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Therapeutic Role of Vitamin D3, K2, Garlic Extract, Calcium and Spirulina Algae Powder on Induced Hyperlipidemia in Rats.

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THE study aims to determine the therapeutic role of vitamin D3, k2, garlic extract, spirulina and calcium on induced hyperlipidemia. The experiments included 90 male albino rats and were divided into two groups, the first group were given a standard diet for two months and the second were fed on fodder with 4% cholesterol added for two months. The groups were treated for a month with statin (40mg/kg) vitamin D3 (1000IU/kg) , k2(100mg/kg of feed), garlic extract (400 mg/kg) , spirulina (1000g/kg) and calcium (800mg/70kg). the results showed increase in the level of cholesterol , triglyceride, LDL ,VLDL and decrease the level of HDL in the hyperlipidemia group compared with the control group and decrease in the level of cholesterol and triglyceride in treatment groups which treated with statin, vitamin D3, k2 , garlic extract, calcium and spirulina compared with the non-treatment hyperlipidemia group with increase in the level of HDL in treatment groups which treated with statin, vitamin D3,vitamin k2, garlic extract, calcium and spirulina compared with non-treatment hyperlipidemia group

Keywords: hyperlipidemia, vitamin D3, vitamin K2, spirulina, Calcium.

Introduction

Cholesterol was isolated for the first time from human gallstones for more than two centuries, fatty cholesterol has been of interest to scientists and doctors, due to its physiological and pathological importance, As cholesterol is strongly linked to the risk of atherosclerosis and cardiovascular diseases (CVD)[1]. Hyperlipidemia A common disease in developing countries, this disease refers to abnormally high levels of blood lipids, including lipids or lipoproteins in the blood due to abnormal metabolism of fats, resulting in nutritional disorders, obesity and genetic diseases such as familial hypercholesterolemia or other diseases such as cardio vascular, diabetes mellitus, strokes, liver and kidney failure, this disease is usually without Symptoms are detected by routine blood analysis[2] [3]. In advanced stages of the disease, patients may suffer from multiple complications such as high blood pressure and angina pectoris there are two main types of hyperlipidemia the first type is primary hyperlipidemia, genetic cause. The other is called secondary hyperlipidemia, which results from other factors such as obesity, thyroid dysfunction, and alcoholism, Hypothyroidism and chronic renal failure [2]. Hypercholesterolemia is the most common form of dyslipidemia and is associated with an increased risk of cardiovascular disease and a number of studies have shown that hyperlipidemia, in addition to its known role in atherosclerosis, may directly affect the heart, leading to an increase in the incidence of ischemia [4]. According to the present studies and reviews, hyperlipidemia is started from endothelial damage of the blood vessels, leading to the loss of nitric oxide in the damaged site, this will be resulting in increase in the inflammatory response around the affected area and accumulation of the lipids in the deepest layer of the endothelial wall,

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macrophage cell will engulf the lipids forming what is called (the foam cell) with cholesterol content the formation of foam cell will cause necrosis, apoptosis, and mitochondrial dysfunction. At the same time, the cells of the smooth muscle encapsulate the foam cell producing fibrotic plague and inhibit destroying of the foamy cells. On other hand, stimulation of the platelets activity with the tissue factors resulting in plague rupturing and thrombosis development of plague occur either rapidly resulting in obstruction of the blood vessels or slowly that cause stenosis of the blood vessels. In both mechanisms, lipid plague remains the mainstay of the development of CVD and deterioration of patient health status.[5]

Material and Methods

Ethical approval number: UM.VET.078

Experimental animals

Experiments were Carrie in the laboratory animal house of the College of Veterinary Medicine. University of Mosul. In this study, 90 male white albino rats, whose weight ranged between 250 and 350 gram, 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.

Experimental design

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 feed for two months, the second group included 55 rats that were fed on feed with 4% cholesterol added for two months [6].After the end of the treatment period, the 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 below

