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Effect of tocotrienol administration on reproductive efficiency and testicular tissue in obesity induced male rats

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ABSTRACT

Obesity is a major public health problem for all age groups across the world. Diet, rather than hereditary alterations, is thought to have a significant role in the obesity pandemic. Obesity can considerably harm male reproductive health, resulting in lower libido, erectile dysfunction, and subfertility or infertility. Tocotrienols (T3s) are among the compounds that have metabolic effects due to their nutritional properties as a food supplement. The study aimed to identify the role of tocotrienol in ameliorating the risks of a high-fat diet on some reproductive hormones and efficiency. The eighteen male adult rats were randomly assigned to three separate sets of six each. The control group was fed a low-fat diet (LF ten percent kcal from fat), the high-fat diet (HFD) group was fed a high-fat diet (HF sixty percent kcal from fat), and the high-fat diet with tocotrienol (HFDT) group was fed a high-fat diet administered tocotrienol (60 mg/kg BW) dissolved in olive oil (1 mL/kg) for 12 weeks. LH hormone showed a significant decrease in concentration for the HFD and HFDT groups when compared with the control group. Tocotrienol supplement introduced its ability to improve motility and account for dead and abnormal sperm values. The histopathological examination of testes in rats fed a high-fat diet and supplemented with tocotrienol revealed normal spermatogenesis. The tocotrienol supplement ameliorated the deleterious effect of obesity in the experiment and appeared significantly less than the control group.

Keywords: tocotrienols, high fat diet, reproductive

Introduction

Obesity has emerged as a major global health problem, with its prevalence rapidly growing among a huge section of the world's population in recent years [1]. According to the researchers, one-third of the world's population is already overweight or obese, and by 2025, the worldwide obesity prevalence will be 18% in men and more than 21% in women [2]. Obesity is a complicated illness with various etiologies, each with its own set of severe symptoms and complications [3,4]. High-fat diets are commonly used to induce obesity in animals [5,6] because they generate harmful metabolism effects, implying that diet is a major contributor to the obesity epidemic [7]. Obese males may experience diminished libido, erectile dysfunction, subfertility, and, in rare cases, hypogonadism [8]. Although research has shown that the function of adipose tissue in several neuro-endocrine networks has advanced recently, the pathogenetic pathways that connect excessive fat accumulation to hypothalamic-pituitary-gonadal HPG dysfunction are still not completely understood. Moreover, increased sex steroid metabolism in adipose tissue depots might result in aberrant androgen and estrogen plasma levels, which may alter the reproductive axis in obesity [9]. The previous researches have been focused on the effects of obesity on the reproductive health of

women. It is also conceivable that the harmful effects of obesity on male fertility have been overestimated [10]. Tocopherols and tocotrienols are two groups of physiologically active compounds found in vitamin E. Each class has four isoforms: alpha (α), beta (β), gamma (γ), and delta (δ), each with a distinct biological function [11,12]. Tocotrienols (T3s) are among the chemicals with metabolic effects owing to their nutritional value as a dietary supplement. It is widely regarded as safe at low dosages for pathophysiology relief in animal models and upcoming human studies [13]. Lee et al. [14] found a substantial increase in sperm motility, viability, and count in groups given a high-dose (1500 mg/kg tocotrienol) therapy for 42 days (6 weeks), although testes weights were not significantly impacted by any treatment. A study found that administering palm tocotrienol-rich fraction (TRF), which contains approximately 60% tocotrienol, for 7 days in male rats treated with corticosterone (CORT), increased reproductive organ weight and testosterone levels. This suggests that TRF can prevent testicular germ cell degeneration and Leydig cell loss during stress [15]. The research linked to looked at the effects of plant parts high in tocotrienols and made up of a combination of tocotrienol isomers as well as tocopherol. Tocotrienol generated from annatto (*Bixa orellana*) bean is unusual as it mostly comprises δ-tocotrienol (about 90%) and γ-tocotrienol (approximately 10%), with the lack of α-tocopherol [16].

As a result, the purpose of this study is to focus on the negative effects of obesity on reproduction and to determine the function of tocotrienol generated from annatto in mitigating obesity risks on some reproductive hormones and efficiency.

