Effect of vanadium (IV) chloride supplementation on appetite-related hormone levels in rats with experimentally induced diabetes

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Abstract

Vanadium is one of the essential trace elements for mammals, which has important functions in the metabolism of carbohydrates. There is limited data about the effects of vanadium (IV) chloride supplementation on appetite-related hormones in literature. Our

target was to analyse the impacts of vanadium supplementation on appetite-related hormone (leptin, nesfatin-1 and apelin) levels in rats with experimentally induced diabetes. Twenty eight male Sprague-Dawley albino rats were used in this study. After formation of diabetes, 0.3 mg/mL of vanadium was added to drinking water of rats for four weeks. A substantial increment was found in serum glucose ($P < 0.001$), HbA1c ($P < 0.001$), HOMA-IR ($P < 0.01$), and leptin ($P < 0.001$) levels and a significant decrease in insulin, apelin and nesfatin-1 concentrations in diabetic rats. Moreover, blood glucose, HbA1c, HOMA-IR, and leptin levels decreased in diabetic + vanadium group, but nesfatin-1 (1.74 ± 0.94 ng/mL), apelin (1.74 ± 0.94 ng/mL), and insulin concentrations were found higher compared with the diabetic group. As a result of this study, vanadium has increased the levels of circulating nesfatin-1 and apelin levels in diabetic rats while decreasing blood glucose and leptin concentrations. But further studies are requirred to determine the blood glucose lowering effects of vanadium and its relationship with appetite related hormones in diabet.

Keywords: Appetite-related hormones, rats, streptozotocin, vanadium

1. Introduction

Diabetes mellitus is an endocrine disease which is common all around the world and results from the ineffectiveness of peripheral and absolute or relative lack of endogenous insulin. It emerges due to genetics, environmental factors and lifestyle changes and has different types. Diabetes-related complications considered common and among the most significant health problems globally. Classical clinical symptoms observed in diabetic patients are polydipsia, polyphagia and polyuria. As time passes,

hyperglycemia, which is also seen in diabetics, causes damage, dysfunction and failure of many vital organs [1].

Leptin is a proteo hormone consisted of 167 amino acids (aa). Its basic impact regarded to energy exhaustion and control of food intake, involved as an anorexigenic factor that lows appetite. Additionally, leptin, adipocyte-derived hormone has been indicated to modulate the innate and adaptive immune response, both in normal and pathological conditions [2].

It has been reported that apelin hormone, an adipokin produced from adipose tissue, plays a critical role in food intake, formation of fluid-electrolyte balance and energy metabolism [3]. However, due to the effects on nutrition and energy metabolism, alterations in physiological mechanisms and diseases has not been enlightened completely for the reason that it is a newly discovered adipokin.

Also another appetite-related hormone, Nesfatin-1 is a saturity hormone that was identified in 2006. It is found in hypothalamus and formed of 82 aa. It is present in brain and further in peripheral tissues like adipose tissue, pancreatic islets, stomach, liver, and testis. Redundance of nesfatin-1 results loss of appetite and lowers body weight. Additionally to this anorexigenic impact, it promotes cardiac function, decreases blood glucose level, and causes fear and anxiety-like behavior. Thereby it is a multi functional peptide with anorectic impacts [4]. Vanadium (V) is an essential element for animals, and has very important roles in biological systems. A small amount of V is needed for the growth and development of mammals [5]. It can be found in many nutrients such as mushrooms, seafood, soy beans, some cereals, corn flakes, parsley, green beans, carrots, oats, cabbage, and sunflower [6]. Vanadium also affects carbohydrate metabolism in

many ways including glucose transportation, glycolysis, glucose output and glycogen synthesis [7]. Since it is a very common global disease, scientists are studying the effects of vanadium compounds on diabetes [8].

Although it has been shown that vanadium compounds have a blood glucose lowering effect [9], there is currently no study about the effects of vanadium (IV) chloride on appetite-related hormone concentrations. Our study is conducted to examine the effects of vanadium (IV) chloride supplementation on some of appetite-related hormones like leptin, apelin, and nesfatin-1 in rats with experimentally induced diabetes.

