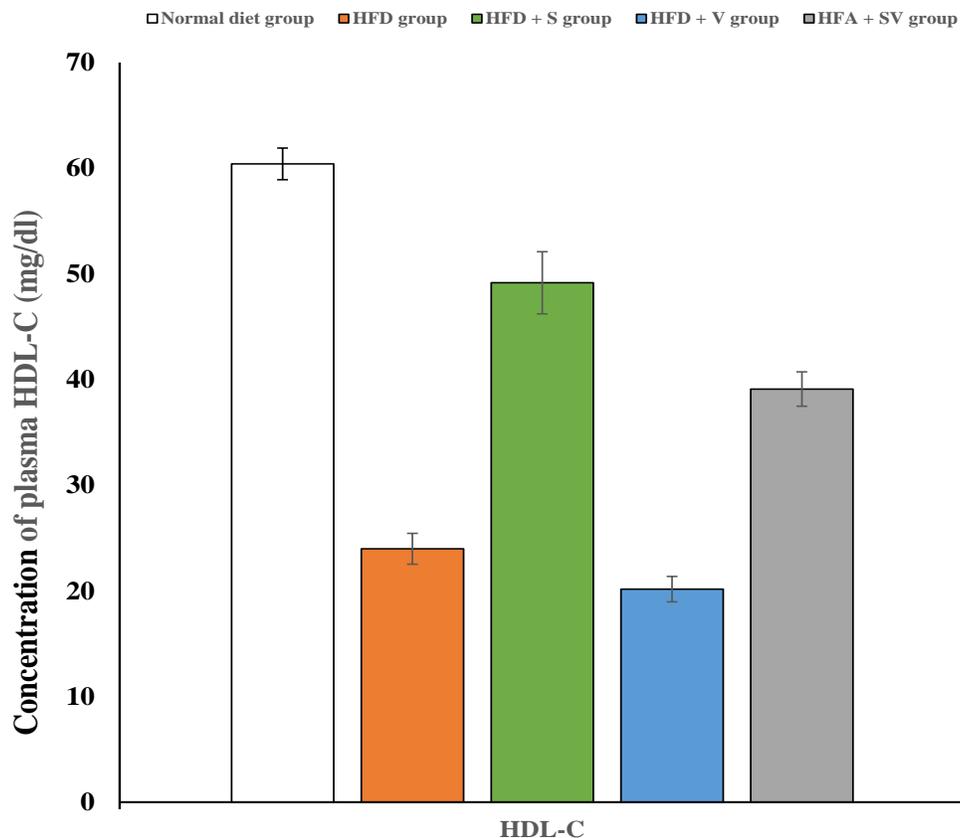


Figure 1. Changes in rat's serum HDL-C concentrations by high fat diet alone and in the presence of simvastatin (30 mg/kg BW) or vandium (15 mg/kg BW), or both.

Table 2. Effect of vanadium chloride or simvastatin or both on liver function in serum of rats fed with high fat diet*.

	Control diet group	HFD group	HFD + S group	HFD + V group	HFA + SV group
AST (U/L)	103.46 ± 4.81	188.50 ± 2.81 ^a	126.83 ± 5.19 ^b	261.66 ± 10.74 ^b	175.83 ± 4.06 ^b
ALT (U/L)	26.51 ± 1.54	113.98 ± 9.90 ^a	49.60 ± 2.94 ^b	251.50 ± 9.58 ^b	109.85 ± 7.71 ^b
GGT (U/L)	4.54 ± 0.18	11.00 ± 0.38 ^a	6.28 ± 0.17 ^b	17.16 ± 0.68 ^b	7.71 ± 0.25 ^b
Bilirubin (mg/dl)	0.57 ± 0.01	1.43 ± 0.04 ^a	0.79 ± 0.03 ^b	2.52 ± 0.09 ^b	1.00 ± 0.03 ^b
ALP (U/L)	366.83 ± 7.38	499.66 ± 9.96 ^a	423.66 ± 5.30 ^b	666.16 ± 14.63 ^b	481.33 ± 8.85 ^b

*The values are expressed as mean ± SE. ^aMean values of HFD group are significantly different in comparison with the control ($P \leq 0.05$); ^bmean values are significantly different in comparison with HFD group ($P \leq 0.05$).

