

## Effect of Tocotrienols Derived from Annatto on Lipid Profile and Some Adipokine Hormones in Rats Fed a High-Fat Diet

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### Abstract

Obesity is a metabolic condition that causes people to develop a variety of diseases and has emerged as a serious global public-health problem. Tocotrienol, a member of the vitamin E family that comes from the annatto bean (*Bixa orellana*), is special in that it doesn't contain alpha-tocopherol and instead mostly consists of delta-tocotrienol (approximately 90%) and gamma-tocotrienol (about 10%). This investigation aimed to ascertain whether annatto tocotrienol could improve certain biochemical indicators and metabolic hormones in male rats fed a high-fat diet. Eighteen adult male rats in total were split into three groups randomly (6 for each). Control group was given a diet low in fat (LF 10 % kcal from fat), High fat diet (HFD) group was fed with high fat diet (HF 60 % kcal from fat), And high fat diet with tocotrienol (HFDT) group was fed with high fat diet additive tocotrienol (60 mg/kg) dissolved in olive oil (1ml/kg) for 12 weeks. Tocotrienol treatment led to a significant decrease in total protein and globulin compared with the high-fat diet group and it significantly increased HDL-C compared with rats fed on a high-fat diet and control groups. While, tocotrienols significantly reduced the level of LDL and insulin hormone in the High fat diet plus tocotrienols group compared to the other groups.

**Keywords:** tocotrienols, high-fat diet, leptin, adiponectin.

## Introduction

Obesity is a metabolic condition that causes people to develop a variety of diseases and has emerged as a serious global public-health problem (1). It is characterized by an excess of fat tissue, which has been recognized as an important endocrine organ that regulates the production of a variety of signaling molecules (hormones, cytokines, growth factors, and chemokines). The disturbance of these signaling molecules may lead to metabolic disorders like diabetic or cardiovascular disease (2).

Tocotrienol, a member of the vitamin E family, has four analogues:  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocotrienol. Tocotrienol is primarily found in rice bran, palm, and annatto (3). Tocotrienol obtained from annatto bean (*Bixa orellana*) is unique in that it mostly includes delta-tocotrienol (about 90%) and gamma-tocotrienol (about 10%), with no alpha-tocopherol (4). The absence of alpha-tocopherol in annatto tocotrienol is advantageous since it interferes with tocotrienol bioavailability due to preferential binding of alpha-tocopherol to alpha-tocopherol transfer protein ( $\alpha$ -TTP) (5). Many studies have addressed the role of tocotrienols as a lipid reducer, depending on the source from which tocotrienols are extracted, the tocotrienol rich fraction (TRF) from palm oil, which consists of a mixture of tocopherols and tocotrienols, has demonstrated both positive (6-8) and negative (9-11) hypocholesterolemic effects. A major factor behind the failure of other studies to show beneficial effects of tocotrienol was the presence of more than 20% tocopherol in palm tocotrienols rich fraction TRF by this may prevent TRF from lowering total blood cholesterol levels or LDL cholesterol levels in some studies (12). A comprehensive analysis examined the numerous biological features of tocotrienols, including the findings of clinical

investigations on palm TRF and pure tocotrienols (13). The consumption of  $\alpha$ -tocotrienol derived from annatto (125, 250, 500, and 750 mg/d) plus the AHA Step-1 diet for 4 weeks was evaluated during the 30-week study period on serum lipid parameters and several cytokines (TNF- $\alpha$ , IL-4, IL-6, IL-8, and IL-10) in hypercholesterolemic subjects (12). The use of high-fat foods to induce obesity leads to increased food consumption, thus increasing body weight and body mass index and a subsequent increase in body fat index adipose tissue (14). Adipose tissue has been identified as an active endocrine organ that influences body homeostasis (15). Some peptide hormones derived from adipose tissue, such as adiponectin and leptin, can selectively affect energy expenditure, food behavior, and insulin sensitivity (16). The effects of annatto tocotrienol on the composition of the body (lean and fat mass), serum adiponectin, leptin, and blood glucose levels were studied in male rats that were treated with buserelin, the hormone testosterone ablation agent, and it was noticed that all groups, except those who were given Annatto tocotrienol at 60 mg/kg, had a significant increase in glucose levels after 3 months ( $P < 0.05$ ) (17).

