International Journal of Biotechnology and Microbiology
www.biotechnologyjournals.com
Online ISSN: 2664-7680, Print ISSN: 2664-7672
Received: 05-09-2023, Accepted: 20-09-2023, Published: 07-10-2023
Volume 5, Issue 4, 2023, Page No. 8-14

Effect of vitamin D3, vitamin K2, aqueous extract of garlic and spirulina algae powder on the levels of adiponectin and resistin in induced hyperlipidemic rats

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Abstract
The study aims to determine the effect of hyperlipidemia and evaluate the therapeutic role of vitamin D3, vitamin k2, garlic aqueous extract, spirulina and calcium. The experiment included 90 male white albino rats and was divided into two groups, the first group included 35 rats that were given a standard diet for two months, the second group included 55 rats that were fed on fodder with 4% cholesterol added for two months. After the end of the treatment period, a lipid profile was examined, and then the animals of the first group were divided into 7 groups and the second group into 11 groups. The groups were treated for a month with statin, vitamin D3, vitamin k2, garlic aqueous extract, spirulina and calcium. The result showed that the Introduce of hyperlipidemia caused a significant decrease in the level of the Adiponectin compared with the control group. results showed a significant increase in the level of the hormone in the hyperlipidemia group treated with statin, vitamin D3, spirulina and calcium. The result showed that the Introduce of hyperlipidemia caused a significant increase in the level of the resistin compared with the control group. results showed a significant decrease in the level of the resistin in the hyperlipidemia group treated with statin, vitamin D3, vitamin k2, garlic aqueous extract, spirulina and calcium.

Keywords: Vitamin D3, Vitamin K2, spirulina, adiponectin, resistin

Introduction

Adiponectin is a secretory protein that regulates a number of metabolic processes, including the regulation of Glucose and Lipid metabolism regulation and also acts as an anti-inflammatory and antioxidant. (Heidari *et al* 2015,) [16] It produced primarily by adipose cells of white adipose tissue and is the most abundant adipokine. adiponectin consists of a nitrogen terminal collagen domain and a carboxyl terminal globular domain. Low levels of adiponectin are expressed in other tissues such as osteoblasts, liver, myoblasts, myoblasts, epithelial cells, and placental tissue (Obata *et al* 2013) [31]. Human adiponectin is encoded by the Adipo gene. The human adiponectin gene contains three exons in addition to the start codon in exon 2 and the stop codon in exon 3. Human adiponectin is 244 amino acids long and consists of four regions that include the signal sequence at the N terminus. It consists of 18 amino acids, a variable region of 24 amino acids, a collagenous domain consisting of 65 amino acids, and a globular-terminal domain, the C domain, consisting of 137 amino acid (Fang and Judd, 2018) [10] The main biological functions of adiponectin include enhanced fatty acid biosynthesis and inhibition of gluconeogenesis in the liver In addition, it enhances glucose uptake in skeletal muscle via signaling pathways. Studies have shown that adiponectin can be used to improve insulin resistance by reducing the amount of intracellular fat through increased oxidation of fatty acid via PPAR_ activation and enhancement of insulin receptor substrate (IRS) signaling in skeletal muscles and the liver (Schindler *et al*, 2017) [41]. Moreover, effects have been found Adiponectin (antioxidant, anti-inflammatory, and atherosclerosis (Thundyil *et al*, 2012) [46]. Resistin A hormone secreted from adipose tissue, which increases resistance to insulin action and affects the balance Glucose in rodents leads to the development of type 2 diabetes (T2DM) .diabetes mellitus It also acts as a link between obesity and diabetes; (Steppan *et a*l 2001) [45] Adipose tissue secretes many polypeptides such as adiponectin, leptin, and resistin, the concentrations of which decrease in obesity and diabetes. (Trujillo and Scherer,2005) [47] Resistin is not limited to type 2 diabetes or obesity, but rather to cardiovascular disease. atherosclerosis, arthritis, high blood pressure, and various malignant cancers. Recent studies have indicated that rasitin has a negative effect on cardiovascular diseases (Filková *et al*., 2009; Zhang *et al*,2017 [50]; Fontana *et al* 2015), resistin is positively associated with metabolic disturbance and increased coronary artery calcification, a quantitative measure of arterial stiffness, as well as other inflammatory markers (Reilly *et al*., 2005) [34]. as well as a study of cases of acute ischemic stroke in patients within 24 hours of the onset of stroke.

