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# Assessment of vitamins D and E acute supplementation on testosterone levels in male rats

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**Abstract**

**Background and objective:** In recent years, vitamin D has been considered an interesting subject of study due to its pleiotropic role including autocrine, paracrine and endocrine function in several target organs and systems. The essential molecule for reproduction in animals and it have an antioxidant properties in all mammalian cells is known vitamin E. This study aimed the study the effects of acute supplementation of both vitamins D and E on developing of testosterone levels and reproductive properties in rats.

**Methods:** Forty rats were divided into four groups, 1st group were feeding by acute supplementation with vitamin D while 2nd group supplementation of vitamin E, 3rd group feeding with acute supplementation of both vitamins, and 4th group was control with normal fed. HPLC technique was used to assessing of vitamins D and E levels while levels of TES were investigated by ELISA.

**Results:** The levels of vitamins D (ng/ml) levels in this experiment (after month of supplementation) of study groups (VDS, VES, VDES, and Control) were 7.27±2.1 ,3.15±1.1, 6.19±0.9, 5.11±1.4, respectively, while vitamin E (ng/ml) levels were 4.13±1.2, 6.4±1.3 ,3.15±1.1, 4.17±1.3, respectively, and the p-value was < 0.05 when comparing between all groups. TES levels (pg/ml) were 13.40±3.9, 16.04±2.6, 17.18±3.5, 11.17±2.7 in study groups and the p-value also <0.05 and significant statistical.

**Conclusion:** Combination of both vitamins D and E as acute supplementation will increase TES levels more than vitamin D or E alone in male rats.

**Keywords:** Vitamin D, vitamins E, Acute supplementation, Testosterone, Rats

## INTRODUCTION

The main activity of vitamin D, belonging to secosteroids group, is the regulation of both calcium and phosphorus homeostasis, and this leads to promoting bone mineralization [1]. The main principal target organs of vitamin D are intestine, skeletal system, kidneys, and parathyroid glands [2]. Therefore, vitamin D plays multiple biological effects on each one of these organs. *Angelis et al., 2017* suggested that the key role of vitamin D in male reproductive system has been suggested, since the expression of VDR and vitamin D metabolizing enzymes was demonstrated in the testis and spermatozoa. Decreasing in levels of vitamin D has a negative impact on semen and hormone function, either in animals or in

humans [3]. An essential lipid-soluble vitamin in the human body is vitamin E. It plays multiple important physiological roles such as it is not only an effective antioxidant and immune-modulator [4]. Vitamin E is essential for reproduction in animals and it have an antioxidant present in all mammalian cells. Thus, *Li D et al., 2006* suggested that vitamin E was stimulated the releasing of both LH and FSH [5]. Therefore the combination of both vitamins, D and E in acute supplementations of rats may be give an reproductive role of this vitamins. The steroid hormone from the androgen group synthesized by the Leydig cells in the testes in males is called testosterone (TES), and also expression on the ovaries in females, and adrenal glands in both sexes. It exerts a wide-ranging influence over sexual behavior, muscle mass and strength, energy, cardiovascular health and bone integrity [6]. TES biosynthesis matches with the spermatogenesis and fetal Leydig cell separation in the male rodents. A few in vivo models including hormone-concealment, hormone-reclamation, and hypophysectomy were set up for the investigation of the hormonal guideline of spermatogenesis by TES [7]. The essential hormone for normal spermatogenesis is testosterone because it stimulates the conversion of round spermatids into elongated spermatids between stage VII and stage VIII of the spermatogenetic cycle [8]. Only a few researches have been conducted to investigate the effect of vitamins D and E on male reproductive function and so it is not quite clear what the effect of vitamins D and E on serum testosterone levels. The aim of this work to study the effects of acute supplementation of vitamins D and E on developing of testosterone levels and reproductive properties in rats.

**Ethical issues**

The study was approved by the institutional animal care and use committee of laboratory animals in veterinary medicine college, Al-Qasim green university.

## MATERIALS AND METHODS:

**Study design**

This study were included forty adult albino male wistar rats (218±27 g) aged (6±1.4 months) were purchased from specific animal house, Iraq. The animals were housed in plastic cages at 24 hours light/dark cycle, 29±1.9 ºC and feeding with standard commercial laboratory chew and water. The rats were randomly assigned into four groups, 1st group were vitamin D supplementation (0.5 mg/kg/day) (VDS), 2nd group were vitamin E supplementation (0.5 mg/kg/day) (VES), 3rd group were both vitamins D and E (0.5 mg/kg/day) (VDES), and 4th is control group was fed rat feed. The period of this experiment was one month and the blood samples collected weekly from all rats to measuring the parameters that included in this study.