The present study aimed to show how annatto tocotrienol affected biochemical markers (lipid profiles, C reactive protein, total protein, Albumin, Globulin, and glucose) and metabolic hormones (leptin, adiponectin, and insulin) in male rats fed a high fat diet. It was hypothesized that annatto tocotrienol might mitigate the negative metabolic effects of a high fat diet, such as hormones and lipid profiles.

## Materials And Methods

### Experimental animals

The present study was carried out at the laboratory animal house in the College of Veterinary Medicine, University of Basrah. Eighteen male rats weighting  $80 \pm 25$  gm and

aged (2 months) were used for the current study. The animals were kept for one week for acclimatization before the beginning of the experiments. All the experimental animals were maintained under optimum conditions ( $24 \pm 2$  °C) and a 12/12-hour light/dark cycle throughout the study. The food and drinking water were administered ad libitum throughout the experimental period. Annatto tocotrienol (70%), delta-tocotrienol (90%), and gamma-tocotrienol (10%) produced by American River Nutrition Inc. (Hadley, MA, USA) were applied to prepare a dose of 60 mg per kg (18), weigh 85.71 mg from tocotrienol, and be diluted with olive oil to 1 ml. The substance was given orally to rats to rats by a gavage needle.

#### **High fat diet and protocol of the study**

A total of eighteen adult male rats were divided into three groups randomly. 1<sup>st</sup> group respected as Control group was given a diet low in fat. (low fat (LF) 10 % kcal from fat) D14031901 which included carbohydrate (67%), fat (4%), protein (19%). 2<sup>nd</sup> group High fat diet (HFD) was fed with HFD (HF 60 % kcal from fat) D14031902 which have carbohydrate (26%), fat (35%), protein (26%) for 12 weeks. The 3<sup>rd</sup> group was fed with High Food Diet plus tocotrienol (HFDT) HFD Tocotrienol (HFDT) was fed with HFD plus tocotrienol 60 mg/kg dissolved in olive oil (1ml/kg) (18).

#### **Blood sample preparation**

Rats were starved for 12–14 hours following the treatment period. Male rats were anaesthetized with exhaled diethyl ether (1.9%), and the jar's capacity was approximately 0.08 mL/L (19). Blood samples (10 ml) were collected from each rat from a cardiac puncture by using a disposable syringe (10 ml). The samples were put into a gel tube (8 ml) and centrifuged at 3000 rpm for 15 minutes for serum separation into clear and non-hemolyzed supernatant, then divided into four parts to store in polyethylene

Eppendorf tubes at  $-20$  ° C for further serological analysis (20).

#### **Biochemical assays**

The Cobas C 311 an analyzer is an independent instrument used to obtain clinical chemistry profiles from rats' serum. This analyzer can determine the levels of some biochemical parameters using ion selective electrodes (ISE). Several biochemical markers have been studied and quantified including triglycerides and cholesterol, total protein, albumin, glucose, C reactive protein, high-density lipoprotein (HDL), low-density lipoprotein (VLDL), and low-density lipoprotein (LDL).

#### **Hormonal assay**

Plasma rat Leptin, adiponectin and Insulin concentrations (ng/ml) were measured using Rat Elisa kit from Bioassay Technology Laboratory in China (Leptin Cat. No. E0561Ra), and Insulin (Cat. No. E0707Ra). Also, adiponectin was measured using the Rat Adiponectin Elisa kit from Bioassay Technology Laboratory in China (Cat. No. E0758Ra).

#### **Statistical analysis**

The data of the current studies have been analyzed using univalent analysis of variance (ANOVA) in the computerized SPSS (Statistical Packages for the Social Sciences) V.23 program. The threshold of significance was set at  $P < 0.05$ . The data was presented in the form of mean  $\pm$  standard error. The least significant difference (LSD) test was used to compare groups.