1. A group of control rats, the normal feed and distilled water were given orally by gavage needle.
2. A group of rats that induced hyperlipidemia by 4% cholesterol addition to the diet for months.
3. A group of rats treated with Rosuvastatin (40mg/kg). orally by gavage needle
4. A group of hyperlipidemic and were treated with Rosuvastatin (40mg/kg)
5. A group of rats treated with vitamin D3 1000 IU / Kg orally by gavage needle [7]
6. A group of hyperlipidemic and were treated with vitamin D3 1000 IU / Kg orally by gavage needle [7]
7. A group of rats treated with vitamin K2 A 100 mg /kg of feed [8].
8. A group of hyperlipidemic and were treated with vitamin K2 A 100 mg /kg of feed [8].
9. A group of rats treated with calcium orally by Gavage needle with a dose calculated depending an health organizations, which recommend taking (800 mg /70 kg) [9]
10. A group of hyperlipidemic rats with calcium. (800mg/ 70kg) orally by Gavage needle [9]
11. A group of rats treated with spirulina algae 1000mg/ kg by Gavage needle [10]
12. A group of induced hyperlipidemic rats with spirulina algae. 1000 mg/ kg by Gavage needle [10]
13. A group of rats treated with garlic aqueous extract 400 mg/kg B.W[11]
14. A group of induced hyperlipidemic rats treated with garlic aqueous extract.
15. A group of induced hyperlipidemic rats treated with vitamin D3 and K2 and Rosuvastatin
16. A group of induced hyperlipidemic rats treated with vitamin D3 and K2 and calcium
17. A group of induced hyperlipidemic rats treated with vitamin D3, K2 and garlic aqueous extract
18. A group of induced hyperlipidemic rats treated with vitamin D3, K2 and Spirulina algae.

Statistical analysis

Data were analyzed by Minitab program system [17] and one way ANOVA test were applied. The means compare by Duncun's multiple range at $P \leq 0.01$.

Results

Cholesterol

The results in Table (1) revealed that intact rats treated with statin ,vitamin k2 And calcium showed significant increase ($P \leq 0.01$) in cholesterol level with no significant changes in cholesterol level in intact rats with vitamin D3, garlic extract and spirulina compare with control group. Induced hyperlipidemia caused rise in cholesterol level compared to control , all hyperlipidemic rat treat rets caused a significant decrease in cholesterol level compare to hyperlipidemia group and return to control level of cholesterol except hyperlipidemic group treated with vitamin k2 still above cholesterol level in control group.

Tri-glyceride

All intact rat treated caused significant increase in TG except statin , spirulina treatment showed no significant changes compare to control group. Induced hyperlipidemia caused increase TG level and all treatments caused significant decrease in TG level compare to hyperlipidemic groups and it's level in all group still above its level in control group.

High-density lipoprotein

All intact rats treated caused no significant change in HDL except significant decrease in HDL level by vitamin D3, garlic extract compare to control group while hyperlipidemia induction caused significant decrease in HDL compare to control level. All hyperlipidemic treatment group chowed significant increase in HDL level compare to hyperlipidemic group it's level return to control level

Low-density lipoprotein

No significant changes in LDL level in all intact rat treated except intact with calcium caused significant increase in LDL level compare to control group. Hyperlipidemia caused significant LDL level rise compare to control in addition rat treated caused significant decrease in LDL level compare to hyperlipidemia group and LDL return to it is level in control group.

Very low-density lipoprotein

All intact rat treated s caused significant increase in VLDL level except treated with calcium treated showed no significant changes compared to control group. Hyperlipidemia caused significant increase in VLDL compared to control group, all hyperlipidemia rats treatment caused significant decrease in VLDL level compared to hyperlipidemia group.

In tractions

Hyperlipidemia rats treated with vitamin D3,+ k2+ statin showed significant decrease in cholesterol, TG, LDL and VLDL levels compares to hyperlipidemia group and all levels return to control group valve except VLDL still above the control valve a compared with significant increase in and return to control level. Vitamin D3+k2+garlic extract treated to hyperlipidemia rats caused significant decrease in cholesterol ,TG, LDL, VLDL level compare to hyperlipidemia group with return of cholesterol ,TG, LDL and VLDL level to control group level with significant increase in HDL level and rise in it is valve above control group. Hyperlipidemia rats treated with vitamin D3,+ k2+ spirulina caused significant decrease in cholesterol, TG, LDL and VLDL levels compares to hyperlipidemia group and return to control valve with significant increase in HDL valve compare to hyperlipidemia group and return to control valve. Rats with induced hyperlipidemia treated with vitamin D3,+ k2+ calcium showed significant decrease in cholesterol, TG, LDL and VLDL levels compares to hyperlipidemia group and return to normal group with significant increase in HDL and return to control valve.