The effect of vanadium chloride alone and with simvastatin on synthetic function of liver of HFD-rats

The HFD alone or with vanadium chloride in groups 2 and 4 respectively, affected the synthetic function of the liver as indicated by significant ($P \leq 0.05$) reduction in the serum levels of total protein and albumin, while a significant improvement in the synthetic function of liver was observed in groups 3 (simvastatin alone) and 5 (simvastatin combined with vanadium chloride). This was indicated by the significant ($P \leq 0.05$) increase in serum

total protein and serum albumin concentrations in those groups as compared to the HFD-group (Figures 2 and 3).

DISCUSSION

Vanadium therapy has been shown to normalize blood glucose levels in diabetic-induced rats and to cure many hyperglycemia-related disorders (Gail et al., 2011). Many other researchers focused on the toxicity of vanadium compounds depending on its oxidation state,

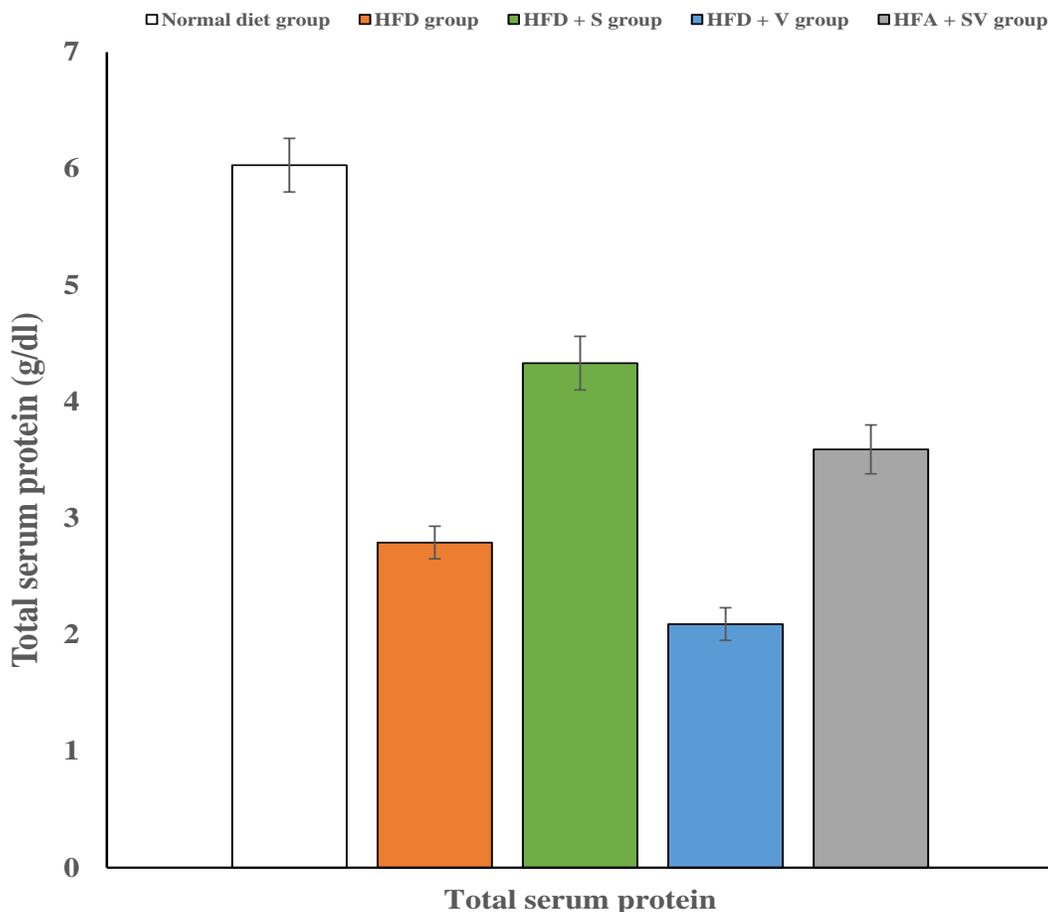


Figure 2. Changes in rat's serum total protein concentrations by high fat diet alone and in the presence of simvastatin (30 mg/kg BW) or vandium (15 mg/kg BW), or both.

administration or exposure route, doses and sensitivity of the organism. Here, the effects of oral administration of 15 mg/kg of vanadium chloride alone and in combination with 30 mg/kg of simvastatin on serum lipids and liver function of rats fed with a high cholesterol diet were investigated. The current study showed that feeding rats with a high cholesterol diet for 4 weeks, results in significant increase in serum concentration of total cholesterol, LDL-cholesterol, VLDL-cholesterol and triglyceride as compared to the control