**Measurement of serum Testosterone**

The levels of serum testosterone (TST) (pg/ml) were measured by Enzyme-linked immune-sorbant assay (ELISA) according to manufacture instructions. The concentration of TES (pg/ml) in the serum samples was determined by comparing the optical density (OD) of the samples to the standard curve (figure 1).

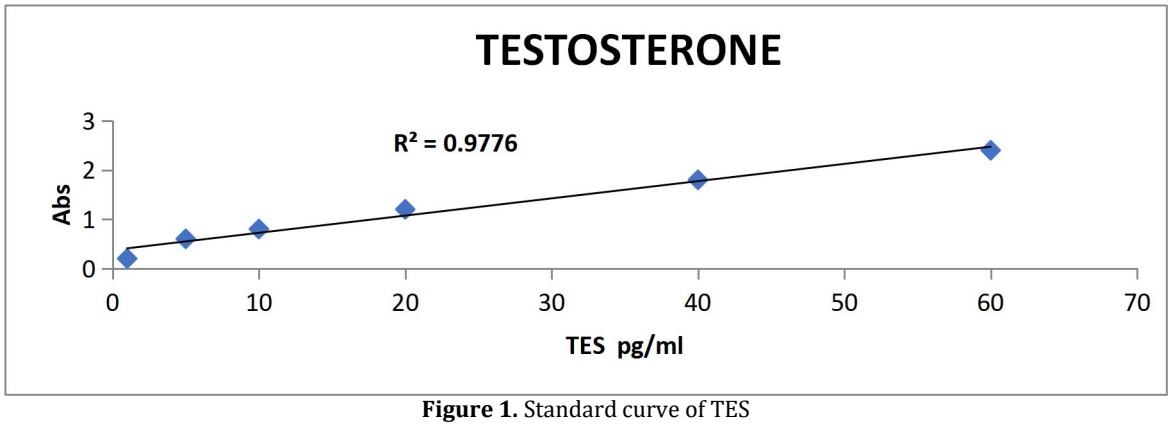


**Figure 1.** Standard curve of TES

**Measurement of serum vitamin D:**

Vitamin D was estimated by high performance liquid chromatography (HPLC). The conditions of HPLC for estimation of vitamin D were:

Stationary phase: C18 column, 5µm, 4.6 ×150mm, mobile phase: water: acetonitrile (30:70) (pH 4.9 ammonium acetate solution), flow Rate: 1.5 mL/min, and detection Type: UV at λ= 273 nm. An auto-sampler injection system were used in this method.

**Measurement of serum vitamin E:**

Vitamin E was estimated by high performance liquid chromatography (HPLC). The conditions of HPLC for estimation of vitamin E were:

Stationary phase: C18 column, 5µm, 4.6 ×150mm, mobile phase: water: acetonitrile (25:75) (pH 4.5 ammonium acetate solution), flow Rate: 1 mL/min, and detection Type: UV at λ= 290 nm. An auto-sampler injection system were used in this method.

**Statistical analysis:**

Results are expressed as mean±SD for ten rats per each group. ANOVA was used to assessing the statistical significance of the data ($p<0.05$) was considered a significant.

## RESULTS

Figure 2, showing the peaks area and retention time of vitamins D of serum in specific sample of VES and VDES groups:

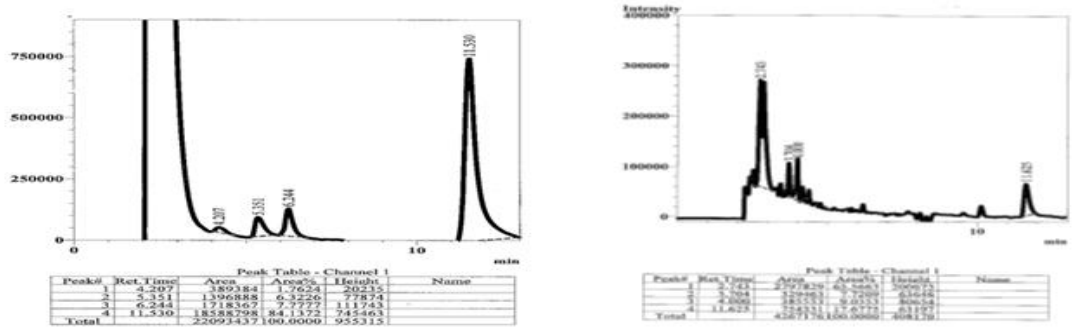

**Figure 2.** HPLC spectrum of sample curves of vitamins D peak area and retention time (RT) in appropriate sample of VDS and VDES groups