2. Material and Methods

2.1.Subjects

In the study, 28 male Sprague-Dawley rats between 12 - 14 weeks old and weighing between 380 - 440 g have been used. The rats were provided tap water and standard pellets of which they ate ad libitum. They were kept in rooms that receives 12 hours of light and 12 hours of darkness at a temperature of 22 ± 2 °C. The study was certified by the animal ethics comittee of Kırıkkale University (Number: 2012 / 03-03). Rats were split into 4 groups and each group had 7 animals.

1. Group I (Control group): This group was given standard feed and water for the duration of the 4 week trial.

2. Group II (Vanadium group): This group was given a standard feed and water containing 0.3 mg / mL of vanadium (IV) chloride (Sigma) for 4 weeks.

3. Group III (Diabetic group): After being melt in 0.1M sitrat buffer at 4.5 pH, streptozotocin (STZ) 60 mg/kg were administered i.p to each rat to induce diabetes. In this group two days after the injection, fasting blood glucose level were measured with a

glucometer (One Touch Lifescan, America)/ Rats with a 250 mg/dL or above blood glucose level were considered diabetics.

4. Group IV (Diabetic + Vanadium group): In this group, 60 mg/kg of STZ were administered i.p to each rat to induce diabetes. Fasting glucose levels were measured two days after STZ injection. Rats who have a blood glucose level of 250 mg/dL or above were regarded as diabetics. After the formation of diabetes, rats were given water containing with 0.3 mg/mL of vanadium (IV) chloride (Sigma) for 4 weeks.

For biochemical analysis, blood samples were taken from the heart of the rats. Then blood specimens were centrifuged in 2500 rpm for 10 minutes, and serums were obtained. Obtained serum samples were stored at - 80 ^{0}C until analyzed.

2.2.Biochemical analysis

In the study, the measurement of blood glucose, triglycerides, total cholesterol, and HDL levels were analysed in the Roche Modular System autoanalyzer with enzymatic colorimetric method, using Roche Diagnostic's reagent. Blood HbA1c levels were measured using the immunoturbidimetric method. Serum VLDL and LDL levels were calculated with Friedewald formule [10].

VLDL = Triglyceride / 5

LDL = Total Cholesterol - [(HDL) + (Triglyceride / 5)]

Triglyceride < 400 mg/mL

Leptin DRG (Rat) ELISA kit (EIA, 4607) was used for the evaluation of serum leptin levels. The lowest concentration of leptin that have been measured is 2 ng/mL, with intra- and inter-assay CV values: 8.1% and 9.6%, respectively. All samples were activated in

duplicate in the test. Serum apelin levels were measured with ELISA using commercial rat kit (YH Biosearch Laboratory, Yehau YHB0112Ra).

Insulin (Diagnostic Products, USA) and nesfatin-1 hormone (Phoenix Pharmaceuticals Inc. USA) concentrations were evaluated with an enzyme-linked immunosorbent assay (ELISA) using a commercial kit. The procedures for detecting the hormone concentrations were done as being suggested in the related catalogues, using a microplate reader (μQuant Elisa reader, Bio-Tek, USA). Insulin resistance was measured by a homeostasis model evaluation of insulin resistance (HOMA-IR) [11] as follows:

HOMA - IR = Fasting insulin level x Fasting blood glucose/ 22.5

2.3.Statistical analysis

Statistical analysis was executed utilizing Student's t-test; P values < 0.05 were regarded statistically important. Data for biochemical analyses are presented as mean ± SD. The correlation of the nesfatin-1 concentration with blood glucose, HbA1c, insulin, HOMA-IR, triglycride, total cholesterol, LDL, and HDL concentration was analyzed using Pearson's rank correlation coefficient.

3. Results

Weekly body weights of animals in study groups are given in Figure 1. After the 4- week trial period of the study, there was a progressive descent in body weights for the diabetic group (330 ± 14 g, $P < 0.01$) compared to control (445 ± 12 g), vanadium (452 ± 10 g), and diabetic + vanadium (360 ± 12 g) groups. Differences in body weights between groups became relevant after STZ administration. There was a progressive reduction in body weight for the diabetic + vanadium in comprison to the control group. There was a measurable prominent difference in body weights for the diabetic + vanadium group in regard of control group ($P < 0.01$)

Changes in serum triglyceride, total cholesterol, VLDL, LDL, and HDL levels were determined also for the demonstration of vanadium's effect on lipid metabolism in this study. Significant increases were found in the serum triglyceride ($P < 0.001$), total cholesterol ($P < 0.001$), and LDL ($P < 0.01$) levels of diabetic rats compared with the control and vanadium groups. On the other hand, serum HDL levels of diabetic rats were found notably ($P < 0.001$) lower than the control and vanadium groups. Moreover, serum triglyceride, total cholesterol and LDL levels of diabetic + vanadium group were found lower ($P < 0.001$), than the ones serum HDL levels of the diabetic + vanadium group were found higher in the diabetic group, but ($P < 0.01$) than the rats in the diabetic group. The supplementation of vanadium (IV) chloride suppressed increases in the triglyceride, total cholesterol, and LDL in the serum of the diabetic rats, but there was no alterations in VLDL levels between the groups in the study (Table).

For demonstration of vanadium's effect on glucose metabolism in the study, changes in blood glucose, HbA1c, insülin and HOMA-IR levels were determined. Significant increases were found in serum glucose ($P < 0.001$), HbA1c ($P < 0.001$), and HOMA-IR ($P < 0.01$) levels of diabetic rats compared to the control and vanadium groups. On the other hand, serum insulin concentrations of diabetic rats were found remarkably ($P < 0.001$) lower than the control and vanadium groups. Moreover, blood glucose (197.6 ± 12.8 mg/dL), HbA1c (2.3 ± 0.04 mg/dL), and HOMA-IR (11.4 ± 0.37 mg/dL) concentrations were found lower in diabetic + vanadium group, but nesfatin-1 (1.74 ± 0.94 ng/mL) and insulin (20.83 ± 0.79 μU/mL) concentrations were found higher when compared to diabetic rats.

Serum nesfatin-1 concentrations of the diabetic group were considerably lower ($P < 0.001$) than those of the control and vanadium groups while the nesfatin-1

concentrations of diabetic + vanadium group were significantly higher ($P < 0.01$) compared with diabetic group (Figure 2). There were not any important differences in nesfatin-1 concentrations among the control and vanadium groups.

Leptin levels were measured as an avarage of 5.56 ng/mL in control group; 5.64 ng/mL in vanadium group; 12.4 ng/mL in diabetic group and 9.1 ng/mL in diabetic + vanadium group (Figure 3). Considerable increases were found in serum leptin levels of the diabetic group in comparison with control group ($P < 0.001$). The levels of leptin in diabetic + vanadium group were superior compared to control and vanadium groups ($P < 0.01$). Leptin levels of the diabetic+vanadium group were expressively lower in comparison with the diabetic rats ($P < 0.01$).

Apelin levels were measured as an avarage of 198.54 pg/mLin control group; 201.08 pg/mL in vanadium group; 125.7 pg/mL in diabetic group and 159.25 pg/mL in diabetic + vanadium group (Figure 4). The results of apelin in diabetic group were considerably lower in compliance with control group ($P < 0.001$). The apelin levels of the diabetic + vanadium group were found higher ($P < 0.01$) compared with the diabetic rats. Besides, the levels of apelin in diabetic + vanadium group were considerably less in compliance with the control and vanadium groups ($P < 0.01$).

4. Discussion

Vanadium is an essential trace element for mammals and has important functions in the metabolism of carbohydrates. The body weight of rats in diabetic and diabetic + vanadium groups decreased in the 4 week study trial (Figure 1). The main reason for weight loss seen in the diabetic group may be related to the inadequate use of glucose in cells and with the increase in leptin levels and decrease in nesfatin-1 and apelin

concentrations as we determined in the study. Therefore, consumption of glycogen stores in the liver and muscles and disruption of glucose oxidation due to the insulin insufficiency triggers the use of non-carbohydrate sources. Thus, catabolism of proteins and fats increases [12].