### **Results**

The effects of tocotrienol supplementation on serum lipid profiles are presented in table (1). In comparison to other groups in the experiment, rats on a high-fat diet had considerably higher blood triglyceride and VLDL concentrations. Additionally, tocotrienol supplementation resulted in a significant increase in HDL-C when compared to animals fed a high-fat diet and

control groups ( $P \leq 0.05$ ). In contrast to the other groups, the high-fat diet plus tocotrienols group's level of LDL was considerably lower after tocotrienol supplementation ( $P \leq 0.05$ ).

According to Table (2)'s results, male rats on high-fat diets had blood levels of total protein that were noticeably higher than those of the control and high-fat diet plus tocotrienols groups ( $P \leq 0.05$ ). The high-fat diet with the tocotrienols group, showed a decrease in globulin levels ( $P \leq 0.05$ ) in contrast to the other groups. The groups also showed significantly higher levels of glucose and C-reactive protein than the control groups. ( $P \leq 0.05$ ). The effects of tocotrienol on insulin, adiponectin, and leptin are displayed

in Table 3. The study results indicate a statistically significant increase in leptin hormone levels in the high-fat diet and high-fat diet plus tocotrienols groups as compared to the control group. The high-fat diet plus tocotrienols group did not differ significantly from the HFD group ( $P \leq 0.05$ ). Furthermore, compared to the control group, the HFD and high-fat diet plus tocotrienols groups had significantly higher levels of Adiponectin ( $P \leq 0.05$ ). The HFD group had higher insulin hormone levels than the control group ( $P \leq 0.05$ ), while the high-fat diet plus tocotrienols group had lower insulin hormone levels than the HFD group.

**Table 1: Effect of tocotrienols supplement on lipid profile of male rats fed a high-fat diet (Mean ± SE) n= 6**

Groups	cholesterol mg/dl	TG mg/dl	HDL-C mg/dl	LDL-C mg/dl	VLDL-C mg/dl
control	59.833 ±1.3	28.666±3.7b	20.500±1.83b	13.466±.96a	5.500±.76 b
HFD	66.166 ±7.25	80.333±2.6a	26.166±2.797b	10.833±1.71a	15.933±.494a
HFDT	64.166 ±5.30	33.166±3.9b	38.500±3.59a	4.333±.49 b	6.666±.80 b
Significant	N.S	*	*	*	*

Values with different letters in the column are significantly different at ( $P \leq 0.05$ ). N.S.=not significant. \* = significant. HFD= High fat diet, HFDT= High fat diet plus tocotrienols.

**Table 2: Effect of tocotrienols supplement on total protein, Albumin, Globulin, C reactive protein and glucose in rats fed a high-fat diet . (Mean ± ED ) n= 6**

Groups	Tprotein mg/dl	Albumin mg/dl	Globulin mg/dl	CRP mg/l	Glucose mg/dl
control	54.166±.9b	32.166±.872	22.166±.60a	.580±.009b	133.166±6.08b
HFD	58.833±1.72a	34.333±.881	24.500±1.23a	1.296±.21a	230.333±34.50a
HFDT	51.500±1.17b	31.833±1.16	18.833±.47b	1.066±.11a	219.500±25.13a
Significant	*	N.S	*	*	*

Values with different letters in the column are significantly different at ( $P \leq 0.05$ ). N.S.=not significant. \* = significant. HFD= High fat diet, HFDT= High fat diet plus tocotrienols.

**Table3: Effect of tocotrienols supplement on Leptin, Adiponectin and Insulin of male rats fed a high-fat diet. (Mean ± SE ) n= 6**

Groups	Leptin (ng/ml)	Adiponectin (ng/ml)	Insulin (ng/ml)
control	1.9024±.17 b	7.0373±.63 b	8.0588±.48 b
HFD	3.3969±.21 a	11.1500±.39 a	10.2923±.44 a
HFDT	3.1307±.23 a	11.7568±.47 a	8.7488±.28 b
significant	*	*	*

Values with different letters in the column are significantly different at ( $P \leq 0.05$ ). \* = significant. HFD= High fat diet, HFDT= High fat diet plus tocotrienols.