Discussion

Perhaps the reason for the decrease in the level of cholesterol, TG, LDL, VLDL and increase the level of HDL in the treatment groups is due to the ability of statins to competitively inhibit the Hydroxyl methyl glutaryl coenzyme-A (HMG-CoA), which is The specific step in the biosynthesis of cholesterol leading to a decrease in the level of low-density lipoprotein and lipids in the blood [12]. Also statins have a critical role in lowering the level of cholesterol, VLDL, LDL, as cholesterol is inhibited by inhibiting the mevalonate pathway by inhibiting the enzyme HMG-COA reductase, regulating LDL receptors in liver cells, and inhibiting VLDL production in the liver. It also inhibits triglycerides by increasing the activity of the lipoprotein lipase enzyme in tissues, The mechanism of statins raising the level of HDL is unknown, while some studies indicated the ability of statins to increase the formation of apolipoprotein A (ApoA) a protein encoded by the *APOA1* gene, which is one of the components of HDL, which leads to an increase in its formation [13-15]. Vitamin D3 improved the lipid profile in polycystic ovarian patients, as the study showed the ability of the vitamin to reduce the level of cholesterol and low-density lipoproteins and raise the level of high-density lipoproteins [16]. The study of some authors [17] showed that giving vitamin D3 at a dose of 50,000 IU twice a week to infertile patients due to polycystic ovaries reduced the level of cholesterol and low-density lipoprotein. The results of another study on HIV patients who suffer from vitamin D3 deficiency showed that giving them vitamin D3 supplements at a dose of 4000 IU per day reduced total cholesterol and low-density lipoprotein [18].Some studies [19] demonstrated the ability of vitamin D3 to reduce levels of total cholesterol and low-density lipoproteins and to reduce the development of atherosclerosis in rats with type 2 diabetes, and explains the reason that in the case of vitamin D3 deficiency It increases monocytes ability increase to transport LDL from the blood and increases the formation of foam cells, and administration of vitamin reduces the ability of

monocytes to transfer OX- LDL. Other studies confirmed that vitamin D3 has the ability to inhibit the HMG-CoA reductase enzyme as well as has an effect on bile acids in the intestine [20, 21] The results of the study were in agreement with the studies of [22] Who stated that giving vitamin K2 to chronic renal failure patients reduced cholesterol levels and the study of [23] rabbits with induced hyperlipidemia, treatment with vitamin K2 show reduced in blood lipids and hydrogen peroxide level. The study of [24] indicated that the use of vitamin K2 reduced total cholesterol and triglycerides in rats induced hyperlipidemia. In addition the study of [25] indicated the ability of vitamin k2 supplementation to improve insulin sensitivity in the body by reducing blood lipids and triglycerides level in patients with type 2 diabetes. The study of [26] indicated that vitamin K2 treated in mice with experimental hyperlipidemia reduced the level of cholesterol and triglycerides with an increase in HDL level and with an improvement in the atherosclerotic factor by 20%, the study indicated vitamin K2 ability to regenerate endothelial progenitor cells, as damage to the lining of blood vessels is one of the most important causes of the formation and development of atherosclerosis. Several studies have shown that vitamin K2 has the ability to inhibit the effectiveness of the HMG-CoA reductase enzyme, and they explained the reason that vitamin K2 has a side chain (geranylgeraniol (GGO) similar to the structure of geranylgeranyl-pyrophosphate (GGPP) found in statins, which is key enzyme in the inhibition process, It is believed that vitamin K2 It has the same properties as statins in inhibiting the enzyme limiting step for cholesterol synthesis [27-30]

The results of the study agreed with the study of [31] which showed the ability of the garlic to reduce the of blood lipids level and thus reduce the incidence of vascular diseases and confirmed that the components of garlic have the ability to treat cases of hyperlipidemia because it contains organosulfur compounds (OSCs) these two groups possess the ability to reduce the enzyme HMG-reductase CoA. The study of [32] also indicated the ability of garlic to reduce cholesterol levels. and the study of [33] [34] confirmed the ability of garlic components such as allicin reduce biosynthesis process of cholesterol and its absorption and increase the secretion in bile. Studies showed that garlic has the ability to inhibit enzyme HMG coA reductase activity by reducing levels of HMG mRNACOA by disabling sterol regulatory element- bindingprotein-2 (SREBP-2). [35,36] study confirmed that food fortified with spirulina algae had clear results in lowering blood lipids, especially cholesterol, and raising HDL levels in hyperlipidemia rats. As indicated by the author [37] after eight weeks of intervention in spirulina treated diabetic patients were shown significant decreases in the total cholesterol, LDL-cholesterol, triglyceride, and MDA serum levels. the mechanism that explains the role of spirulina to reduce hypercholesterolemia and lipid disorders has not yet been identified, but some researchers found that the addition of these algae to the diet reduced the intestinal absorption of cholesterol as well as the reabsorption of bile acids from the intestine[38,39]. The results of the current study agreed with the study of [40] about spirulina ability to inhibit the HMG-CoA reductase enzyme is related to the medicinal properties and phytochemical components of spirulina such as phenols and flavonoids [41,42]. Spirulina also has the ability to reduce the expression of RNA HMG COA 1 regulatory element-binding protein in liver tissue and these genes have a role in the formation of fat and production [43]

Other studies indicated that spirulina algae contains C-phycocyanin protein, which has a role in increasing the effectiveness of Peroxidase and SOD, which suppress free radicals and prevent peroxidation of fats [44,45] and the results of the study agreed with many studies in the ability of calcium with vitamin D3 to improve the lipid profile [46-48]. A study [49] showed that calcium and vitamin D3 supplementation has the ability to lower cholesterol and triglyceride levels and raise the level of HDL, and a study by [49] showed that calcium has the ability to lower the LDL level. The study indicated Denke *et al.* and a study by [50] showed that calcium has a role in increasing the excretion of saturated fats with the stool and contributes to weight reduction and reduces the level of cholesterol and LDL in the | blood. A study by [51] indicated that consuming large amounts of calcium increases the rate of fat oxidation and prevents its accumulation in the body. The effect of calcium on the level of fats in the blood can be clarified through two mechanisms, The first suggests that calcium binds to fatty acids and bile and prevents the absorption of fats in the intestine. The second mechanism suggests that calcium affects the activity of fat cells by decreasing levels of parathyroid hormone and vitamin D3 As laboratory studies showed that the high level of parathyroid hormones reduces fat breakdown and causes obesity[52-54] .

Conclusions

Vitamin D3 and vitamin k2 improved the lipid profile the study showed the ability of the vitamin D3 and k2 to reduce the level of cholesterol, triglyceride and LDL and raise the level of HDL. The

study showed the ability of the garlic extract, calcium and spirulina to reduce the level of blood lipids.

Conflict of interest

Regarding the research, writing, and publication of this paper, the authors acknowledged no conflicts of interest.

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TABLE 1. Values are expressed as the arithmetic mean ± standard deviation and the number of rats is 5 for each group.

Treatment	Cholesterol mg/dl	Triglyceride mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
Control	180.27± 4.48^{d}	157.44±2.94^{f}	50.594±0.643^{b}	98.0±4.05^{cd}	31.480± 0.630^{f}
Hyperlipidemia	291.5±29.6 ^{a}	186.37±7.33^{a}	39.720± 4.260^{d}	214.05±31.20 ^{a}	37.24±1.461^{a}
intact + statin	193.54±9.98^{bc}	156.11±2.68^{f}	49.460± 4.420^{bc}	112.860±12.86^{bc}	31.22±0.554^{f}
Hyperlipidemia+ statin	184.5±5.34 ^{cd}	165.51 ± 3.91^{de}	49.789±0.527^{bc}	114.9±30.20 ^{b}	33.288± 0.724^{cd}
intact + vita D3	187.49±14.36 ^{bcd}	163.91±2.90^{e}	48.990± 1.547^{c}	105.71±15.32^{bc}	32.772± 0.581^{e}
Hyperlipidemia+ vita D3	192.26±14.58^{bc}	173.4±3.73^{b}	50.828±1.238^{b}	107.16±13.67 ^{bc}	34.66± 0.747 ^{b}
intact + vita k2	190.78±21.47^{bc}	165.14± 3.31^{de}	49.622± 1.067^{bc}	108.1± 22.50^{bc}	33.024 ± 0.653^{de}
Hyperlipidemia+ vita k2	191.52± 10.70^{bc}	163±2.37 ^{e}	51.218± 0.989^{b}	107.54±10.83 ^{bc}	32.754 ±0.477^{e}
intact + aqueous extract of garlic	189.74±14.30^{bcd}	162.24±3.11^{e}	47.940±4.410^{c}	109.38±16 ^{bc}	32.42± 0.622 ^{e}
Hyperlipidemia+ aqueous extract of garlic	185.55±8.77^{bcd}	165.68± 4.97^{de}	51.11±2.25^{b}	101.1±9.88^{bcd}	33.12± 1.01 ^{de}
intact + spirulina	183.32±3.40^{cd}	162.88±1.832^{e}	51.669±0.955^{b}	99.04±3.59^{cd}	32.56± 0.358^{e}
Hyperlipidemia+ spirulina	184.76±14.42 ^{cd}	167.64±1.789 ^{cd}	49,884±1.425^{bc}	101.52±14.39^{bcd}	33.52± 0.349^{cd}
intact + calcium	196.52±10.24^{b}	158.55±4.26^{f}	51.577±1.378^{b}	113.43± 10.01^{b}	31.7±0.860^{f}
Hyperlipidemia+ calcium	182.63±3.36^{d}	173.58±4.8^{b}	48.366±0.572^{c}	99.56±3.51^{cd}	34.7±0.962 ^{b}
Hyperlipidemia + Vita D3+ Vita K2+ Statin	186.84± 8.88^{bcd}	165.90±2.97^{de}	50.558 ±1.116^{b}	103.0 ±9.280^{bcd}	33.160±0.586^{de}
Hyperlipidemia+ Vita D3+ Vita K2+ aqueous extract of garlic	179.51±5.40^{d}	173.50± 6.45^{b}	53.260 ± 3.330^{a}	91.60 ±5.350^{d}	34.660 ± 1.268^{b}
Hyperlipidemia+ Vita D3+ Vita K2+ Spirulina	181.01±5.12^{d}	157.67±2.73^{f}	49.220 ± 2.390^{bc}	100.29± 6.560^{bcd}	31.520±0.540^{f}
Hyperlipidemia+ Vita D3+ Vita K2+calcium	179.84±5.17 ^{d}	169.04 ± 2.92^{c}	50.828 ± 1.949^{b}	97.42 ± 5.550^{cd}	33.780 ± 0.618^{c}