group. However, the HDL-cholesterol decreased in the case of the HFD treated group as compared to the control. These results are in agreement with those emphasized by Zhang and his co-worker (2013) who demonstrated the same pattern of changes in lipid profile in the HFD-induced model of hyperlipidemia. It has been reported that exposure to large quantities of fructose and fats leads to rapid stimulation of lipogenesis with accumulation of triglycerides and contributes to hepatic insulin resistance (Schaalan et al., 2008). High intake of dietary saturated fatty acids and cholesterol also increases the level of

plasma cholesterol, particularly that within the LDL fraction. Apo-B is required for assembly and secretion of LDL and VLDL, and increases the plasma's total cholesterol and triglyceride (TG) levels (Vallim and Salter, 2010).

The HFD-rats' oral ingestion with 30 mg/kg body weight of simvastatin significantly decreases serum total cholesterol, LDL-C, VLDL-C and triglycerides, while causing significant increase in HDL-C as compared to the HFD-group. The results are in agreement with Corsini et al. (1992) who reported that statins are used for lowering hypercholesterolemia through its inhibitory effect on the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase enzyme that catalyzes the rate-limiting step of the cholesterol synthesis in the liver and other tissue. It has been suggested that lipophilic statins, such as simvastatin, have absorptivity by tissue and are more effective in inhibition of extra-hepatic HMG-CoA reductase than other hydrophilic statins, such as pravastatin (McKenney, 2003). Also, it has been reported that the HDL-C level was higher in simvastatin-treated