In experimental diabetes, vanadium compounds were found more effective in lowering the level of glucose than free vanadium [13]. It was also shown that vanadyl sulphate reduces exogenous insulin requirements, glycosuria and food uptake depending on the dose used [9,14]. In similar studies, it has been shown that vanadium compounds have a lowering effect on blood glucose and HbA1c levels, and an increasing effect on insulin levels in experimental diabetes [9,15]. As in other studies, we observed similar reduced levels of serum glucose ($P < 0.001$), HbA1c ($P < 0.001$), HOMA-IR ($P < 0.001$), and elevated levels of insulin ($P < 0.001$) in diabetic rats after vanadium supplementation in our study (Table). This blood glucose lowering effect of vanadium may be related to vanadium's insulin stimulating effect on pancreatic β cells and increasing insulin activity on the insulin receptors of cells [16].

Hypertriglyceridemia and hypercholesterolemia are very common conditions in diabetic patients [17]. It has been determined that there are changes in the levels of triglyceride, total cholesterol, HDL, LDL and VLDL cholesterol in diabetes mellitus [17-19]. In diabetes, lipoprotein lipase and hepatic lipase enzyme activities are responsible for changes in plasma lipid profile such as hyperlipidemia and hypercholesterolemia [20]. Lipoprotein lipase and hepatic lipase activities increase under the influence of insulin. Lack of insulin or insulin insensitivity decreases the levels of these enzyme activities and chylomicron catabolism. As a result, serum triglyceride levels increase and HDL cholesterol levels decrease [21,22]. The lower serum triglyceride, total cholesterol, LDL

levels, and higher HDL levels were documented in diabetic + vanadium group in the study (Table, may be related to vanadium's insulin-like effect that increases the activities of lipoprotein lipase and hepatic lipase enzymes. Similar to the findings in our study, the application of vanadium sulfate, vanadium (III) dipicolinate and vanadium (IV) dipicolinate orally or intraperitoneally decreases the levels of serum triglyceride, free fatty acid and total cholesterol [19,23], but vanadium (V) dipicolinate did not change the lipid profile [5]. It has been shown in other studies that oral vanadium sulphate applications in diabetic rats decreased levels of blood glucose, total cholesterol, VLDL and LDL cholesterol, while increasing the levels of HDL cholesterol [9,24].

Nesfatin-1 is a proteo-hormone produced in the brain of mammals and is liable for the production of body fat and the control of appetite. Excess nesfatin-1 in the brain leads to a loss of appetite, less frequent hunger, and a drop in body fat and weight. [25]. Stengel et al. has indicated that excessive nesfatin-1 in the brain causes a loss of appetite, less often hunger, and a decrease in body fat and weight [4]. The act of nesfatin-1 in the pathogenesis of diabetes are not presently well understood. Recently, it has been demonstrated that nesfatin-1 also effects glucose metabolism by an immediate mechanism to enhance insulin secretion and insulin sensitivity in skeletal muscle, adipose tissue and liver [26]. Li et al. stated that serum nesfatin-1 values were significantly lower in diabetic patients [27]. However, Zhang et al. indicated a positive correlation between plasma nesfatin-1 concentrations and discomposed glucose tolerance [28]. In diabetes, symptoms such as hyperphagia and increase in appetite are observed. In this study, nesfatin-1 concentrations were considerably depressed in the diabetic rats compared with the control group ($P < 0.001$) and this may be related to diabetic hyperphagia and increase of appetite that is observed in diabetes. There was also a small rise in serum nesfatin-1

concentrations in the diabetic + vanadium group (Figure 2). This may be related with the stimulation of nesfatin's insulin-releasing effect from pancreatic beta cells by vanadium.