Materials and Methods

Experiment Animals and Study Design

The study was conducted at the College of Veterinary Medicine, University of Basrah. The animals were acclimatized in the animal house one week prior to the experiment and were kept under optimal conditions (24±2°C) and a light/dark cycle (12/12 hours) throughout the study. Food and drinking water are provided ad libitum during the study period. Obesity induction in laboratory rats was carried out using diet-induced obesity (DIO) in rodents (HF 60% fat) D14031902, whereas for the control group (LF 10% fat) D14031901 was created according to Research Diet Inc. The experiment involved 18 male rats weighing 80±25 grams and aged 2 months. They were randomly separated into three groups as following:

1. Group 1 (Control group): six rats were fed a low-fat diet and drench with olive oil 1 ml/ kg B.W. by gavage for 12 weeks.

2. Group 2 (high fat diet group HFD): six rats were fed a high-fat diet and drench with an oral dose of olive oil 1 ml/kg by gavage for 12 weeks.

3. Group 3 (protective group HFDT): six rats were fed a high-fat diet and drench with an oral dose of 60 mg/kg according to [17] of tocotrienol supplement dissolved in olive oil (1ml/kg) by gavage for 12 weeks.

Blood sample preparation

At the end of the study, rats were fasted for 12-14 hours after the treatment period. The rats were anesthetized with 1.9% inhaled diethyl ether, which has a container that can hold 0.08 mL/L [18]. Samples of blood (3 mL) were obtained from each rat's heart (cardiac puncture) and centrifuged at 3000 rpm for 15 minutes to separate serum. The levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone were measured using bt-laboratory kits. (Catalog numbers: EA0015Ra for FSH, EA0013Ra for LH, and EA0023Ra for testosterone).

Sperm analysis

In the present study, we assessed sperm count according to Evans and Maxwell (1987), whereas sperm motility was assessed according to Chemineau et al. [19]. The aberrant spermatozoa percentage was determined on the same slide as the viability of epididymal sperm, employing 200 sperm under a light microscope at 100X power [20].

Bioassay for histopathology

The testes were carefully removed, washed with normal saline, and preserved in 10% buffered formalin for 24 hours.

The specimens were dehydrated using a graded series of ethanol, cleaned with two changes of xylene, and then embedded in paraffin wax. Sections with a thickness of 5μm were cut using a rotary microtome and put on clean slides for histological evaluation. The sections were stained with Hematoxylin and Eosin (H&E) and evaluated under a light microscope [21].

Statistical analysis

The current studies' data were analyzed with univalent analysis of variance (ANOVA) in the computerized SPSS (Statistical Packages for the Social Sciences) V.23 program. P<0.05 was considered statistically significant. The data was reported as mean ± standard error to compare groups, the least significant difference (LSD) test was used.

Results

The effect of obesity on reproductive hormones and role of tocotrienol in improving the concentrations represented by table (1). The results investigated there was no significant effect of

obesity on testosterone and FSH hormones concentration among the studied groups when tocotrienol added together with feed of animals with high fat diet, except for LH hormone that showed significant decrease in their concentration for HFD and HFDT groups when compared with control group.

Table (1) Effect of tocotrienols administration on some sex hormones of male rats in experiment. (Mean ± SE)

Groups	Testosterone (ng/ml)	FSH (mlU/ml)	LH (mlU/ml)
control	0.94±0.14	0.73±0.05	2.14±0.23 a
HFD	0.90±0.15	0.60±0.02	1.25±0.05 b
HFDT	0.94±.14	0.69±0.05	1.48±0.13 b
Significant	N.S	N.S	*

The letter indicates significant variation at (P≤0.05). N.S.=not significant. star = significant. HFD: high fat diet, HFDT: high fat diet plus tocotrienols

At the end of the experiments, sperm viability was estimation in the scarified rats of experiment. As showed in table (2) sperm account and motility recorded sever significant reduction in their values for the rat's induced obesity feed on high fat diet, tocotrienol supplement introduced its ability to improve motility and account of sperm values. In contrast, dead and abnormal sperms in table (2) investigated the deleterious effect of obesity on sperm viability, by recorded high significant percent for dead and abnormal sperms when compared with all studied groups. While tocotrienol supplement ameliorated the deleterious effect of obesity in experiment and appeared significantly less than control group.