Materials and Methods

1. Experimental animals

Experiments were carried out in the laboratory animal house of the College of Veterinary Medicine. University of Mosul. In this study, 90 male white rats, whose weight ranged between 250 and 350 g, were used. They were placed in special plastic cages under appropriate conditions of a temperature of 25 C and a light cycle of 12 hours of light and 12 hours of darkness. They were fed a standard diet and were provided with water openly. The cages were covered with sawdust and changed every three days.

2. Experiment design

The experiment included 90 male white rats and was divided into two groups, the first group included 35 rats that were given a standard diet for two months, the second group included 55 rats that were fed on fodder with 4% cholesterol

added for two months (Alhamami et al. 2006) [1]. After the end of the treatment period, a lipid profile was examined, and then the animals of the first group were divided into 7 groups and the second group into 11 groups. The groups were treated for a month as shown

1. group of control rats, the normal diet and distilled water were given orally
2. A group of rats that induced hyperlipidemia
3. A group of rats treated with Rosuvastatin (40mg/kg)
4. A group of rats that induced hyperlipidemia and were treated with Rosuvastatin (40mg/kg)
5. A group of rats treated with vitamin D3
6. A group of rats that induced hyperlipidemia and were treated with vitamin D3 were dosed with vitamin D3 at a dose of 1000 IU (kg) by Gavage needle [5]
7. A group of rats treated with vitamin K2
8. A group of rats that induced hyperlipidemia and were treated with vitamin K2 A dose of 100 mg /kg of feed weight, as stated in the study [6].was used, and the dose was given with the feed
9. A group of rats treated with calcium by Gavage needle calculated depending an health organizations, which recommend taking (800 mg /70 kg (Hassan, 2006)
10. group of induced hyperlipidemic rats with calcium.
11. A group of rats treated with spirulina algae 1000mg/ kg by Gavage needle (Hasanen et al. 2015)
12. A group of induced hyperlipidemic rats with spirulina algae.
13. A group of rats treated with garlic aqueous extract 400 mg/kg B.W(Ebrahimi et al. 2015)
14. A group of induced hyperlipidemic rats treated with garlic aqueous extract.
15. A group of induced hyperlipidemic rats treated with vitamin D3, vitamin K2 and Rosuvastatin
16. A group of induced hyperlipidemic rats treated with vitamin D3 and vitamin K2 and calcium
17. A group of induced hyperlipidemic rats treated with vitamin D3, vitamin K2 and garlic aqueous extract
18. A group of induced hyperlipidemic rats treated with vitamin D3, vitamin K2 and Spirulina algae..

3. Determination of adiponectin and Resistin hormone concentaration

Adiponectin and Resistin concentration was estimated using an analysis kit produced by Sun long Chinese origin, used the sandwich ELISA kit

Statistic analysis

Data were analyzed by Minitab program system .17. and anova test were applied. The means compare by Duncun's multiple rang under the level of significantly 0.01

Results

The results of the level of adiponectin hormone shown in Table (1) showed that the Introduce of hyperlipidemia caused a significant decrease ($P<0.01$) in the level of the hormone compared with the control group. In addition to a significant decrease of $P<0.01$ in the group of healthy rats treated with statin compared with the control group, and the results showed a significant increase in the level of the hormone in the hyperlipidemia group treated with statin when compared with the hyperlipidemia group, and the level of the hormone appeared without a significant difference from the control group and the group of treated rats Vitamin D3 with a significant increase in the level of the hormone in the hyperlipidemia group treated with vitamin D3 compared with the hyperlipidemia group, and the level of the hormone in the hyperlipidemia group treated with vitamin D3 was almost equal to its level in the control group. Treatment of healthy rats with vitamin K2 caused a significant decrease ($P<0.01$) in the level of adiponectin compared with the control group, and treatment of hyperlipidemia rats with vitamin K2 did not cause a significant difference from its level in the hyperlipidemia group. No significant difference was observed in the level of the hormone when compare a group of healthy rats treated with garlic aqueous extract with a control group, and no significant difference was observed between the group of hyperlipidemia treated with the extract and a hyperlipidemia group. Also, no significant difference was observed in the level of the hormone between the group of healthy rats treated with spirulina with the control group, while a higher Significant in its level in hyperlipidemia group treated with spirulina compared with hyperlipidemia group. Calcium treatment at a probability level of $P<0.01$, and the hormone in the hyperlipidemia group treated with calcium recorded a level equal to its level in the control group. The results in Table () showed that the intervention group between vitamin D3, vitamin K2, and stanin did not cause a significant difference in the level of the hormone adiponectin when compared with the hyperlipidemia group, the hyperlipidemia group treated with stanin alone, and the hyperlipidemia group treated with vitamin K2 alone, and a significant decrease occurred when compared with the hyperlipidemia group. Hyperlipidemia treated with vitamin D3 alone

Significantly increased ($P<0.01$) the level of the hormone in the group of intervention between vitamin D3 and K2 and the aqueous extract of garlic compared with the hyperlipidemia group, the hyperlipidemia group treated with the extract alone, and the hyperlipidemia group treated with vitamin K2, but when compared with the hyperlipidemia group treated with vitamin D3 alone notes There is no significant difference in the hormone level As for the intervention group between vitamin D3, vitamin K2 and spirulina, when compared with the hyperlipidemia group, we notice a significant increase in the level of the hormone in the overlap group at a probability level of $P>0.01$, but when compared with the hyperlipidemia group treated with spirulina alone, we notice that there is no significant difference in the level of the hormone There was no significant difference between them, as well as no significant difference in the level of the hormone between them when compared with the hyperlipidemia group treated with vitamin D3 at the probability level of $P < 0.01$ While there was a significant increase in the level of the hormone in the intervention group when compared with the hyperlipidemia group Treatment with vitamin K2 alone at a $p<0.01$ level As for the intervention group between vitamin D3, vitamin K2, and calcium, when compared with the hyperlipidemia group, we notice a significant increase in the level of the hormone in the interaction group, but when compared with the hyperlipidemia group treated with vitamin D3 alone, we notice that there is no significant difference in the level of the hormone between them, and when compared with the hyperlipidemia group and treatment with vitamin K2 alone, we notice a significant increase in the intervention group and at the level of

probability P>0.01 and when compared with the hyperlipidemia group treated with calcium alone, we notice that there is no significant difference in the level between them at the probability level of P<0.01. vitamin D3, vitamin K2 and calcium to its level in the control group

