

EFFECTS OF HIGH DOSE VITAMIN D₃ INDUCED HYPERVITAMINOSIS D ON SERUM BIOCHEMICAL PARAMETERS IN *ORYCTOLAGUS CUNICULUS*

Laiba Malik¹, Nageen Quddus¹, