# EVALUATION THE ROLE OF SUPPLEMENTATION OF VIT. D ON SOME PHYSIOLOGICAL PARAMETERS, SEMEN QUALITY IN HYPOVITAMINOSIS D MALE RABBITS INDUCED BY FUROSEMIDE

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(Received 8 August 2020 ,Accepted 28 September 2020).

**Keywords**: Vit. D., Parathyroid hormone, Semen quality.

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## ABSTRACT

This study was planned to assess the improvement effect of supplementation Vit.D on adult male rabbits with hypovitaminosis D was induced by put the male rabbits in dark room un exposure to sunlight, eaten restricted food for experiment period and administration of furosemide drug (0.5 mg/kg.B.W.). Vitamin D treated rabbit group was given Vitamin D. two doses (high and low doses).Thirty adult male rabbits of (1500-2000 gm) body weight were used in this study and animals were divided into five main groups, each of 6 rabbits. Control group treated with normal saline, second group the male rabbits put in dark room un exposure to sunlight, eaten restricted food and treated with furosemid 0.5mg/kg B.W. I.M for 16 days, third group the male rabbits put in dark room un exposure to sunlight, eaten restricted food and treated with 0.5 mg/Kg B.W. of furosemid I.M for 16 days +200000IU/2ml of Vit.D in every week for 1month, fourth group the male rabbits put in dark room un exposure to sunlight, eaten restricted food and treated with 0.5mg/Kg B.W.of furosemide I.M for 16 days+100000IU/1ml of Vit.D in every week for 1month, fifth group put the male rabbits in dark room un exposure to sunlight, eaten restricted food and treated with both 0.5 mg/kg B.W. of furosemid I.M for 16 days + exposure to sunlight for 5 hours in day until 1 month. The taken results exposed that hypovitaminosis D is associated by a significant ($P \leq 0.05$) decrease in body weight

and body weight gain as compared with control (-ve) group. Also the results indicate a significant (P≤0.05) decrease in serum Vitamin D, Calcium, total protein and testosterone concentrations compared with control(-ve) and another treated groups. A significant(P≤0.05) increase in serum concentrations of urea, creatinine, uric acid, Phosphate, Parathyroid hormone (PTH), Follicle stimulating hormone(FSH) and Luetinzing hormone(LH) have been shown in serum of hypovitaminosis D groups compared to control(-ve) group. Semen analysis showed a significant decrease (P≤0.05) of epididymal sperm concentration, sperm motility, and viability and a significant increase (P≤0.05) of sperm abnormalities were recorded in hpovitaminosis D groups compared with control(-ve) and another treated group. Also, histological examination on the parathyroid gland, kidneys and testis showed histological changes of hypovitaminosis D male rabbits treated with furosemide while when treated Vit.D groups and group exposure to sunlight showed alimentative in list previous parameters also histological examination.

## INTRODUCTION

Vit.D is a lipophil secosteroid that are naturally known for Ca-P and bone metabolism and pivotal target organs are intestine, skeletal system, renal and parathyroid glands (1). Vit.D lifes in two forms; ergocalciferol (vitamin $D_2$) and cholecalciferol (vitamin $D_3$) (2). These two forms differ in one double-binding and one methyl group. While Vit.$D_2$ is found in vegetable sources like mushrooms, Vit.$D_3$ can be originate of animal sources like fat fish, cod oil of liver, yolk of egg and fortified food like dairy products (2) or by endogenous skin synthesis that needs exposure of sunbeams. Vit.D supplements might contain both forms, although in Norway, vitamin $D_3$ is almost exclusively used in over-the-counter Vit.D supplements. Despite of its creating formation, pre-vitamin D suffers hydroxylation to 25-hydroxyvitamin D (25(OH)D) by 25-hydroxylase, a cytochromeP450-dependent enzyme.

In 21st century, Vit. D deficit has happened to a global trouble changing 1 billion people (3). Severe Vit.D insufficiency reasons osteomalacia or rickets, osteoporosis and bone breaks as of diminished calcium absorption (4). At the side of its found character in skeletal strength, little Vit.D grade is conferred as a danger aspect in next of kin to numerous non-skeletal health results for example CVD (5),

fatness (6), diabetes (7), asthma (8), manifold sclerosis (MS) (9), cancer diseases (10) and overall mortality (11, 12).

Diuretics are broadly applied in cardiovascular disease. Furosemide (Furo) influence on parathyroid hormone circulating (PTH) and whether such deeds are concluded by the results of these composites on Ca secretion (34). The aimed of this work was studying the physiologic effect of Vit.D on parathyroid gland activity and fertility of male through comparing the effect of deficiency and supplementation.

## MATERIAL AND METHODS

This study was performed on thirty male rabbits weighing (1500-2000gm). The rabbits were put in the animal house of College of Veterinary Medicine / University of Basrah.

Twenty four rabbits were induction hypovitaminosis D. This rabbits put in plastic cages in dark room un exposure to sunlight with restricted food for hypovitaminosis D and drinking *ad libitum* under 20-25°C controlled temperature condition.

Rabbits were divided into five main groups, each of 6 rabbits. Control(-ve) group treated with normal saline, second group control positive (+ve) male rabbits put in dark room un exposure to sunlight, eaten restricted food for experiment period and treated with furosemid 0.5mg/kg B.W. I.M for 16 days(induction hypovitaminosis D), and remain until end experiment, third group male rabbits put in dark room un exposure to sunlight, eaten restricted food and treated with 0.5 mg/Kg B.W. of furosemid I.M for 16 days +200000IU/2ml of Vit.D every week for 1moth, fourth group male rabbits put in dark room un exposure to sunlight, eaten restricted food and treated with 0.5mg/Kg B.W.of furosemide I.M for 16 days+100000IU/1ml of Vit.D every week for 1moth, fifth group male rabbits put in dark room un exposure to sunlight, eaten restricted food and treated with both 0.5 mg/kg B.W. of furosemid I.M for 16 days + exposure to sunlight for 5 hours in day until 1 month.

At the end of experiment, rabbits were collection blood from heart puncture for estimation studied parameters after that sacrificed, testis and epididymis were taken out for semen analysis. Epididymal sperm were counted as illustrated by (13). The individual and massive epididymal sperm motility was then approximation as described by (14). Abnormal and dead sperms percentage were then records in the

stained slide by eosin- nigrosin stain as illustrated by (13).Statistical Analysis was carried out by one-way covariance (ANOVA) test. By SPSS program V. 21.(15).

## RESULT

**1-Effect of Supplementation of Vit.D on Body Weight and Body Weight gain in Hypovitaminosis D Male Rabbits.**

The current study in Table (1) discovered a significant increase (P≤0.05) in final body weight in group drench 100000IU/1ml of Vit. D than control(+ve) while the results illustrated no significant changes (P>0.05) final body weight of group drench 200000IU/2ml of Vit.D. and exposure to sunlight than control(-ve) and control(+ve).

The results of body weight gain that demonstrated a significant (P≤0.05) high in group drench 100000IU/1ml of Vit. D than another groups but no significant changes (P>0.05) of body weight gain in male rabbits treated with 100000IU/1ml of Vit. D than control (-ve).

**Table(1)**Effect of supplementation of Vit.D on Body Weight and Body Weight gain in Hypovitaminosis D Male Rabbits. Mean±SD N=6

| Parameters / Treatments | Intial Body Weight (g) | Final Body Weight (g) | Body Weight Gain (g) |
|---|---|---|---|
| Control(-ve) (Normal saline 0.9%NaCl) | ١٦٣٠.00±١١٣.66 a | 1816.70±132.91 ab | 186.67±67.72 a |
| Control(+ve) Hypovitaminosis D By Furosemide | 1686.70±146.24 a | 1753.30±146.65 b | 66.66±28.04 c |
| Hypovitaminosis D+ 200000IU/2ml of Vit.D | 1726.70±121.92 a | 1848.30±121.55 ab | 121.67±25.62 b |
| Hypovitaminosis D+ 100000IU/1ml of Vit.D | 1700.00±187.08 a | 1948.30±179.49 a | 198.33±31.88 a |
| Hypovitaminosis D+ Exposure of Sunlight | 1683.30±194.07 a | 1793.20±177.40 ab | 109.83±50.32 bc |
| LSD | NS | 195.00 | 55.00 |

Different letters indicate differences among groups at (P≤0.05) level vs. control. NS=Non significant.

**2-Effect of Supplementation of Vit.D on Urea, Creatinin, Uric acid and Total protein in Hypovitaminosis D Male Rabbits.**

The results in Table (2) indicate a significant (P≤0.05) raise in serum urea, creatinine, uric acid levels of hypovitaminosis male rabbits treated with furosemide (control (+ve)) group as contrast with the control (-ve) group and another treated groups. While no significant (P>0.05) differences in the group serum urea, creatinine, uric acid concentrations of male rabbits treated with 200000IU/2ml of Vit.D, 100000IU/1ml of Vit.D and group exposure to sunlight.On other hand, a significant (P≤0.05) decrease in serum total protein concentration of hypovitaminosis male rabbits treated with furosemide (control (+ve)) as compared with the control (-ve) and treated with200000IU/2ml of Vit.D. But no significant (P>0.05) differences in serum total protein concentration of hypovitaminosis male rabbits treated with furosemide (control (+ve)) as contrast with of male rabbits treated with 100000IU/1ml of Vit.D and exposure to sunlight.

**Table (2):** Effect of Supplementation of Vit.D on Urea, Creatinin, Uric acid and Total protein in Hypovitaminosis D Male Rabbits.(Mean±SD) (N=6)

| Parameters / Treatments | Urea mg/dl | Creatinine mg/dl | Uric acid mg/dl | Total Protein g/dl |
|---|---|---|---|---|
| Control(-ve) (Normal saline 0.9%NaCl) | 35±1.41 c | 0.29±، .03 b | 5.00±2.00 b | 6.00±0.83 a |
| Control(+ve) Hypovitaminosis D By Furosemide | 52.16±8.61 a | 5.66±0.87 a | 8.3±2.94 a | 4.00±0.89 bc |
| Hypovitaminosis D+ 200000IU/2ml of Vit.D | 36.50±2.88 bc | 0.37 ±، .051 b | 4.16±1.72 b | 6.41±0.73 a |
| Hypovitaminosis D+ 100000IU/1ml of Vit.D | 42.66±4.58 b | 0.51± 0.055 b | 4.66±0.81 b | 5.25±0.52 b |
| Hypovitaminosis D+ Exposure of Sunlight | 39.50 ±7.39 c | 0.59±0.077 b | 5.33±1.86 b | 4.50±1.04 b |
| LSD | 7.66 | 5.06 | 3 | 1.16 |

Different letters represent differences among groups at (P≤0.05) level vs. control

**3-Effect of Supplementation of Vit.D on Vit.D Ca, P and PTH Concentrations in Hypovitaminosis D Male Rabbits.**

The results of concentrations of Vit.D and Ca in male rabbits treated with furosemide alone are represented in the Table(3). The results indicate a significant ($P \leq 0.05$) decrease progressively in serum Vit.D and Ca concentrations of male rabbits treated with furosemide as control (+ve) than control (-ve) and another treated groups. While treated with furosemide led to significant ($P \leq 0.05$) elevate in serum PTH and P than control (-ve) and another treated.

**Table(3)**:Effect of Supplementation of Vit.D on Vit.D, PTH, Ca and P Concentrations in Hypovitaminosis D Male Rabbits. (Mean±SD) (N=6).

| Parameters / reatments | Vit. D ng/ml | PTH Pg/ml | Ca mg/ml | P mg/ml |
|---|---|---|---|---|
| Control(-ve) (Normal saline 0.9%NaCl) | 48.93±6.40 a | 5.68±0.11 b | 13.70±1.35 a | 5.73±0.56 b |
| Control(+ve) Hypovitaminosis D By Furosemide | 17.83±1.72b | 15.16± 2.22 a | 9.45±1.44 b | 10.98±0.03 a |
| Hypovitaminosis D+ 200000IU/2ml of Vit.D | 60.46±2.78 a | 6.93± 0.94 b | 13.28±2.04a | 5.30±0.13 b |
| Hypovitaminosis D+ 100000IU/1ml of Vit.D | 41.16±4.40 a | 6.66±0.57b | 13.28±1.98a | 5.76±0.88 b |
| Hypovitaminosis D+ Exposure of Sunlight | 33.01±6.38ab | 7.16±0.81 b | 12.25±2.31a | 6.63±1.67 b |
| LSD | 19.30 | 8 | 2.79 | 4.35 |

N=number of animals., different letters refer to differences among groups at ($P \leq 0.05$) level vs. control

**4-Effect of Supplementation of Vit.D on FSH, LH and Testosterone in Hypovitaminosis D Male Rabbits.**

The data in the table (4) showed a significant (P≤0.05) increase in the serum FSH concentration of hypovitaminosis D male rabbits (control(+ve)) than the control group(-ve) and another treated. However there were no significant(P>0.05) variations in serum FSH level among groups such as group treated 200000IU/2 ml of Vit. D, treated with 100000IU/1ml of Vit. D, group exposure to sunlight and control(-ve).

The results also showed prominent role of furosemide induction hypovitaminosis D in causing a significant (P≤0.05) increase in serum LH concentration as compared to the control group(-ve) and another treated except group drench 100000IU/1ml of Vit.D(low dose). While, significant (P≤0.05) decline in LH concentration were observed in control(-ve) group than another treated groups.

Moreover, a significant (P≤0.05) decrease in serum T concentration was recorded in control(+ve) hypovitaminosis D as compared to the control(-ve) and group treated 200000IU/2ml of Vit. D(high dose).While no significant (P>0.05) change in serum T concentration observed as compared to control (+ve) and group treated 100000IU/1ml of Vit.D and group exposure to sun light.

**Table (4):** Effect of Supplementation of Vit.D on FSH, LH and Testosterone in Hypovitaminosis D Male Rabbits. (Mean±SD) (N=6)

| Parameters / Treatment | FSH (mlU/ml) | LH (mlU/ml) | Testosterone(T) ( ng/ml) |
|---|---|---|---|
| Control(-ve) (Normal saline 0.9%NaCl) | ٥.٥٠±٢.٤٢ b | 3.30±1.35 d | 9.80±1.30 a |
| Control(+ve) Hypovitaminosis D By Furosemide | ١١.٨٣±1.94 a | 11.08±1.42 a | 5.57 ±1.95 b |
| Hypovitaminosis D+ 200000IU/2ml of Vit.D | 6.34 ±1.75 b | 6.05±1.41 b | 8.44±2.06 a |

| | | | |
|---|---|---|---|
| **Hypovitaminosis D+ 100000IU/1ml of Vit.D** | **5.30±٢.٠٥٠ b** | **3.45±1.02 a** | **6.00±1.78 b** |
| **Hypovitaminosis D+ Exposure of Sunlight** | **٦.٠٨±1.93 b** | **٥.٥٣ ±1.93 c** | **6.50±1.64 b** |
| **LSD** | **٥.48** | **2.07** | **2.36** |

Different letters indicate differences among groups at (P≤0.05) level vs. control,

**5-Effect of Supplementation of Vit.D on Physical Properties of Semen Analysis in Hypovitaminosis D Male Rabbits.**

The current study in Table (4-11) illstrutled a significant raise (P≤0.05) in mass activities, concentrations of sperm, total sperm and live sperm of male treated with 200000IU/2mland 100000IU/1ml of Vit.D as than to control (-ve) and another treated groups. However significant decrease (P≤0.05) were noted in semen volume**,** mass activities, concentration of sperm, total sperm and live sperm of male treated with than to control (-ve) and another treated groups.

There are a significant decrease (P≤0.05) in sperm abnormalities of male treated with 200000IU/2ml of Vit.D than to control (-ve) and another treated . However, significant increase (P≤0.05) were observed in sperm abnormalities of hypovitaminosis D male rabbits than control(-ve)group and another treated groups.

**Table(5):** Effect of Supplementation of Vit.D on Physical Properties of Semen Analysis in Hypovitaminosis D Male Rabbits. (Mean ±SD) (N=6)

| Groups / Parameters | Control(-ve) (Normal saline 0.9%NaCl) | Control(+ve) Hypovitaminosis D By Furosemide | Hypovitaminosis D+ 200000IU/2 ml of Vit.D | Hypovitaminosis D+ 100000IU/1 ml of Vit.D | Hypovitaminosis D+ Exposure of Sunlight | D |
|---|---|---|---|---|---|---|
| **Semen colour** | **Creamy** | **Creamy** | **Creamy** | **Creamy** | **Creamy** | **-** |
| **Mass activities** | ++++ | + | ++++ | +++ | ++ | - |
| **Sperm motility%** | **86.33 ±3.98 a** | **47.00± 5.05 d** | **76.50±2.25 b** | **69.66±3.44 c** | **66.66±6.25 c** | **6.83** |
| **Sperm concentration ($\times10^6$/ml)** | **118.33 ± 4.50 a** | **55.50±0.14 e** | **96.16±0.28 b** | **89.16 ± 0. 68 c** | **75.00±0.13 d** | **16** |
| **Live-dead sperm ratio** | **92.50:7.50 ±1.87 a** | **73.50:6.50 ±2.66 c** | **91.50:8.50 ±6.77 a** | **90.50:9.50 ±4.03 a** | **82.66:17.34 ±3.88 b** | **7.83** |
| **Sperm abnormalities** | **15.33±1.03 d** | **57.00±14.56 a** | **16.66±3.88 d** | **35.83±5.63 c** | **41.20±6.37 b** | **15.80** |

N=number of animals., different letters refer differences among groups at P≤0.05 vs.

## 6-Sperm Morphology

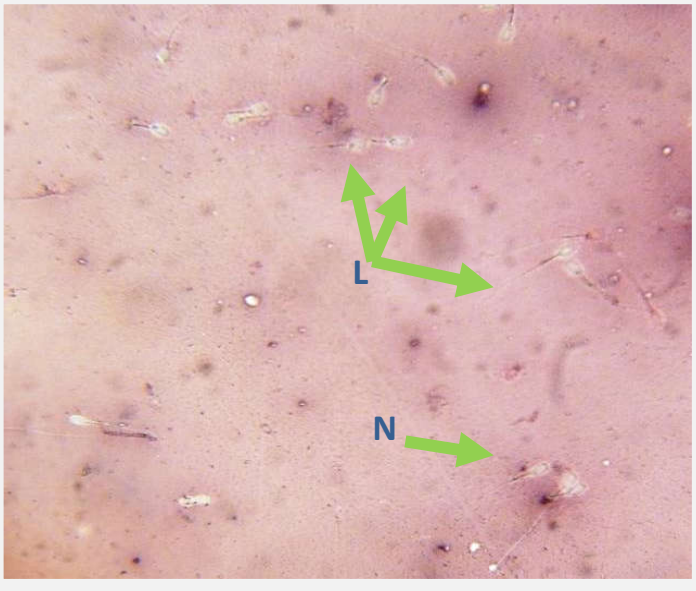



**Fig.(1):-**Sperm of rabbits (control(-ve)). Showing normal sperm(N), Live(L), Stain with(N&E) 100X.

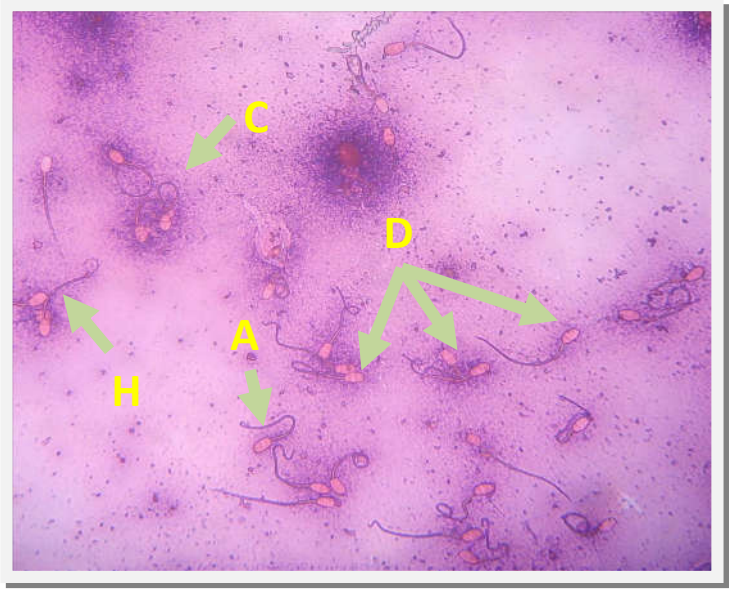



**Fig.(2):-** Sperm of rabbits (control(+ve)) treated with furosemide. Showing abnormal shape sperm(A), most sperms dead (D), some sperm tailless, many sperm with coiled tail (C), sperm presented with Double head(H), Stain with(N&E) 100X.

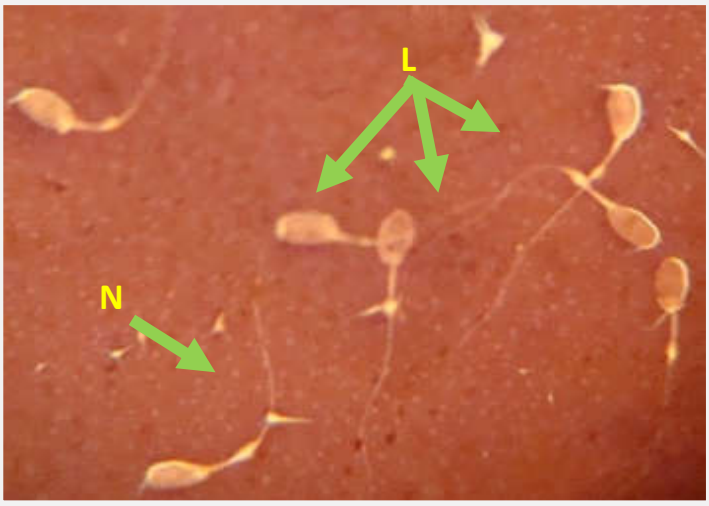



**Fig.(3):-**Sperm of rabbits treated with furosemide and 200000 IU/2ml (high dose) of Vit.D. Showing normal sperm(N), Live(L), Stain with(N&E) 400X.

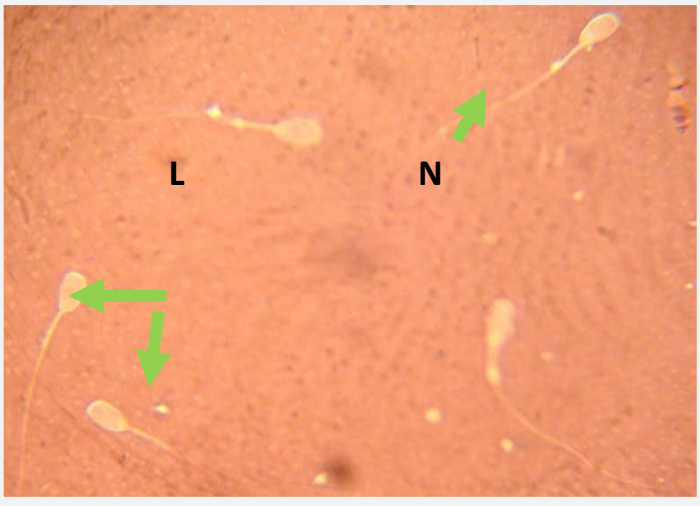



**Fig.(4):-**Sperm of rabbits treated with furosemide and 100000 IU/1ml (low dose) 0f Vit.D. Showing normal sperm(N), Live(L), Stain with(N&E) 400X.

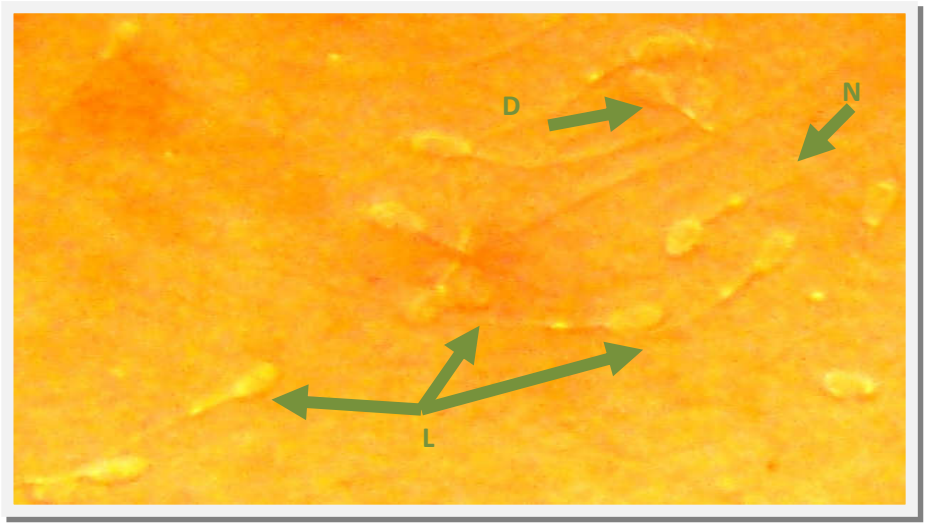



**Fig.(5)**:-Sperm of rabbits treated withfurosemide and exposure to sunlight. Showing normal sperm(N), Live(L) and some sperm dead, Stain with(N&E)400X.

**7-Histological Examination:**

Parathyroid Gland: Section of the parathyroid gland of male rabbits (control group) (-ve).normal structure of parathyroid gland. There are two types of cells within the parathyroid gland, the **chief cells** and the **oxyphil cells** (dark and light cells) as shown in figure (6). While section of the parathyroid gland of male rabbits (control group)(+ve) treated with furosemide induced hypovitaminosis D shows hyperactivity with normal limits of structure as shown in figure (7). Also another groups treated with Vit.D 200000 IU/2ml, 100000 IU/1ml and exposure to sunlight showed normal parathyroid gland structure as shown as in figure (8),(9) and (10).

Kidneys: As shown in figures (11) kidneys of male rabbits of control (-ve) group showed normal architecture of kidneys, normal glomeruli, normal renal cortical tubules and normal epithelial cells lining of the renal tubules. While kidneys of male rabbits treated with furosemide (control +ve) revealed histological changes as shown in figure(12). The changes included infiltrations of inflammatory cells, congestion of renal vascular, glomerular congestion. But kidneys of male rabbits treated with 200000 IU/2ml of Vit.D (high dose) showed normal architecture as shown in figures (13). In adding, the renal of rabbits treated with 100000 IU/1ml of Vit.D(low dose) revealed hemorrhage in the renal pelvis as shown in figures (14). Also the kidneys of rabbits exposure to sunlight revealed interstitial medullary hemorrhage in figure(15).

Testes: Section of testis of control (-ve)group rabbits showing normal structural design of seminiferous tubules as shown in figure(16).

While section of testis of rabbits treated with furosemide alone for induced hypovitaminosis. Showing vacuolation of spermatognia and suppression of spermatogenesis, widening of inter seminiferous tubules, decrease in the number of interstitial leydig cells as shown in figure (17). But, section of testis of male rabbits treated with Vit.D. 200000 IU/2ml (high dose). Showing normal of spermatogenesis in seminiferous tubuled as shown in figure(18).

Also section of testis of rabbits treated with Vit.D. 100000 IU/1ml (low dose) showing normal seminiferous tubules and suppression of spermatogenesis as shown in figure(19). Also section of testis of male rabbits group exposure to sunlight, showing normal seminiferous tubules, marked suppression of spermatogenesis as shown in figure(20).

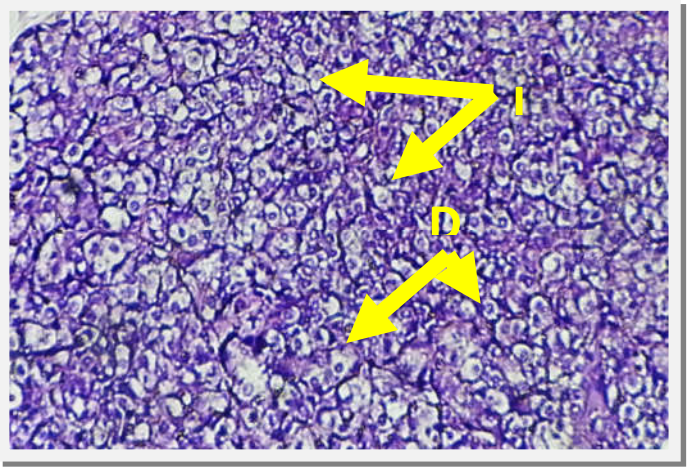



**Fig.(6):**Section of Parathyroid gland of male rabbits control (-ve) groups reveal normal parathyroid gland normal structure of chief and oxyphil cells (dark(D) and light(L) cells. Stain (H&E) 400X.

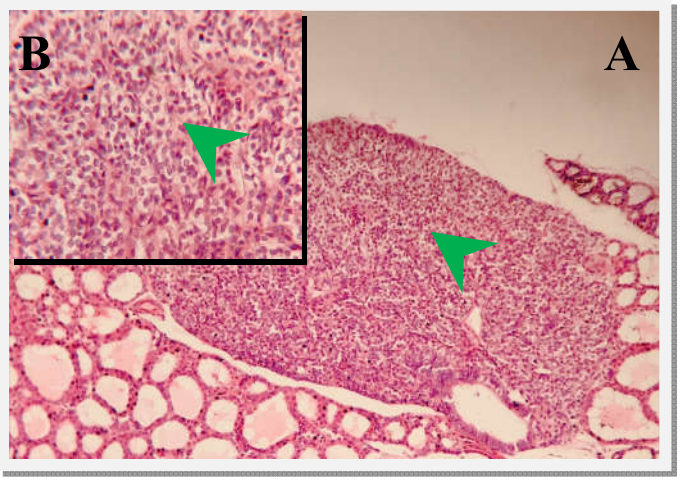



**Fig (7)**: Section of Parathyroid gland of male rabbits control (+ve) groups shows appears hyperactivity (greenarrow).H&EA)125X B) 500X

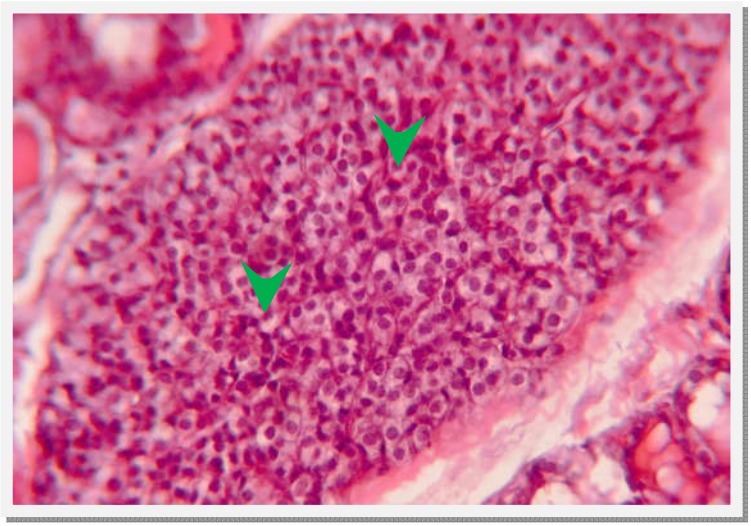


**Fig.(8):**Section of Parathyroid gland of male rabbits with 200000IU/2ml of Vit. D.(high dose), reveal normal parathyroid (green arrow). A) 500X H&E

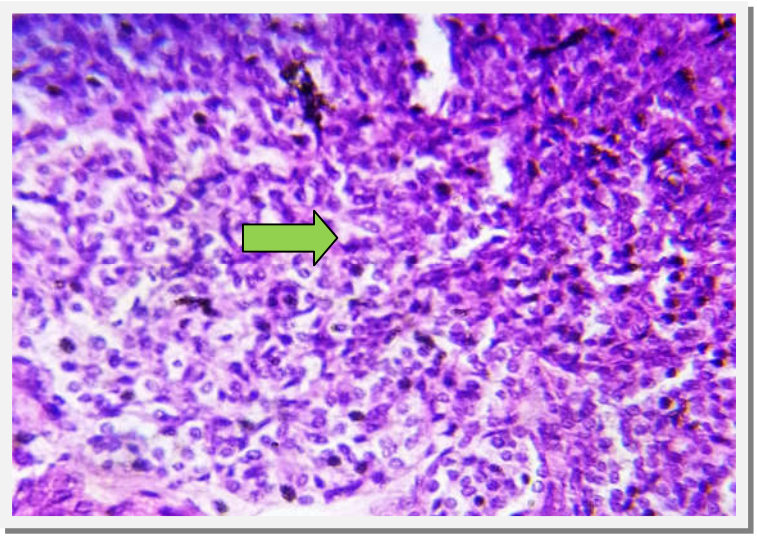


**Fig (9)**:Section of Parathyroid gland of male rabbits treated with 100000 IU/1ml of Vit. D.(low dose) shows appears within normal limits (green arrow). H&E A)125X B)500X

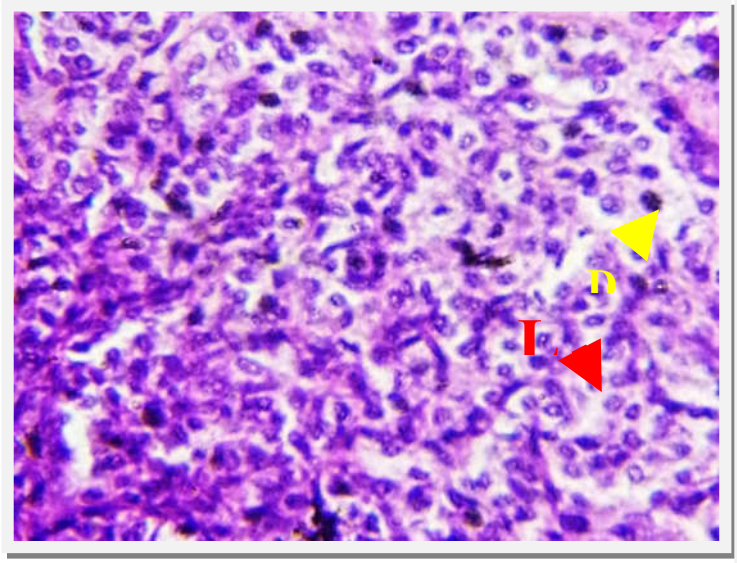
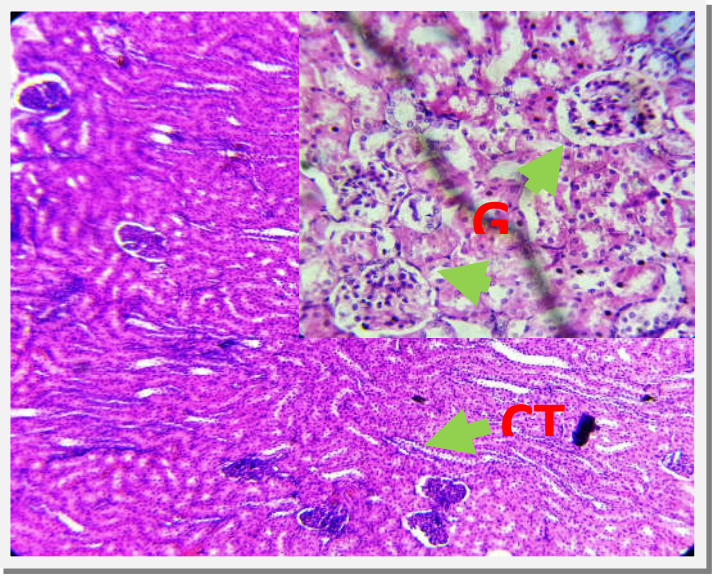



**Fig.(10):**Section of Parathyroid gland of male rabbits exposure to sunlight shows appears within normal contain chief and oxyphil cells (dark(D) and light(L) cells) hyperactivity limits (green arrow).H&EA)125X B)500X




**Fig(11):**Section of kidney of male rabbits control (-ve). Showing normal glomeruli (G) and normal renal cortical tubules (CT), stain (H&E).A)125X B)500X

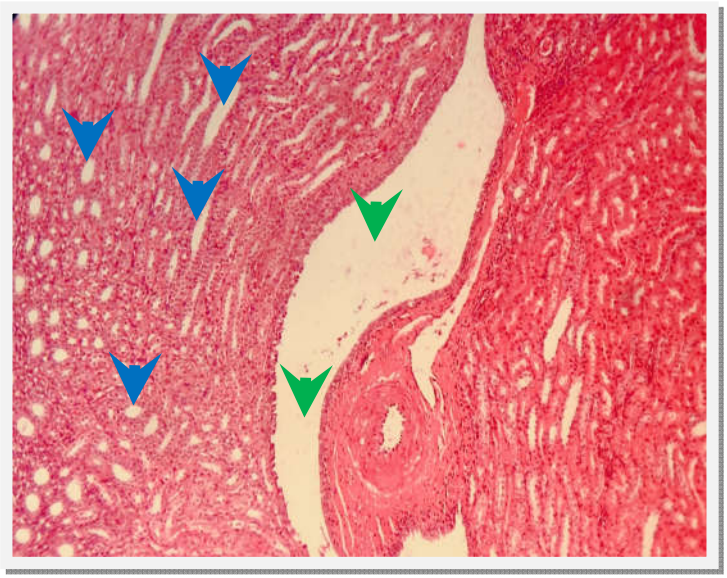


**Fig. (12):** Section of kidney of male rabbits treated with 0.2ml of Vit.D (high does), reveal normal collecting tubules in the medulla (blue arrow), normal renal pelvis (green arrow).125 X H&E

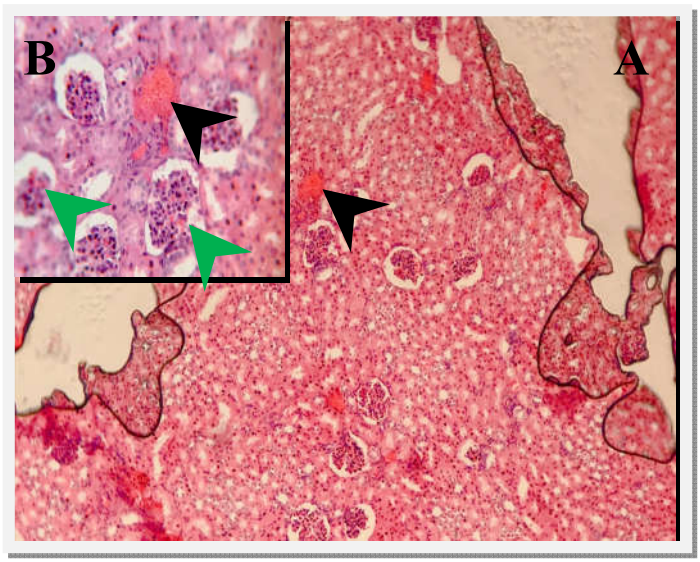



**Fig.(13):** Section of kidney of control(+ve) group shows glomerular congestion (green arrow), renal vascular congestion (black arrow). A)50 XB) 500X

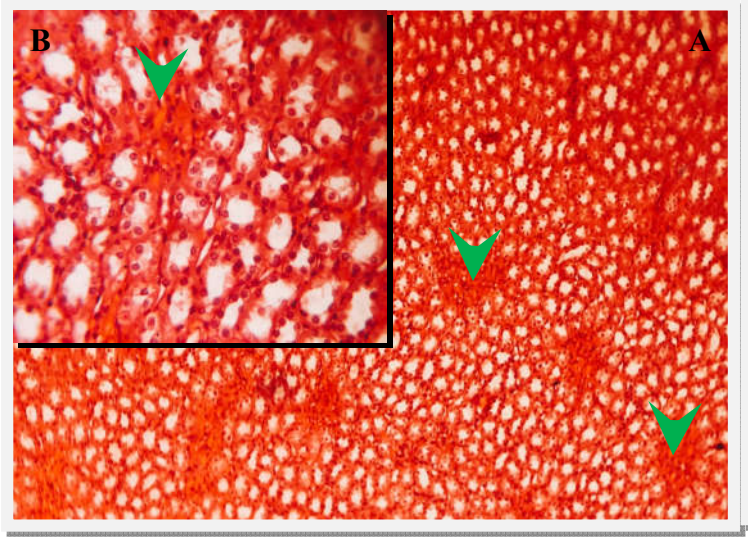



**Fig (14)** Section of kidney of male rabbits exposure to sun light treated group, reveal interstitial medullary hemorrhage (green arrow). A) 125 X B) 500 X H&E

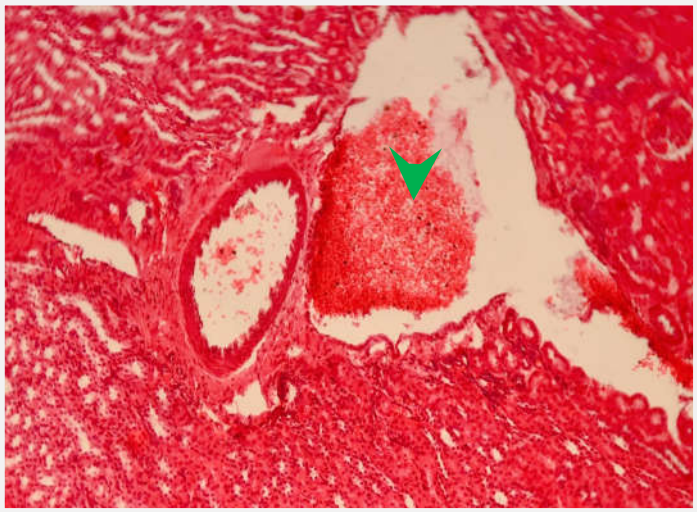


**Fig. (15):** Section of kidney of male rabbits treated with 0.1ml of Vit.D (low does), reveal hemorrhage in the renal pelvis (green arrow). A) 125 X H&E

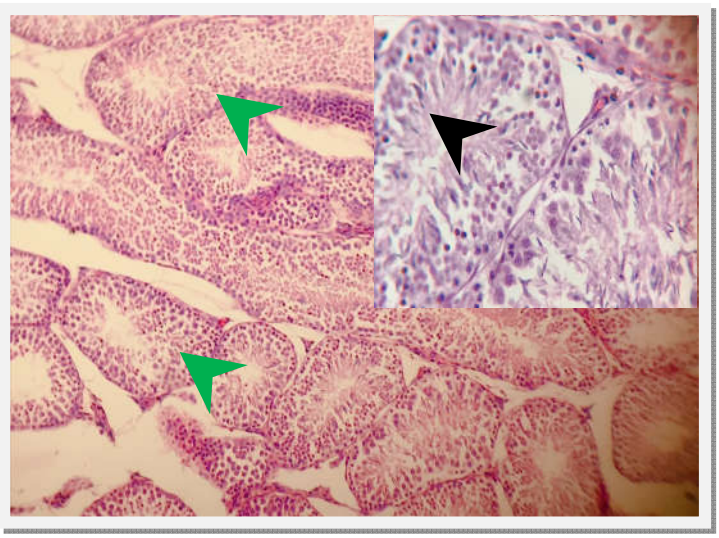


**Fig.(16)**:Section of testes of male rabbits of control (-ve) group shows normal seminiferous tubules (green arrow), normal spermatozoa (black arrow). H&E A) 125X B)500X

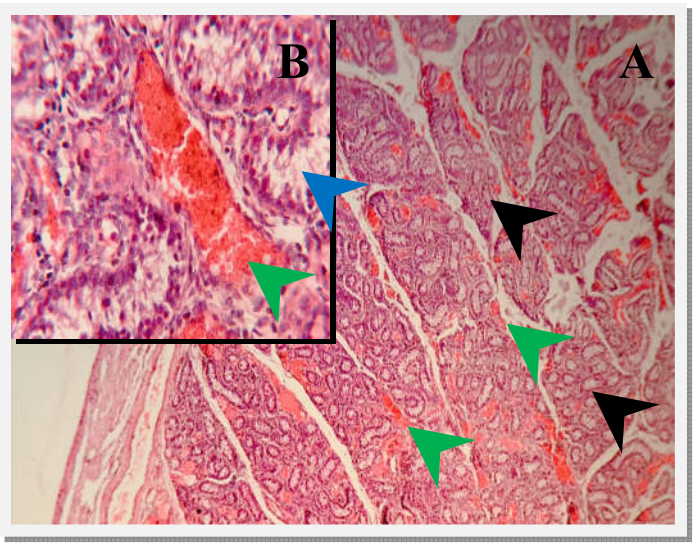



**Fig (17)** Section of testes ofmale rabbits of control (+ve) group shows marked vascular congestion (green arrow), marked atrophy of some seminiferous tubules (black arrow), marked suppression of spermatogenesis (blue arrow). H&E A)50X B)500X.

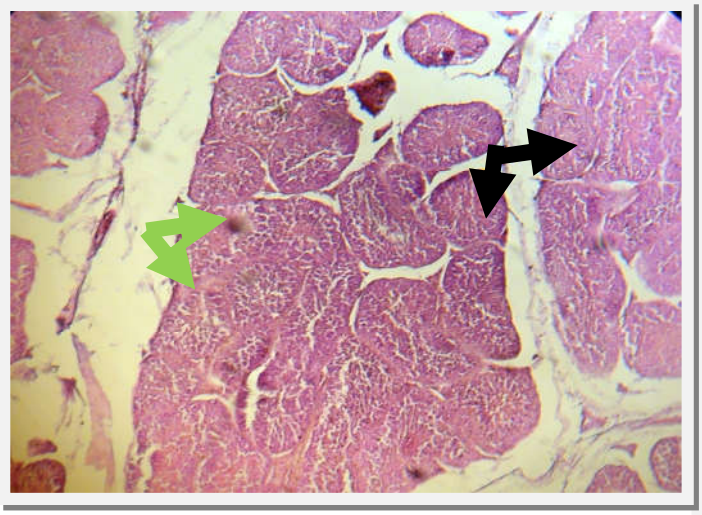


**Fig.(18):** Section of testis of male rabbits treated with 0.2ml of Vit.D (high does) reveal normal seminiferous tubules (green arrow), normal spermatozoa (black arrow). H&E 100X.

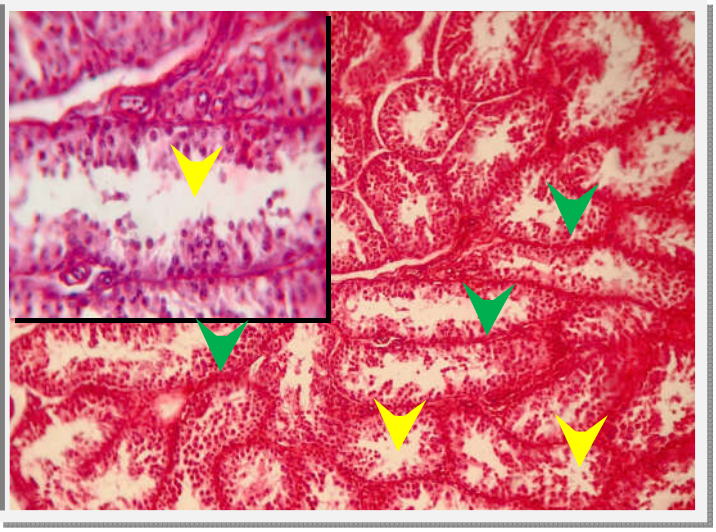


**Fig: (19)** Section of testis of male rabbits treated with 0.1ml of Vit.D (low does), reveal normal seminiferous tubules (green arrow), Marked suppression of spermatozoa (yellow arrow). A)125 X ,B)500 H&E

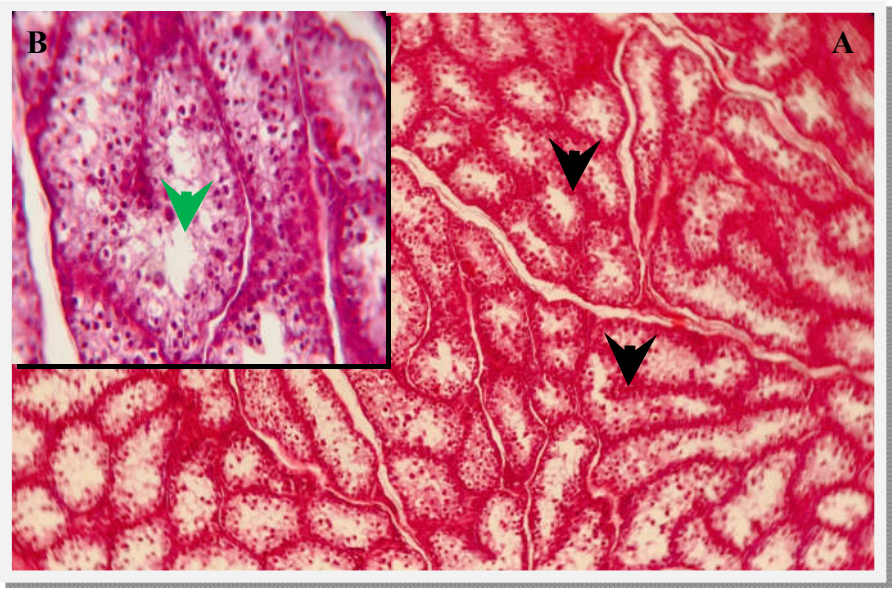



**Fig.(20)**: Section of testes of male rabbits group exposure to sun light shows normal reveal normal seminiferous tubules (black arrow), suppression of spermatozoa (green arrow). A)125 X, B) 500X H&E

## DISCUSSION

Vitamin D is the main steroid hormone which vitally essential for good bone- and overall health the full time life in mishmash with Ca, P and PTH. It helps in the absorption of Ca as of the intestine and reabsorption as of tubules of kidneys and in combination with Ca mobilization as of bones by PTH(16). This delicate balance of Vit.D, Ca, P and PTH is disturbed when impaired kidney progresses and this leads to highly risk of not only bone disease but also cardiovascular disease(17).

Vit.D insufficiency raises the danger of expanding rickets, osteomalacia, osteoporosis moreover add to the hazard of various non-skeletal adverse health outcomes including CVD, autoimmune diseases and overall mortality. In animals, Vit. D is mainly created in the skin following environmental exposure and only a little quantity is get during the diet. The lifestyle, humans have a mixture of Vit.$D_2$ and $D_3$ available as of ambient ultraviolet (UV) rays, usual dietary intake of Vit. $D_3$-rich foods (yolks of egg and oil of fish), fortified foods which usually have vitamin $D_2$ fortification(18). The extended shortage of 1,25 dihydroxy Vit.D together with parathyroid abnormality leads to renal osteo-dystrophy (ROD) and CRDmineral bone disorder (CKD-MBD), which can be evidenced by any one or mixture of abnormalities of Ca, P, PTH, and Vit.D metabolism, leading to elevated in PTH2, bone disease, vascular or soft tissue calcification.(19)and (20).

Parathyroid hormone is essential in Ca and P hemostasis and also plays a role in the conversion of Vit.D to its active form (1, 25 dihydroxycholecalciferol) in the renal. A reduce in extracellular Ca concentrations or an increase in P concentrations guides to a PTH discharge as of the parathyroid gland, which in turn elevates renal reabsorption of Ca, renal activation of Vit.D, urinary P secretion, and bone resorption. In turn, Vit.D promotes intestinal absorption and renal reabsorption of Ca and P(21),(22).

Diuretics are generally used with hypertension. We required to explore, in hypertension, the influence of furosemide (Furo) on circulating parathyroid hormone (PTH) and whether such actions are established by the results of these complexes on Ca excretion.Furosemide elevated the hazard of $HPT_2$. This effect was independent of eGFR or Ca excretion. The use of LD, present preferred in advanced stages, should be reappraised.

The present study revealed in Table (1) a decrease in body weight and body weight gain in control (+ve) group along the experimental period. The decrease in the body weight and body weight gain may be attributed to the postulated that furosemide-induced reductions in body weight suggested reductions in body fluid volume (23). Furosemide causes rapid and substantial urine loss, a 2% to 4% loss in body weight, and, only 30 minutes after administration, a 13% reduction in plasma volume(24). This profound increase in urination after furosemide dealing with in horses, combined with its ability to cause increased excretion of Na, Ca, K and Cl, as well as evidence in animals that furosemide dealing wirth may contribute to raised danger of fracture.

The taken of furosemide caused serum Vit. D and Ca concentrations were significantly reduced. This may reflect the activities of parathyroid hormone , which may impair Ca regulation effects on thyroid gland and these changes may be connected with effects on parathyroid gland therefore may cause disturbance in serum concentration of Ca and P. The affect of serum Ca concentration in hypovitaminosis rabbits reflect an adverse image on bone mineralization and this reveal the importance role of thyroid hormones in regulation of growth and bone metabolism (25).

The obtained results revealed that a significant increase of creatinine and urea concentrations in the serum of male rabbits treated with furosemide Control(+ve). These results are attributed to deleterious effect of furosemide on kidney functions and these results is supported by histological findings of kidney.

Hypovitaminosis leads to adverse effects in various tissues. The present cross-sectional study was performed to determine whether furosemide has deleterious effects on renal function. furosemide-induced kidney impairments in male rabbits.

In hypovitaminosis D directing to gout has been reported {26),(27). The raise in uric acid in hypovitaminosis state may result from either enlarged production due to myopathy associated with hypovitaminosis D or due to reduced renal clearance of uric acid(28). Though the observed decrease in renal function, renal dysfunction induced hypovitaminosis D may lead to adverse clinical. In this study, the histological changes of the kidney especially the basement membrane of renal cortical tubules thickening have been confirmed and agreed with hypovitaminosis D in animals. The reason is low concentrations of Vit.D in active form, which is responsible for absorption and reabsorption of Ca, due to the kidney losing their function, an enzyme is not produced, 1-α hydroxylase responsible for convertingVit.D from formula in active 25-hydroxycholecalciferol to active 1,25-dihydroxycholecalciferol(29),(30).

The obtained results revealed benefit effect of Vit.D supplementation plus furosemide and exposure to sun light on serum concentrations of Vit.D, Ca and P in male rabbits. Serum Vit.D was arrived to normal range in male rabbits after Vit.D supplementation and exposure to sunlight. Also Ca and P concentrations get better compared to treated with furosemide alone which may be attributed to Vit.D supplementation has a potent stimulatory effect on bone formation through it was effected on the activity of the hormones regulating Ca and P in blood concentrations and parathyroid hormones to their normal concentrations.

Vit.D has a stimulatory effect on bone formation and mineralization thus vit.D plays an vital role in the progress of the skeletal system. This result agrees with(31), (32)who found that Vit.D is beneficial to body weight. This result is supported by histological findings of parathyroid gland and kidneys after Vit.D supplmentation. The enhancement of histological parathyroid gland which including chief cell is responsible on secretion PTH as well as kidneys are responsible on excretion of Ca and P by glomerular.

The Vit.D receptor and Vit.D metabolizing enzymes in male reproductive system, particularly in the testis, suggests the happening of Vit.D synthesis and regulation as well as function in the testis. The role of Vit D in the modulation of

testis functions, including hormone production and spermatogenesis, has been explored in animals. .

In fact, VitD was shown to be positively associated to sperm motility, and to exert direct actions on spermatozoa, together with driven modulation of intracellular Ca homeostasis and activation of molecular pathways involved in sperm motility, capacitation and acrosome reaction. The present study confirmation on the role of Vit. D in male fertility, by reporting trial studies in animals deal with the association connecting Vit. D and testis role. This results are agreement with (33)who indicate by the estimation of both VitD and the alternate marker 25-hydroxy-Vit.$D_3$ circulating points, on semen quality and fertility have been explored in vivo studies in animal models.

## تقييم دور اضافة فيتامين د على بعض المعايير الفسيولوجية وفعالية الغدة جار الدرقية ونوعية السائل المنوي في ذكور الأرانب المستحث فيها نقص فيتامين د بواسطة الفوروسيميد

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### الخلاصة

صممت هذه الدراسة لتقصي التأثير العلاجي لفيتامين د على المعايير الفسلجية وصفات السائل المنوي في ذكور الأرانب المستحدث فيها نقص فيتامين د، وقد أجريت الدراسة في البيت الحيواني لكلية الطب البيطري- جامعة البصرة على30 من ذكور الأرانب المحلية ,قسمت حيوانات التجارب بصورة عشوائية إلى خمسة مجاميع. الأولى هي مجموعة السيطرة السالبة عوملت بالمحلول الفسيولوجي (٣مل)لمدة ٣٠ يوم والمجموعة الثانية هي السيطرة الموجبة وضعت الارانب في اقفاص داخل غرفة مظلمة غير معرضة للشمس وتغذيتها محدودة وكذلك عوملت ب furosemide (0.5mg/kgB.W.) لمدة ١٦ يوم لاستحداث نقص فيتامين د ،و المجموعة الثالثة ايضا وضعت الارانب في اقفاص داخل غرفة مظلمة غير معرضة للشمس وتغذيتها محدودة وكذلك عوملت ب furosemide (0.5mg/kgB.W.) لمدة ١٦ يوم + ( 200000 IU/2ml) كل ١٠ ايام لمدة شهر ،كذلك المجوعة الرابعة وضعت الارانب في اقفاص داخل غرفة مظلمة غير معرضة للشمس وتغذيتها محدودة وكذلك عوملت ب furosemide (0.5mg/kgB.W.) لمدة ١٦ يوم + (100000 IU/1m)) كل ١٠ ايام لمدة شهر بينما المجموعة الاخيرة وضعت الارانب في اقفاص داخل غرفة مظلمة غير معرضة للشمس وتغذيتها محدودة وكذلك عوملت ب furosemide (0.5mg/kgB.W.) لمدة ١٦ يوم + التعرض للشمس خمس ساعات في اليوم لمدة شهر. بعد انتهاء التجربه تم قياس وزن الجسم ومعدل الزيادة الوزنية لجميع الحيوانات وتم سحب عينات الدم بعد ذلك تم التضحية بالحيوانات لاستخراج الاعضاء المدروسة وجمع السائل المنوي من جميع الحيوانات. بينت النتائج ان هنالك انخفاض معنوي في وزن الجسم

ومعدل الزيادة الوزنية وكذلك انخفاض معنوي في مستويات الكالسيوم وفيتامين د وهرمون Testosterone وكذلك انخفاض العدد الكلي للنطف وعدد النطف الحية وقلة فعاليتها وحركتها للأرانب المستحث فيها نقص فيتامين د مقارنة مع مجموعة السيطرة والمجاميع المعالجة بفيتامين د والمعرضة لاشعة الشمس بينت النتائج هناك زيادة معنوية في مستوى اليوريا والكرياتنين و حامض اليوريك والفسفور وهرمون البراثايرود وكذلك هرموني FSHو LH وهناك تغيرات نسجية بالنسبة للغدة جار الدرقية والكلى والخصى لذكور الارانب المستحث بها فيتامين د. بينما اظهرت نتائج المجاميع المعاملة بفيتامين د و المجموعة المتعرضة لاشعة الشمس هناك تحسن واضح بكل المعايير المذكورة سابقا وكذلك المقاطع النسجية .

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