Leptin is an adipokinin comprised of 164 aa and discovered by Zhang et al. in 1994 [28]. Previous research findings reveal that leptin plays a crucial role in the hormonal regulation of the energy balance. Several contradictory results have been stated for leptin levels in humans with diabetes: increased [29,30], decreased [31], or not changed [11]. Tuominen et al. reported that hyperinsulinemia had increased serum leptin levels in humans with normal body weight [32]. Segal et al. and Kamoda et al. informed that insulin resistance distincted from adiposity had increased plasma leptin levels and also elevated blood glucose concentrations had stimulated leptin production [33,34]. From the point of their view, this increase may be related to the induction of ob gene expression. Serum leptin concentrations were notably higher ($P < 0.001$) in diabetic group compared with control group (Figure 3) in our study. Besides, serum leptin levels were reduced in the groups of vanadium ($P < 0.01$) and diabetic + vanadium ($P < 0.05$) in comparison to the diabetic rats. Increased leptin concentrations may be related with leptin's anorexigenic effect that reduces appetite and cause weight loss during disease.

The most important factor affecting leptin secretion is body weight. Especially acoording to the fat and body mass index there is direct proportion between the total mass of adipose tissue and serum leptin levels. Aktaş et al. reported that leptin levels increased in obese patients, in other words body fat index is controlled by leptin [35]. In our study while serum triglyceride and cholesterol levels were high in diabetic group also leptin levels were high compared with the diabetic + vanadium group. This may be related to the direct proportion between leptin and body fat index.

Adipose tissue has a function as being an energy store but also is an endocrin organ that produces several adipokines to blood circulation. The apelin hormone is one of them and has effects on energy metabolism, cardiovascular system, insulin sensivity, and vascular responses via its local and sistemic effects [3]. In obese animal and human trials it is reported that plasma apelin levels were high due to the increasing body fat [36]. Alipour et al. stated that deficiency of insulin oscillation induces increase of apelin concentration [37], although Erdem et al. (2008) and Zhang et al. determined that plasma apelin levels were considerably lower in diabetic group compared with the control [38,25]. In a contrast study of 4 obese mice; apelin levels were significantly higher in models with hyperinsulinemia. At the same study it was stated that dropped insulin levels may correlate with the reduction of apelin secretion from adipocytes in insuline addicted mice [36]. In another study it was reported that apelin-36 inhibited the insulin secretion that glucose stimulated in mice [39]. Comparably, in this study apelin levels were found considerably lower in diabetic group in comparison with control group ($P < 0.001$) and were higher in diabetic + vanadium group compared with diabetic rats (Figure 4), ($P < 0.05$). According to the obtained results, it is considered that low apelin levels may be related with decreasing of insulin secretion in diabetes being associated with the decrement of apelin gene expression.

These results indicated that vanadium increased the levels of circulating nesfatin-1 and apelin concentrations in diabetic rats while decreasing blood glucose concentration. There are restricted information in literature about the roles of appetite related hormones in the pathophysiology of diabetes and the effect of vanadium on these hormones. Therefore, there is need for further studies to determine the blood glucose lowering effects of vanadium and it's relationship with appetite-related hormones in diabetes. For the

treatment of diabetes different intra peritonal doses of vanadium may be designated and applied In the future studies and also different forms of vanadium may be utilized in future studies.

Acknowledgements

This study is supported by scientific foundation of Çankırı Karatekin University (ÇAKÜ- 2012/04).

Table. Changes in appetite related hormones and biochemical parameters levels in experimental groups

Parameters	**Group I** **n=7**	**Group II** **n=7**	**Group III** **n=7**	**Group IV** **n=7**
Glucose (mg/dL)	78.2 ± 8.73^{a}	81.9 ± 6.04^{a}	378.4 ± 23.9^{b}	197.6 ± 12.8^{c}
Glucose levels 2 days after STZ injection (mg/dL)	75.1 ± 6.75^{a}	83.2 ± 5.24^{a}	278.6 ± 25.9^{b}	281 ± 10.5^{c}
HbA1c (mg/dL)	1.6 ± 0.04^{a}	1.7 ± 0.02^{a}	3.1 ± 0.06^{b}	2.3 ± 0.04^{c}
Insulin (µU/mL)	26.45 ± 1.02^{a}	27.61 ± 0.86^{a}	12.03 ± 0.55^{b}	20.83 ± 0.79^{c}
HOMA-IR (mg/dL)	5.5 ± 0.78^{a}	5.8 ± 0.67^{a}	11.4 ± 1.17^{b}	8.7 ± 0.92^{c}