The results shown in Figure showed that induced hyperlipidemia caused a significant increase of the hormone level compared with the control group at the probability level of ≥P 0.01. The results also showed that there was no significant difference between the group of healthy rats treated with the statin. Compared with the control group at the probability level P < 0.01, while the results showed a significant decrease in the level of resistin hormone in the hyperlipidemia group treated with a statin compared with the hyperlipidemia group. As for the group of healthy rats treated with vitamin D3 at a dose of 1000 IU kg, it was observed that there was no There was a significant difference in the hormone level at the probability level of ≥0.01 compared to the control group, while it was observed that there was a significant decrease in the hormone level in the hyperlipidemia group and the treatment with vitamin D3 when compared to the hyperlipidemia group. Also, treating the rats with vitamin K2 at a dose of 100 mg kg resulted in no difference. Significantly in the hormone level when compared with the control group was set at a probability level of <P<0.01, with a significant decrease in its concentration in the hyperlipidemia group treated with vitamin K2 compared to the hyperlipidemia group at the same dose used The results shown in Table () showed that the use of garlic extract at a dose of 400 mg/kg led to a significant decrease of <P>0.01 in the level of resistin hormone in the group of healthy rats treated with the extract compared to the control group, in addition to a significant decrease in its level in the hyperlipidemia-treated group. extract when compared with the hyperlipidemia group. It was also observed that there was no significant difference in the hormone level in the group of healthy rats treated with spirulina at a dose of 1000 mg kg when compared to the control group. As for the hyperlipidemia group treated with spirulina, a significant decrease was observed when compared with the hyperlipidemia group at Probability level <0.01, and the hormone level returned to its level in the control group when the rats induced hyperlipidemia were treated with aqueous extract of garlic and spirulina. As for the group of healthy rats treated with calcium only, the results showed a significant decrease in them when compared to the control group at the probability level <0.01P as well. A significant decrease occurred in the hyperlipidemia group treated with calcium when compared with the hyperlipidemia group, and the hormone level in the hyperlipidemia group treated with calcium returned to its level in the control group. As for the intervention groupbetween vitamin D3, vitamin K2, and statin medication, the results in Table (1) showed a significant decrease in the level of the hormone in the intervention group compared with the hyperlipidemia group and the hyperlipidemia group treated with statin alone, as well as when compared with the hyperlipidemia group treated with vitamin D3 Alone, we notice an increase in the hormone level in the intervention group, but when compared to the hyperlipidemia group treated with vitamin K2 alone, we notice that there is no significant difference between them, and from the results it is noted that the individual treatments have a better effect than using them together. As for the intervention group between vitamin D3 and K2 and aqueous extract of garlic, it was noted that there was a significant decrease in the hormone level in this group compared with the hyperlipidemia group, with no difference appearing

Significant compared with the hyperlipidemia group treated with garlic extract alone, and when compared with the hyperlipidemia group treated with vitamin D3, a significant increase was found in the intervention group, with a significant decrease when compared to the hyperlipidemia group treated with vitamin K2 alone. As for the intervention group between vitamin D3, vitamin K2, and spirulina, when compared to the hyperlipidemia group, there was a significant decrease in the hormone level in the interaction group, with no significant difference when compared to the hyperlipidemia group treated with spirulina alone, with a significant increase when compared to the hyperlipidemia group treated with vitamin D3 alone. When compared to the hyperlipidemia group treated with vitamin K2 alone, a significant decrease was found in the level of the hormone in the intervention group, and no significant difference was observed in the intervention group between vitamin D3 and K2 and the aqueous extract of garlic and its level in the intervention group between vitamin D3, K2 and spirulina compared with the control group**.** As for the intervention group between vitamin D3, vitamin K2, and calcium, when compared to the hyperlipidemia group, it was noted that there was a significant decrease in the intervention group in the hormone level, while when compared to the hyperlipidemia group treated with vitamin 03 alone, a significant increase was found in the interaction group, while we did not record a significant difference. When comparing the intervention group with the vitamin K2 group alone, while when comparing the intervention group with the calcium group alone, a significant increase in the hormone level was found in the intervention group**.**

Discussion

Adiponectin is one of several hormones secreted by adipose tissue. Collectively known as adipocytokines, adiponectin in a healthy liver regulates the metabolism of glucose and fat. Which reduces gluconeogenesis and stimulates glycolysis and fatty acid oxidation. (Mao *et al.,* 2006) [26] The current study agreed with the study of Choi *et al* (2020) [5], which confirmed a decrease in the level of adiponectin in the atherosclerosis and type II diabetes. The reason may be due to a change in the lipid profile that depends on inflammation and blood glucose levels (Pischon *et al* 2004) [39]. It has been shown that a decrease in adiponectin stimulates phagocytic cells to become more effective in secreting cytokines that cause heart failure and its after effects Adiponectin is an anti-inflammatory and anti-atherosclerotic (Rasul *et al.,* 2011 [33]; Koncsos *et al*., 2010) [21]