Table (2) Effect of tocotrienols administration on seminal analysis in experiment

Groups	Sperm motility %	Sperm account x 10^6	dead sperm	abnormal sperm
control	83% ±1.35 a	178.66±1.49 a	7%±0.73 d	13% ±1.35 b
HFD	61% ±2.27 d	157.16±2.03 c	14%±0.87 a	28%±1.75 a
HFDT	76% ±2.08 b	179.33±4.34 a	9%±0.30 c	17%±1.80 b

Significant	*	*	*	*

The letter indicates significant variation at (P≤0.05). N.S.=not significant. star = significant. HFD: high fat diet ,HFDT: high fat diet plus tocotrienols.

Histopathological changes in the testes

The microscopic finding of testes in control rat showed normal structure of seminiferous tubules and spermatogenesis and supporting cells that arranged in the lining of testes (figure 1). The rats fed high fat diet exhibited histological changes in testis represented by suppression of spermatogenesis and vacuolation of seminiferous tubules (figure 2). The histopathological examination of testes in rats fed a high fat diet and supplemented with tocotrienol revealed normal spermatogenesis (figure 3).

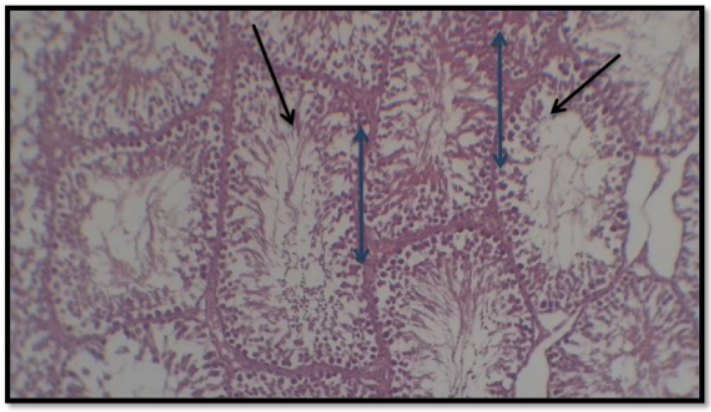

Figure (1): Histological micrographs of the control group's testis parenchyma revealed normal architecture, including normal seminiferous tubules (black arrow) and normal spermatogenesis (blue arrow). H & E stain. 100X

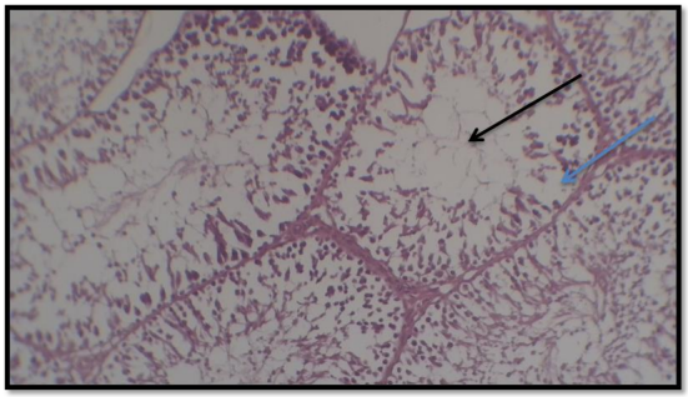

Figure (2) A histological micrograph of the HFD group's testis with inhibition of spermatogenesis (black arrows) and vacuolation of seminiferous tubules (blue arrow). H & E stain. 100X

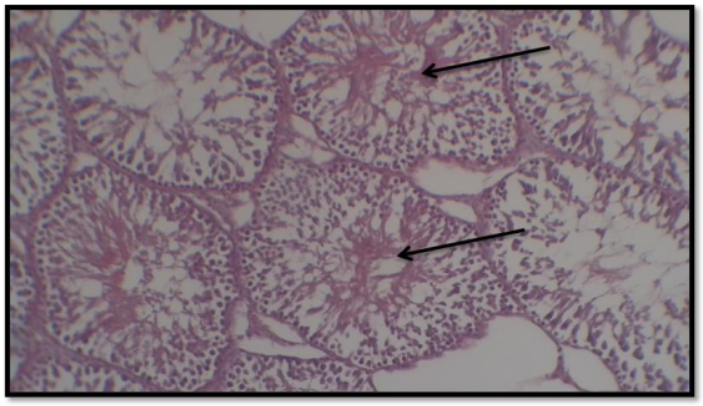

Figure (3): Histological micrograph of testis of HFDT group normal spermatogenesis (black arrows). H&E stain. 100X