Leptin (ng/mL)	5.56 ± 0.58^{a}	5.64 ± 0.77^{a}	12.4 ± 1.85^{b}	9.1 ± 1.25^{c}
Nesfatin-1 (ng/mL)	2.05 ± 1.08^{a}	2.24 ± 0.86^{a}	1.41 ± 1.01^{b}	1.74 ± 0.94^{c}
Apelin (pg/mL)	198.54 ± 69.2^{a}	201.08 ± 75.5^{a}	125.7 ± 42.6^{b}	159.25 ± 60.5^{c}
Triglyceride (mg/dL)	95 ± 6.16^{a}	90.6 ± 8.52^{a}	167.1 ± 17.25^{b}	146.3 ± 9.78^{c}
Cholesterol (mg/dL)	65.6 ± 12^{a}	68.3 ± 5.4^{a}	102.0 ± 22.3^{b}	87.4 ± 15.1^{c}
VLDL (mg/dL)	8.1 ± 2.0^{a}	8.4 ± 1.5^{a}	18.7 ± 5.6^{b}	16.6 ± 4.3^{b}
LDL (mg/dL)	34.5 ± 1.7^{a}	38.1 ± 2.1^{a}	68.9 ± 6.3^{b}	59.7 ± 3.2^{c}
HDL (mg/dL)	41.2 ± 1.27^{a}	39.8 ± 1.35^{a}	26.2 ± 0.94^{b}	37.7 ± 1.12^{a}

1 $^{a, b, c,}$ differences are statistically significant among groups marked with different letters
2 on the same line ($P < 0.05$) (Group I = control, Group II = vanadium, Group III = diabetic,
3 Group IV = diabetic + vanadium)

4

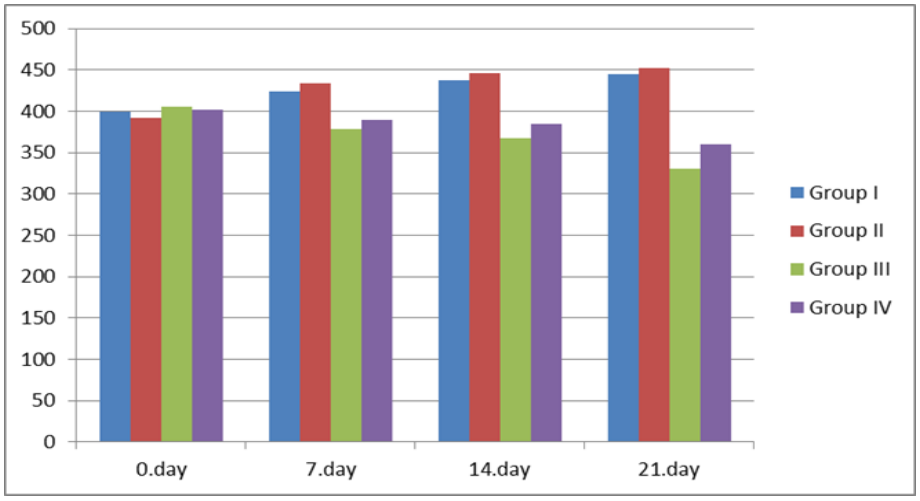

Figure 1. Weekly body weight changes of rats in experimental groups (Group I = control, Group II = vanadium, Group III = diabetic, Group IV = diabetic + vanadium)