The effect of statins and vitamin D3 on the level of adiponectin can be attributed to the ability of statins and vitamin to lower the lipid level, reduce the inflammatory process and raise the level of the hormone. Study by Baigent *et al*., (2010) [2] reported statin have the ability to competitively inhibit the enzyme hydroxyl methyl glutaryl coenzyme A (HMG CoA), which is the specific step in the biosynthesis of cholesterol, which leads to a decrease in the level of low-density lipoprotein and lipids in the blood. Behmanesh *et al* (2019) [3] reported that vitamin D3 improved the lipid profile in polycystic ovarian patients. Riek *et al*. (2018) [35] demonstrated the ability of vitamin D3

to reduce levels of total cholesterol and low-density lipoprotein and to reduce the development of atherosclerosis in patients with type 2 diabetes mellitus. The vitamin also has anti-inflammatory properties (Brito *et al* ., 2020) [4] Studies show that vitamin D3 has the ability to reduce the inflammatory process by multiple mechanisms such as its ability to reduce the Th1 helper cell response and increase the Th2 response as well as increase the production of anti-inflammatory cytokines (Papapostoli *et al*. 2016) [37]. The reason for reducing the level of the hormone adiponectin using vitamin D3 may be due to its ability to improve insulin resistance and prevent type 2 diabetes, as the study of Choi *et al* (2020) [5] showed an increase in the level of the hormone adiponectin in patients with type 2 diabetes, and the study indicated Pramono *et al*. (2020) [38] Vitamin D3 has a role in lipid and glucose metabolism, thus preventing obesity, improving insulin sensitivity, and preventing type 2 diabetes*.*

The results of our study did not correspond with the study of Hussein *et al* (2018) [17], as the study showed the ability of vitamin K2 to raise the level of adiponectin, and attributed the reason for this to the ability of the vitamin to improve blood sugar and increase the efficiency of beta cells in diabetic mice. A study by Lee *et a*l. (2008) [24] indicated that the use of spirulina in patients with type 2 diabetes caused an increase in the hormone adiponectin, and the study attributed the reason to the ability of spirulina to improve the lipid profile, raise the level of antioxidants and reduce the inflammatory process, and may be attributed to the ability of spirulina to raise the level of adiponectin For its ability to treat obesity, as the study of Heo and Chhoun (2018) indicated that spirulina has a role in the treatment of obesity due to diet, that the level of adiponectin decreases in obese periods due to their feeding on a diet rich in fats, as spirulina activates AMP activated protein kinase (AMPK) pathway and sirtuin 1 (SIRTI), thereby disrupting some physiological processes associated with obesity, such as promoting lipid synthesis and preventing lipid oxidation A study by Rutkowski *et al* (2017) [36] showed that the level of adiponectin increased with the level of calcium in the blood. The study of Nobre *et al* (2011) [29] showed that the use of calcium supplements can affect the regulation of metabolism, which leads to weight loss and improvement of insulin resistance, which contributes to raising the level of adiponectin. Calcium also has the ability to lower the level of lipid in the blood and protect against obesity. the stydy Morvaridzadeh *et al*(2021) [28] reported that calcium and vitamin D3 supplementation has the ability to lower cholesterol and triglyceride levels and raise HDL levels. A study by Derakhshandeh Rishehri *et al* (2022) [7] showed that calcium has the ability to lower LDL levels. study of Christensen *et al* (2009) [6] indicated that calcium has a role in increasing the excretion of saturated fats with feces and contributes to weight reduction and reduces the level of cholesterol and LDL in the blood. The study of Gonzalez *et al.,* (2012) [12] indicated that consuming large amounts of calcium It increases the rate of fat oxidation and prevents its accumulation in the body.