## Discussion

In the present study, the high fat diet group had significantly higher serum triglycerides compared to other groups (Table 1). In previous investigations, high fat intake raised the amounts of fat in enterocytes (21), which may alter the expression of nutrient transporters and impede intracellular lipid synthesis. The current study's findings were consistent with those of Allen et al., who showed the HF-fed mice had higher amounts of triglyceride droplets in the liver and higher serum triglycerides compared to other groups (22). The increased concentration of triglycerides in rats fed a high-fat diet may be due to decreased triglyceride clearance as a result of decreased lipoprotein lipase activity (23). In addition, our findings are in agreement with Garcia et al., who found that rats given a high-fat diet had no change in their cholesterol levels (24). However, this finding disagreement with previous study's who discovered that the concentration of cholesterol in rats fed a high-fat diet increased when compared to normal healthy control rats (25-27 ). The difference in effect may have been due to the difference in the percentage of fat added to the diet and the duration of the intervention.

Hypertriglyceridemia, one of the diagnostic criteria for the metabolic syndrome, appears to be present in rats fed the high-fat diet. The overproduction of triglyceride-rich very low-density lipoproteins (VLDL) as a consequence of increased liver-derived free fatty acid flow (caused by an increase in adipose tissue mass) may be the cause of elevated circulating triglyceride levels (28). Hypertriglyceridemia is another indicator of insulin resistance (28). deterioration of glucose tolerance, which is linked to insulin's inability to promote the uptake and metabolism of glucose by tissues that are insulin-sensitive. There is evidence linking a high-fat diet to an increased number of lipid droplets in hepatocytes. By incorporating fats into bile and producing VLDL, or very low-density lipoproteins, the liver regulates lipid metabolism (29). However, dyslipidemia, which manifests as elevated blood triglycerides, low HDL levels, LDL-C, and excess VLDL synthesis along with a blockage in their release, increases the risk of hepatic fat accumulation, ultimately resulting in non-alcoholic fatty liver disease (NAFLD) (30).

The use of tocotrienols improved triglyceride levels compared to the HFD group. Tocotrienols, which are significant food

nutrients, have been shown to alter several metabolic syndrome characteristics, including blood pressure, blood glucose levels, and lipid profiles (31). Instead of treating each risk factor separately with the higher risks of polypharmacy, treating the metabolic syndrome with tocotrienols may improve its various manifestations, such as obesity, insulin resistance, and cardiovascular disease (32). T3 regulates the expression of genes and proteins for fatty acid synthase FASN, carnitine palmitoyltransferase 1 (CPT1A), and cytochrome P450 3A4 (CYP3A4) thereby reducing the accumulation of TG (33). a theory that suggests that dietary T3's capacity to lower TG production in human hepatoma cells (HepG2) may be the main advantage (34). Our results of the current study were in agreement with Allen et al.'s research, which it was shown that tocotrienols reduced hepatic steatosis and serum triglycerides were observed in  $\delta$ T3-supplemented groups compared to the HF group (22).

Liver function parameters like total protein, albumin, globulin, and albumin to globulin are linked to liver's function, including transporting anions, removing substances, and hepatic synthetic function (35). Lower amounts of serum albumin would suggest liver impairment (36). The current study's results indicated that the high-fat diet group had higher total protein levels than the control group and that this increase was caused by an increase in globulin levels, which in turn led to higher total protein levels. Additionally, tocotrienols decreased the high-fat diet plus tocotrienols group's total protein levels when compared to the HFD, suggesting a mitigating effect of the high-fat diet. Our study somewhat concurs with the Ghasi and colleagues' study, which showed that eating foods high in fat increased total protein and caused a significant decrease in albumin (37).