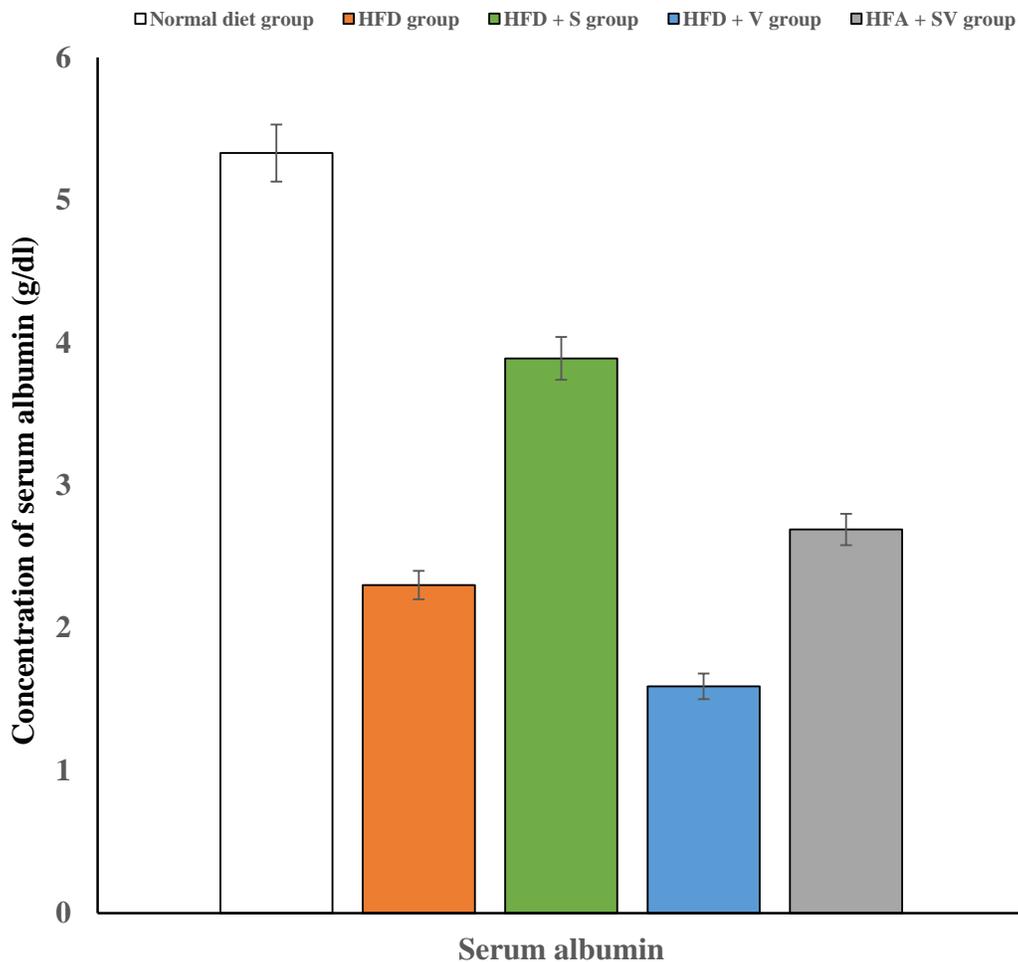


Figure 3. Changes in rats serum albumin concentrations by high fat diet alone and in the presence of simvastatin (30 mg/kg BW) or vandium (15 mg/kg BW), or both.

groups when compared with the control (Yong et al., 2014). The LDL-receptor uptake and clear LDL-C from circulation. This receptor is regulated at the transcriptional or posttranscriptional level (Kong et al., 2006). Simvastatin induced the upregulation of the LDL receptor gene expression. The study showed an increase in the rate of LDL receptor degradation with statin as well. This finding suggests that statins might increase the rate of LDL-C removal from the blood by increasing the rate of its receptor cycling (Ness et al., 1996). Other studies reported that plasma oxidized LDL was significantly reduced in statin-treated rats. The reduction in oxidized LDL is parallel to that of LDL-C (Elisavet et al., 2013).

Unfortunately, vanadium chloride (HFD+S group) did not improve dyslipidemia in rats fed with the high cholesterol diet. Diversely, vanadium chloride worsened the lipid profile as shown by the further significant increase in serum total cholesterol, LDL-C, VLDL-C and triglycerides. In addition, HDL-C unfavourably and significantly decreased as compared to the HFD-group.

This change in blood lipids found was most likely generated by disturbance in the hepatocytes lipid metabolism. It is well known that the *in vivo* effect of various metals, such as vanadium, may result from their interactions with protein-bound essential groups (Mahmoud et al., 2011). These interactions may lead to activation or inactivation of specific enzymes that affect lipid metabolism. It has been reported that vanadium ions may interfere with many metabolic processes at many levels (Garribba et al., 2015). Vanadium-protein interactions are starting points for understanding the effects of vanadium on the biological system (Pessoa, 2015). Phosphate is important because it is involved in a number of biological recognition bio-catalytic systems. Due to the closely and analogously physicochemical properties of vanadate and phosphate, the vanadium ion is able to systematically inhibit many enzymes, such as phosphatase, phosphodiesterases and phosphoglucomutases (Costa Pessoa, 2015). Such enzymes may be involved in acceleration of lipogenic

pathways in tissues that lead to lipid accumulation in blood. The co-administration of simvastatin with vanadium chloride significantly decreases the deleterious effects of vanadium and high cholesterol diet. This is explained by the decline of blood total cholesterol, LDL-C, VLDL-C and triglycerides, and the increase in HDL-C levels in the (HFD+SV) group as compared to the HFD-group.