The results of the current study are consistent with Zhang *et al* (2017) [50] whose results stated that elevated levels of the resistin are associated with vascular disease and atherosclerosis. The results of the study by Gao *et al* (2016) [11] also showed an increased level of expression of the resistin*.* In mice induced coronary arteritis. Kawanami *et al* (2004) [20] reported that resistin has the ability to stimulate the expression of adhesion molecules such as Vascular cell adhesion protein1 (VCAM-1) And intercellular Adhesion Molecule 1(ICAM-1) also showed that the balance between adipocytokine such as resistin and adiponectin determines the state of inflammation and insulin resistance, as adiponectin works to inhibit adhesion molecules activator mediated by resistin. The results of our current study agreed with the study of Ichida *et al* (2006) [19], as the results of the study stated that atorvastatin reduced the level of resistin in mice with type 2 diabetes. The study by Zhao and Zhang (2003) [49] explained that statins have the ability to activate peroxisome proliferative activated receptor gamma receptors PPAR-γ in fat cells and monocytes, causing inhibition of the expression of the resistin gene. The mechanism of the effect of vitamin D3 on the hormone resistin may be indirect. The decrease may be through improving insulin resistance in the body's tissues and thus reducing its level, as a study by Pramono *et al* (2020) [38] indicated that this vitamin has a role in the metabolism of fats and glucose, thus preventing obesity and improving sensitivity Insulin and the prevention of type 2 diabetes, or by lowering the level of blood lipid and preventing vascular diseases, as it is accompanied by an increase in the hormone level. Rønn *et al*., (2021) [40] study indicated that vitamin K2 is a contributing factor in the phosphorylation of bone matrix protein osteocalcin, as it Increasing the concentration of this un carboxylated protein (undercarboxylated osteocalcin (ucOC) increases insulin sensitivity. The results of a Kurnatowska *et al* (2015) [23] study showed that vitamin K2 supplements are associated with a reduced risk of vascular disease and atherosclerosis. Vitamin K2 plays an important role in cardiovascular disease by regulating calcium homeostasis through activation of the anti-calcification protein matrix Gla protein. This protein is associated with various signs of cardiovascular disease including increased arterial and vascular stiffness, valve calcification, insulin resistance and indicators of heart failure (Hariri *et al* 2021) [14]. Vitamin K2 contributes to the carboxylation of the MGP protein, which is expressed by endothelial cells, fibroblasts, and smooth muscle cells, and its function is to bind calcium crystals and prevent their effect on elastin fibers (Evrard *et al*., 2015) [8]. The study of Li *et al*., 2018 reported Vitamin K2 has the ability to reduce insulin resistance through the participation of vitamin K-dependent-protein osteocalcin and its anti-inflammatory and blood lipid-lowering properties. The study by Koziot-kozakowska and Maresz (2022) [22] confirmed that vitamin K2-based proteins have several important effects on bone health, arteriosclerosis, insulin sensitivity, body weight, and brain health. Thus, it contributes to reducing the level of resistin. The results of the current study indicated the effect of garlic extract and spirulina algae on the hormone level

Perhaps the reason for this positive effect is due to the ability of the garlic plant and spirulina to lower the level of lipids in the blood and thus reduce the incidence of vascular diseases. The Nickavar study (2022) [30] confirmed that the components of garlic have the ability to treat cases of hyperlipidemia because it contains organic sulfur components (Organosulfur compounds (OSCs) with effective blood lipid-lowering activity such as ajoenes, y-glutamyl-S-alk (en)ylcysteine. These two groups have the ability to reduce the enzyme HMG CoA reductase. The