Different letters vertically indicate the presence of a significant difference, and similar letters vertically indicate the absence of a significant difference at the probability level of P≤0.01.

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الدور العلاجي لفيتامين D3، K2، مستخلص الثوم ، الكالسيوم ومسحوق طحالب السبايرولينا على فرط دهون الدم المستحدث في الجرذان

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تهدف الدراسة إلى تحديد الدور العلاجي لفيتامين D3، K2، مستخلص الثوم، السبيرولينا والكالسيوم على ارتفاع نسبة الدهون في الدم. شملت التجارب 90 من ذكور الجرذان البيض وتم تقسيمهم إلى مجموعتين، المجموعة الأولى أعطيت نظاماً غذائياً قياسياً لمدة شهرين والثانية غذيت على علف مضاف إليه 4% كولسترول لمدة شهرين. عولجت المجموعات لمدة شهر باستخدام الستاتين (40 ملغم/كغم)، وفيتامين D3(1000 وحدة دولية/كغم)، وk2 (100 ملغم/كغم من العلف)، ومستخلص الثوم (400 ملغم/كغم)، والسبيرولينا (1000 غم/كغم)، والكالسيوم (800 ملغم / 70 كغم). أظهرت النتائج ارتفاع مستوى الكولسترول، الدهون الثلاثية، LDL، VLDL وانخفاض مستوى HDL في مجموعة فرط شحميات الدم مقارنة مع مجموعة السيطرة وانخفاض في مستوى الكولسترول والدهون الثلاثية في مجموعات العلاج التي عوملت بالستاتين وفيتامين D3، k2 ومستخلص الثوم والكالسيوم والسبيرولينا مقارنة مع مجموعة فرط شحميات الدم غير المعاملة مع زيادة مستوى HDL في المجموعات المعاملة بالستاتين وفيتامين D3 وفيتامين K2 ومستخلص الثوم والكالسيوم والسبيرولينا مقارنة مع مجموعة فرط شحميات الدم غير المعاملة.
الاستنتاج: فيتامين D3 وفيتامين K2 لهما دور في تحسين مستوى الدهون أظهرت الدراسة قدرة فيتامين D3 وk2على خفض مستوى الكوليسترول والدهون الثلاثية والبروتين الدهني منخفض الكثافة (LDL) ورفع مستوى البروتين الدهني عالي الكثافة (HDL). وأظهرت الدراسة قدرة مستخلص الثوم والكالسيوم والسبيرولينا على خفض مستوى الدهون في الدم.

الكلمات الدالة: فيتامين D3، فيتامين K2، السبايرولينا، الكالسيوم.