Discussion

This study employed a high-fat diet-induced obesity model. Regarding the effects of HFD-induced obesity on levels of reproductive hormones in male rats, serum LH considerably reduced in the HFD and HFDT groups compared to the control group, and no significant changes in the serum testosterone and FSH levels were identified across the three groups, although reduce they value in in HFD and HFDT groups but these change non-significant. the impact of obesity on the LH hormone may be resulted from several mechanisms, including increased estrogen production in excess fat [22] , which can affect the balance of sex hormones in the body. In addition, obesity-related inflammation can affect the hormonal system [23] and reduce the secretion of LH hormone. Tocotrienols are antioxidants that may help reproductive health, though their impact on FSH and LH levels are not completely understood. A study on female mice found that δ-tocotrienol supplementation increased FSH and LH levels, suggesting a potential for improved hormonal regulation in females [24]. In this study, tocotrienol increased LH hormone levels, although the difference was not statistically significant when compared to the high-fat diet group. In this study, testosterone hormone levels were not significantly different between the HFD and control groups. The results of the current investigation were consistent with the findings [25]. Male rats given a high fat diet for 6 weeks exhibited no significant change in serum testosterone levels between HFD animals and control animals, but there was a substantial rise in MDA levels and a significant drop in SOD in the HFD group when compared to the control. The current study disagreed with Vigueras-Villaseor *et al*.,

[26], who reported that Sprague-Dawley rats given HFD from birth to 90 days had decreased testosterone levels. Also disagreed with Bakos et al. [27], who discovered that male mice on HFD (for 9 weeks) had lower testosterone levels than the control group.

Current investigation revealed that sperm account and motility recorded a severe and significant reduction in their values for rats induced obesity. This was consistent with Ghanayem et al. [28] and Bakos et al. [27], which found that mice given HFD had worse sperm motility, fertilization rate, and pregnancy rate. This impact may be due to increased damage to the DNA of sperm and reactive oxygen species within cells [28]. Obesity can cause oxidative stress and increase testicular oxidative stress [25]. And this result may be due to a high-fat diet and elevated testicular malondialdehyde (MDA) levels [29].

Tocotrienol supplement introduced its ability to improve motility and account for sperm values. Vitamin E treatment has a protective effect against oxidative stress and testicular damage [30]. In this study dead and abnormal sperms in table (2) investigated the deleterious effect of obesity on viability of sperm, by recorded high significant percent for dead and abnormal sperms when compared with all studied groups, and this finding agreed with [31], who found It seemed that the high diet cholesterol rats group have reduced sperm concentration, motility, dead and abnormal sperm compared with control values. While tocotrienol supplement ameliorated the deleterious effect of obesity and this finding agreed with Jegede et al. [32], who discovered that Red Palm Oil RPO has the ability to decrease the harmful effect of lead on testicular cells, preventing probable male infertility. Also, this resulted was consistent with a previous study examining the effects of vitamin E who showed that vitamin E is able to compensate the toxic effects of p-NP on testis weight, sperm number, sperm motility and estrogen level, and increases sperm viability in developing rat [33].

In the current study, the high fat diet suffered from suppression of spermatogenesis and vacuolation of seminiferous tubules as a result of the harmful effects of obesity which may be resulting from increased the amount of oxidative stress in the reproductive system, which is stress brought on by an increase in the number of molecules containing free oxygen as well as Malondialdehyde (MDA) concentrations in testes were elevated by a high-fat diet [29]. This result agreement with [34], who showed that constant ingestion of excessive dietary fat in rats may create observable changes in rat testes, reducing sperm function and leading to reproductive issues.

In our study, the histological micrograph of the testis of the HFDT group showed normal spermatogenesis which indicates the protective roles that tocotrienols play against the damage caused by a high-fat diet. This was consistent with the study of Taib and his colleagues, which showed that

palm oil tocotrienol-rich fraction (TRF) has potential to reduce oxidative stress under various pathological conditions, such as exposure to organophosphates (fenitrothion), which have been reported to cause testicular oxidative damage [35].

Conclusion

LH hormone concentration decrease in high fat diet and high fat diet plus tocotrienol. Administration of tocotrienol introduce its ability to improve motility and count for dead and abnormal sperm values. Histopathological examination of testes in rat fed diet high in fat and administrated with tocotrienol revealed normal spermatogenesis. The tocotrienol administration ameliorated the deleterious effect of obesity in experiment end appeared significantly less than the control group.

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