The present study showed that feeding rats with a high cholesterol diet alone (HFD-group), or with oral ingestion of 15 mg/kg of body weight vanadium chloride (HFD+V group), causes structural and functional damage to the liver. High cholesterol diets significantly increased serum activity levels of ALT, AST, GGT and ALP as compared to the control group, which indicates damage in the structural integrity of hepatocytes. In addition, the synthetic and excretory functions of the liver deteriorated in HFD groups as indicated by the significant decrease in the serum total protein and albumin concentration as well as a significant increase in the level of total bilirubin, respectively, as compared with the control group. These results are in accordance with those obtained by Chen et al. (2010). It was reported that rodents fed high fat diets demonstrate visceral adiposity, hyperglycaemia, dyslipidaemia, hyperinsulinemia and hepatic steatosis (Beyegue et al., 2012). These symptoms may be considered a liver dysfunction risk. The oral ingestion of vanadium chloride with a high cholesterol diet in the HFD+V group does not protect the liver as expected. This is indicated by further significant increase in the serum activity of ALT, AST, GGT and ALP and further significant increase in the serum concentration of total bilirubin as compared to the HFD-group. Also, the synthetic dysfunction of liver becomes more significant as indicated by the decreased concentration of total serum protein and serum albumin as compared to the HFD-group. The results are consistent with the findings of Mahmoud et al. (2011). It has been reported that vanadium is a potentially toxic environmental pollutant that can interact and inhibit many enzymes and therefore may have negative effects on the liver (Mahmoud et al., 2011). According to the work done by Stohs and Bagchi (1995), vanadium induces lipid peroxidation and oxidative stress, which may be considered as a potent factor in hepatotoxicity.

On the other hand, the oral ingestion of 30 mg/kg of BW simvastatin alone or with vanadium chloride by rats fed high cholesterol diets in HFD+S- and HFD+SV groups, respectively, significantly amended hepatotoxic effects of both high cholesterol diet and/or vanadium chloride. This is indicated by significant reduction in all of the above assayed biochemical parameters except serum total proteins and serum bilirubin which significantly increased as compared to the HFD-group. It has been reported that simvastatin has anti-inflammatory properties that may reduce the risk of hepatotoxic effects (Vaughan

et al., 1996). Other studies showed that simvastatin suppresses the production of pro-inflammatory cytokines from hepatocytes and exerted immunomodulatory effects, (Buchwald et al., 1995) which may contribute to the reduction of vanadium and a high fat diet toxicity in liver.

Although, this research was carefully conducted, it still has limitations and shortcomings. First, the research was conducted using treatment for short period of seven days. Seven days is not enough to observe the clear picture of the changes made by vanadate. Second, using a single dose of vanadium does not present the exact level in which vanadium begins to cause negative effects. Since the assessment of the dose, as well as its response, was based on studying one dose level of vanadium, further research is recommended to test longer treatment time of up to, for at least, three weeks as well as test with at least three dosage levels of vanadate.

Conclusion

This study indicated that vanadium did not show reduction of cholesterol levels or other lipid profiles in the experimental animals. The examined vanadium (III) compound was found to cause an elevation of cholesterol, which provides evidence to avoid the use of vanadium as a dyslipidaemia therapy. Since the assessment of the dose and response was based on one dose level of vanadium, further long-term mechanistic study should be conducted to consider at least, three dosage levels in order to confirm these results.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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