As a result of the various biological roles that proteins play, including controlling food intake and body weight, acting as a satiety signal, and aiding in the transport of fats, it is anticipated that protein production will rise in tandem with rising fat consumption and body fat levels (37). This may be due to the type of fat used in the high-fat food (38). The high level of total protein in our current study in the high-fat diet group to induce obesity was inconsistent with Shawky study, which indicated serum protein levels were decreased in HFD fed rats (39) and he indicated that the reason for the decline may be the depletion in the protein levels might be due to localized damage in the endoplasmic reticulum or hazard effect of energy which liberated through the metabolism of HFD (40).

According to the Framingham study, elevated serum proteins are a factor in the development of health disorders in overweight and obese individuals (41). According to Marques et al., serum albumin levels decreased during HF feeding as negative acute phase protein called albumin may be decreased in inflammatory diseases like obesity (42). It was inconsistent with current study Perhaps because our study is a chronic study that spanned 8 and 12 weeks in protective. The use of tocotrienols in our current study led to a reduction in total protein levels, and this was inconsistent with the Lin and his Colleagues study which showed no significant difference was noted between TRF and placebo interventions on total protein, albumin, globulin, total bilirubin levels and the ratio of albumin to globulin (43). Our findings are in line with those of Jayusman et al., who demonstrated that 28 male Sprague-Dawley rats were given TRF (200 mg/kg) 30 minutes before receiving fenitrothion FNT (20 mg/kg) orally for 28 days in a row. The results demonstrated that TRF administration significantly reduced the

total protein level when compared to the group of rats given fenitrothion (44)

CRP is an acute-phase sensitive test for systemic inflammation, infection, and tissue damage (45,46). CRP levels are typically high in those who have overweight, obesity (45), metabolic syndrome (46), overt type 2 diabetes T2D and insulin resistance as co-occurring symptoms (47). Adipose tissue is thought to have a role in moderating the link between elevated CRP and various diseases, while the physiological processes underlying this relationship are still unknown (48). However, because CRP is a part of the nonspecific acute-phase response to many disorders, it cannot be utilized to diagnose illness on its own (49). The results of the current study showed an increase in levels of CRP in the HFD. group compared to the control group, and that tocotrienols led to a decrease in levels of CRP, However, it was not significant when compared with the HFD group. Tocotrienols have better anti-CRP effects than tocopherol. Tocotrienols inhibit the production of inflammatory mediators, tocotrienol being the most effective (50). It has been demonstrated that consuming grape seed oil alongside high tocotrienols levels reduces serum level of high-sensitivity C-reactive protein hs- CRP. The current study showed that glucose levels increased in the HFD group compared to the control group and that the use of tocotrienols as a protective factor from high-fat food led to a decrease in glucose levels. However, this decrease was non-significant compared to the HFD group, and the use of tocotrienols improved the level of insulin when compared with the HFD group. This result agreed with Patel et al. (51), who showed that tocotrienols enhance metabolic functions such as insulin sensitivity and glucose utilization. Reduced plasma triglycerides and non-esterified fatty acids

might be the outcome of this. Since insulin improves glucose uptake and glycolysis, shifts energy production from primarily oxidizing fat to primarily utilizing carbohydrates, suppresses hormone-sensitive lipase, and up-regulates lipoprotein lipase (52), and maintains a constant rate of free fatty acid re-esterification, it is a potent suppressor of circulating non-esterified fatty acid (NEFA) concentrations (53). Adipose tissue secretes polypeptide hormones such as adiponectin and leptin, which contribute to the progression of obesity-related illnesses such as hypertension, atherosclerosis, and type 2 diabetes mellitus T2DM (54). While adipocytes release the majority of leptin, levels of this hormone grow in obesity; yet, at the hypothalamic level, leptin has the opposite effect, increasing energy expenditure and decreasing food intake (55). According to the present study's findings, in the protective experiment, leptin levels were higher in the diet-induced obesity group than in the control group. This might be seen as an effort to overcome leptin action resistance, which can worsen obesity and hyperphagia (42). Additionally, the content of plasma leptin increases proportionately with body fat (56). An imbalance between increased calorie intake and decreased energy expenditure results in obesity. This imbalance can be caused, among other things, by an excessive consumption of saturated fatty acids (57). Leptin levels are higher in thin individuals than in obese individuals (58). However, obese people have a high level of circulated leptin due to leptin resistance (59). The development of the brain, immune function, reproduction, bone density, hemodynamics, respiratory function, sympathetic nerve activity, and insulin levels in the liver are all influenced by leptin, which has receptors in both peripheral tissues and the brain (60).