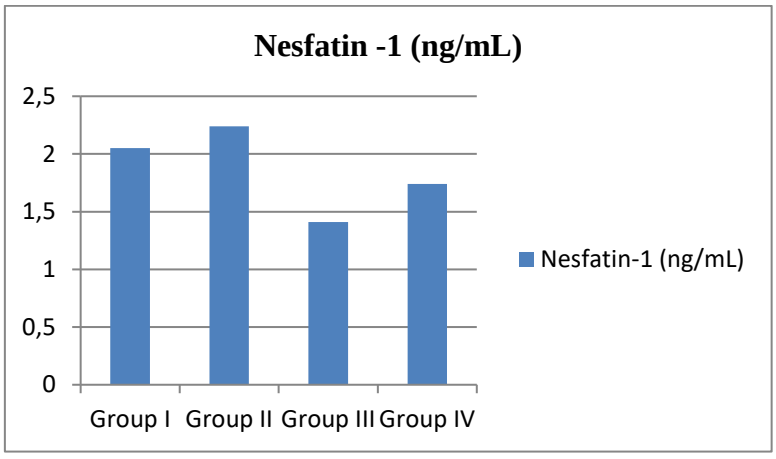

Figure 2. Changes in nesfatin-1 hormone levels in experimental groups (Group I = control, Group II = vanadium, Group III = diabetic, Group IV = diabetic + vanadium)

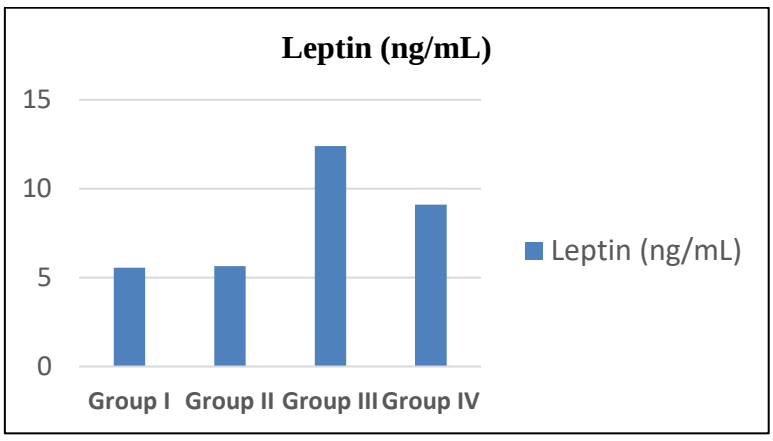

Figure 3. Changes in Leptin hormone levels in experimental groups (Group I = control, Group II = vanadium, Group III = diabetic, Group IV = diabetic + vanadium)

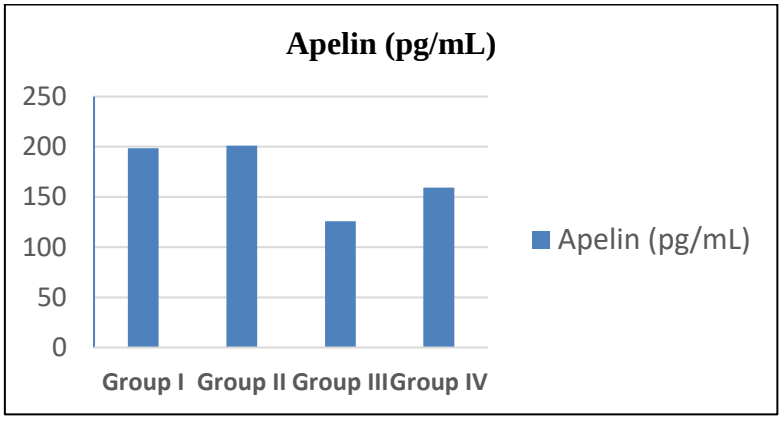

Figure 4. Changes in Apelin hormone levels in experimental groups (Group I = control, Group II = vanadium, Group III = diabetic, Group IV = diabetic + vanadium)

Conflict of Interest

The authors confirm that this article content has no conflict of interest

Supplemental Information

1. Rats were fed adlibitum where they were provided tap water and standard pellets. In standart pellet the ingredients were;

Wheat, barley, Lupins, Soya meal, Fish meal, Mixed vegetable oils, Canola oil, Salt, Calcium carbonate, Dicalcium phosphate, Magnesium oxide, and a Vitamin and trace mineral premix.

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