Sengupta (2018) [44] study also confirmed that food fortified with spirulina algae has clear results in reducing The level of blood lipids, particularly cholesterol, and an increase in the level of HDL in hyperlipidemia rats. .Other studies indicated the presence of C-phiyoocyanin protein in spirulina algae, which has a role in increasing the effectiveness of an enzyme. GSH Peroxidase and SOD, which work to reduce the effect of free radicals and prevent the total oxidation of fats. It also works to reduce the formation of NADP and NADH and inhibits the enzyme NADPH Oxidase, and by this mechanism it reduces the level of blood Lipid (Upasani *et al*., 2003 [48]; Sharma *et al*., 2019) [42]. The study by Singh *et al* (2018) [43] confirmed that calcium has a role in regulating the level of resistin. The reason may be due to calcium's ability to reduce fat levels and prevent obesity. as the Nobre *et al* (2011) [29] study indicated that the use of calcium supplements can affect the regulation of metabolism, leading to weight loss and improving insulin resistance. Other studies have also shown that calcium supplements play a crucial role in adolescence to prevent obesity, as the study showed an inverse relationship between total calcium intake with various obesity indicators (Nappo *et al*., 2019; Jürimae *et al*.,2019). A study by Morvaridzadeh *et al* (2021) [28] showed that calcium and vitamin D3 supplements have the ability to reduce the level of cholesterol and triglycerides and raise the level of HDL. A study Derakhshandeh- Rishehri *et al* (2022) [7] showed that calcium has the ability to reduce the level of LDL. A study by Denke *et al*.,(1993) and a study. Christensen *et al* (2009) [6] that calcium plays a role in increasing the excretion of saturated fats with the stool, contributes to weight loss, and reduces the level of cholesterol and LDL in the blood. A study by Gonzalez *et al* (2012) [12] indicated that consuming large amounts of calcium increases the rate of oxidation of Lipid and preventing their accumulation in the body.

Table 1

treatment	Resistin Pg/ml	Adiponectin ng / L
Control	2483.2±67.4 de	46.23±7.76 ab
Hyperlipidemia	3258± 67.2a	38.52±2.83 cd
Healthy + statin	2456±86.9 de	37.55±1.51 cde
Hyperlipidemia+ statin	3010±79.7 b	44.64±2.1 ab
Healthy+ vita D3	2420.6±88.8 de	44.15±3.97 ab
Hyperlipidemia+ vita D3	1883±78.3 g	44.65±4 ab
Healthy + vita k2	2760.6±93.8 de	35±3.16 de
Hyperlipidemia+ vita k2	2706.4± 90.8 c	37.63±2.36 cde
Healthy + aqueous extract of garlic	2012±71.4 f	45.25±6.24 ab
Hyperlipidemia+ aqueous extract of garlic	2503±96.4 d	36.82±4.85 de
Healthy + spirulina	2500.4±70.1 d	42.1±4.9 bc
Hyperlipidemia+ spirulina	2378±59.5 e	46.25±7.1 ab
Healthy + calcium	2012±71.4 f	32.53± 2.53 e
Hyperlipidemia+ calcium	2513±93.1 d	46.9±8.1 ab
Hyperlipidemia + Vita D3+ Vita K2+ Statin	2872± 63.3 c	34.92 ± 4.05 de
Hyperlipidemia+ Vita D3+ Vita K2+ aqueous extract of garlic	2476.4±99.6 de	43.18 ± 3.04 b
Hyperlipidemia+ Vita D3+ Vita K2+ Spirulina	2486.0± 85.3 de	49.02± 15.45 a
Hyperlipidemia+ Vita D3+ Vita K2+calcium	2846.4±97.8 c	48.6± 1.329 a

Table (1) Values are expressed as the arithmetic mean ± standard deviation and the number of rats is 5 for each group. Different letters vertically indicate the presence of a significant difference, and similar letters horizontally indicate the absence of a significant difference at the probability level of $P \leq 0.01$

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