These results were inconsistent with the Rocha-Rodrigues et al. study, which showed that HFD (71 kcal% fat) over 17 weeks increased epididymal adipose tissue leptin levels but did not change plasma leptin (61).

At the end of the study, animals fed a high-fat diet had higher adiponectin plasma levels. This is in line with previous research that found that rats treated with an HF diet for 24 and 32 weeks experienced increases in Adiponectin plasma levels (62, 63). Adiponectin is known to have an insulin-sensitizing effect, but obesity has been associated with a malfunction in Adiponectin signaling, or Adiponectin resistance (64).

Our current study also showed that the use of tocotrienols reduced the levels of leptin and increased adiponectin, but was not significant in comparison with the diet-induced obese group, and this was consistent with what Kok-Yong found: a study showed that, in male rats receiving buserelin for a 12-week period of therapy, oral administration of annatto tocotrienol at 60 or 100 mg/kg had no effect on levels of adiponectin or leptin (65). Although another investigation found that delta tocotrienol reduced leptin protein content in the tocotrienol group at dose T400 whereas not in the tocotrienol group at dose T1600 groups when compared to high-fat-fed mice, there were no differences in serum levels of the anti-inflammatory adipokine adiponectin between any of the groups (22). In addition, T3 supplementation (600 mg/day) for six months improved liver enzymes, inflammatory markers, oxidative stress, leptin, FLI, and hepatic steatosis significantly more than placebo (66). The difference in the effect of tocotrienols may be due to the different duration of the intervention. We concluded that tocotrienols improve lipid profiles and have anti-glycemic effects.

**Conflict of interest:** All authors declare that there is no conflict of interest.

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## تأثير التوكوترينول المشتق من أناتو على مستوى الدهون وبعض هرمونات الأديبوكين في الجرذان التي تتغذى على نظام غذائي عالي الدهون

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### الخلاصة

السمنة هي حالة استقلابية تؤدي إلى إصابة الناس بمجموعة متنوعة من الأمراض، وقد برزت باعتبارها مشكلة صحية عامة عالمية خطيرة. يهدف هذا البحث إلى التأكد مما إذا كان أناتو توكوترينول يمكنه تحسين بعض المؤشرات البيوكيميائية والهرمونات الأيضية لدى ذكور الجرذان التي تتغذى على نظام غذائي غني بالدهون. تم تقسيم ثمانية عشر جرذًا ذكرًا بالغًا إلى ثلاث مجموعات عشوائية (6 لكل مجموعة). تم إعطاء المجموعة الضابطة نظامًا غذائيًا منخفض الدهون (LF 10%) سعر حراري من الدهون، وتم تغذية مجموعة الغذاء عالي الدهون (HFD) بنظام غذائي عالي الدهون (HF 60%)، وتم تغذية مجموعة الغذاء عالي الدهون مع توكوترينول (HFDT) تمت تغذيته بمضاف توكوترينول الغذائي عالي الدهون (60 ملجم / كجم) المذاب في زيت الزيتون (1 مل / كجم) لمدة 12 أسبوعًا. أدى العلاج بالتوكوترينول إلى انخفاض كبير في إجمالي البروتين والجلوبيولين مقارنة مع مجموعة النظام الغذائي عالي الدهون وزيادة كبيرة في HDL-C مقارنة مع الفئران التي تغذت على نظام غذائي عالي الدهون ومجموعات المراقبة. في حين أن التوكوترينول قلل بشكل كبير من مستوى LDL وهرمون الأنسولين في المجموعة التي تتبع نظام غذائي عالي الدهون بالإضافة إلى التوكوترينول مقارنة بالمجموعات الأخرى.

**الكلمات المفتاحية:** توكوترينول، نظام غذائي عالي الدهون، اللبتين، أديبونيكتين.