Figure 3, showing the peaks area and retention time of vitamin E of serum in specific sample of VES and VDES groups:

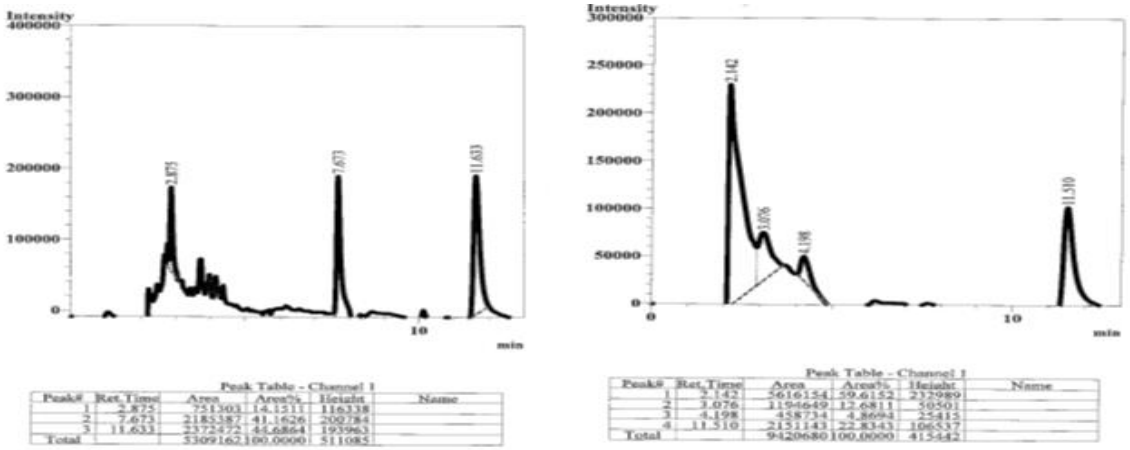

**Figure 3.** HPLC spectrum of sample curves of vitamins E, peak area and retention time (RT) in appropriate sample of VES and VDES groups

Table 1, showing the levels of vitamins D and E (ng/ml), and TES (pg/ml) levels in end of experiment (after month of supplementation) of study groups**:**

**Table 1.** Levels of vitamin D, E and TES levels in study groups

| *Groups* / *Parameters* | VDS N=10 | VES N=10 | VEDS N=10 | Control N=10 |
|---|---|---|---|---|
| D (ng/ml) | 7.27±2.1 | 3.15±1.1 | 6.19±0.9 | 5.11±1.4 |
| P-value= 0.0001 | | | | |
| E (ng/ml) | 4.13±1.2 | 6.4±1.3 | 3.15±1.1 | 4.17±1.3 |
| P-value=0.0001 | | | | |
| TES (pg/ml) | 13.40±3.9 | 16.04±2.6 | 17.18±3.5 | 11.17±2.7 |
| P-value= 0.00001 | | | | |

Table 2, shows the TES (pg/ml) status in VDS and VES groups at the end of this experiment:

**Table 2.** RF status in study groups

| **Groups** / **TST status** | **VES N=10** | **VDS N=10** | **OR(95% CI)** |
|---|---|---|---|
| <10 (pg/ml) | 4 | 7 | 0.285 (0.04 - 1.8) |
| ≥10 (pg/ml) | 6 | 3 | P-value=0.184 |

Figure 4, showing the positive correlation between D, E and TES in both TES and VES groups at the end of this study, respectively:

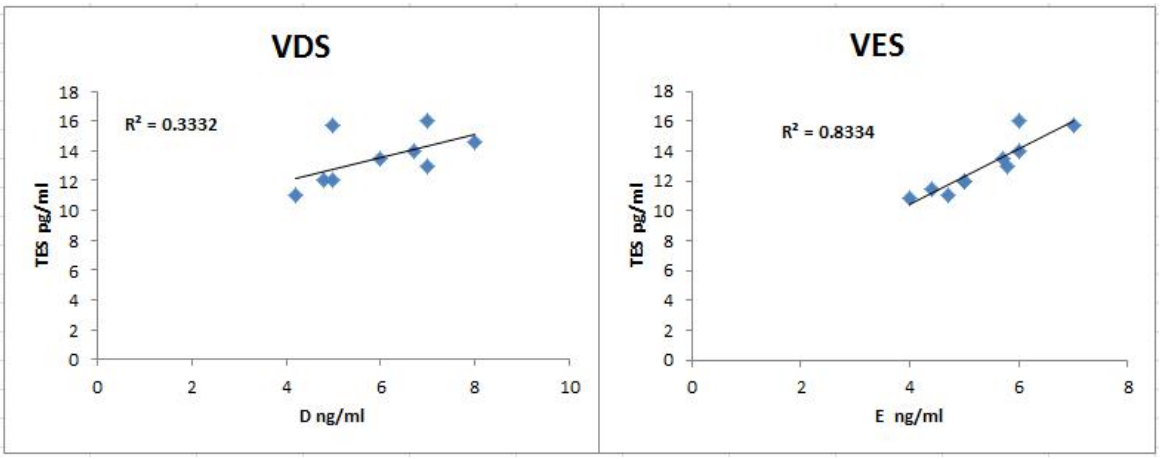


**Figure 4.** Correlations between vitamins D and E with TES levels in VDS and VES group at the end of experiment

Figure 5, suggested the TES levels pg/ml in the period of this study(weekly measured) in all study groups:

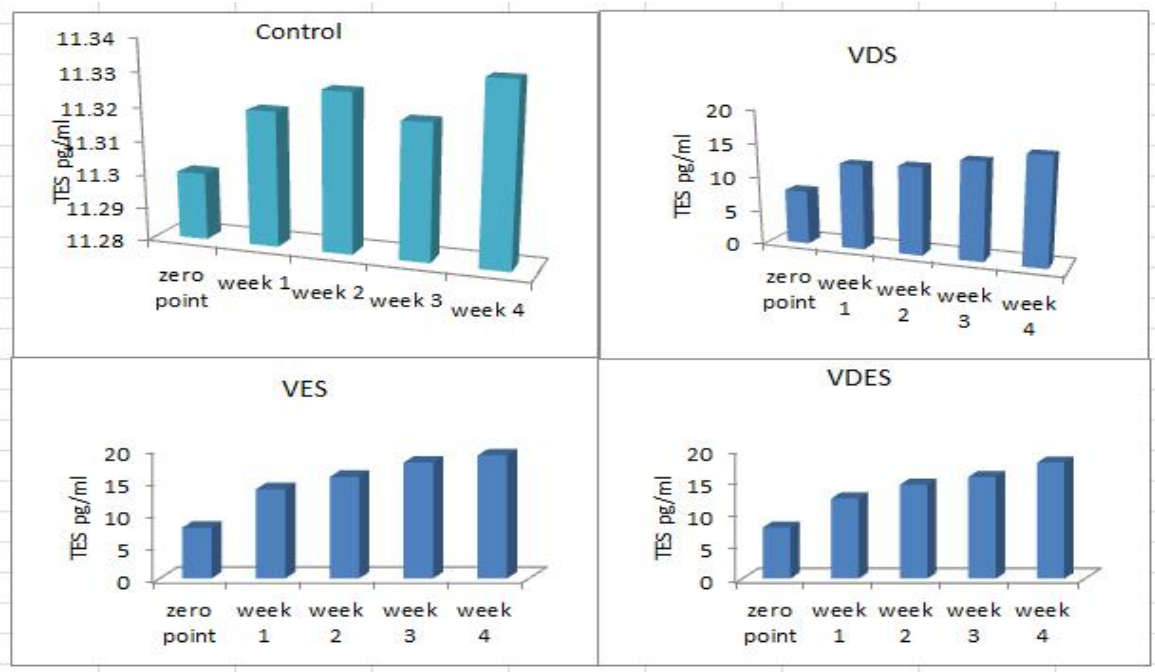


**Figure 5.** TES levels in the end of four weeks of experiment in all study groups

The results of present study suggesting that no statistical significant effects of acute supplementation of vitamins D and E alone or in combination status on weight of experiment rats in all period of study (p-value> 0.05), as showing in figure (6):

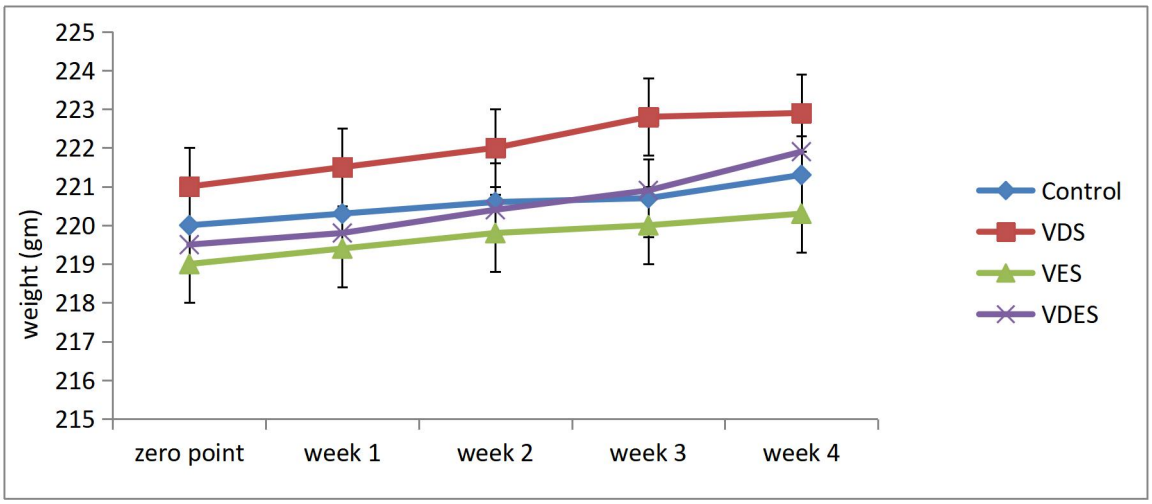

**Figure 6.** Effects of acute supplementation with D and E on weight (gm) of rats

## DISCUSSION

This study examined the adverse effect of acute supplementation of vitamins D and E on developing of testosterone levels and reproductive properties in rats. In this study we showed that vitamins D and E acute supplementation is associated with increasing of testosterone levels in male rats. In addition to human, the different rat examines have likewise recommended that decreased sperm tallies, motility, and diminished mating capacity are related to vitamins D and E inadequacy [9]. It has also not been investigated whether the vitamin D and E acute supplementation in the aged subject would affect the testosterone levels or not. Studies those says that vitamin D have an important role in the success rate of fertilization and suggested a strong correlation between follicular fluid and serum vitamin D levels [10,11]. This study suggested the association between vitamin D and increasing of serum testosterone is in same line with this studies on human [12-13], but not association by other study [14]. The main vitamin that has been reported to act as a free radical scavenger, preventing damage to the cell membrane by removing lipid peroxidation products and also by maintaining mitochondrial integrity and mediating generation of superoxide systems is vitamin E [15]. The present study showing the combination of both vitamins ply a critical role in reproductive status of male rats by stimulating TES levels. *Dennis et al., 2017* were reported that vitamin D have positive effects on the fertility of woman through the regulation of ovarian anti mullerian hormone levels [16]. As previously reviewed [17-20], several lines of evidence in both animals and humans suggest that vitamin D have main role in reproduction. *Sun et al., 2010* works on rodents and say that vitamin D deficiency may be associated with infertility and vitamin D receptor knockout mice exhibit impaired ovarian folliculogenesis and uterine hypoplasia [21]. *Anagnostis et al., 2013* were says that vitamin D can also stimulate steroidogenesis, folliculogenesis and implantation in animals [22]. The role of vitamin D in reproductive status may be due to high expression of its protein receptors on reproductive glands. *Ramlau-Hansen et al., 2011* were investigation that vitamin D receptors are present in human testis and human sperm cells and recently vitamin D–metabolizing enzymes have been found in human testis, the ejaculatory tract, and mature sperm cells, suggesting that vitamin D is important for spermatogenesis and maturation of sperm cells [23]. There was a significant correlation between baseline of vitamins D and E with baseline testosterone levels in VDS and VES groups, but the correlation is highly significant in VES compare to VDS group (p-vale<0.05, CI% 1.2-5.9). The results of present study suggested that vitamin E supplementation is more effects on TES levels than effects of vitamin D. The results of present study not showing any statistical significant effects of acute supplementation of vitamins D and E alone or in combination status on weight of experiment rats in all period of study (p-value> 0.05). The antioxidant activity of vitamins and other free radical scavenger were very important in treatment in may diseases [24-27]. Janssen et al., 2013 were founds a correlation between vitamin D and testosterone levels in human is not unexpected, as a healthy lifestyle is important for both vitamin D and testosterone levels [28,29]. Other study suggest that vitamin E may play an important and potent role in hormone production in the pituitary-gonadal axis in humans and rats [30]. This may be due to vitamin E acute supplementation make more antioxidant status and will increase the expression of TES receptors in reproductive cells compare the antioxidant activity of vitamin D and so, the expression of TES are low that occurs due to acute supplementation with vitamin D.

## CONCLUSION

TES levels in rats are in higher status after acute supplementation with both vitamins D and E compared to single supplementation and the weight not effects by this status.

**Conflict of interest**

Nil

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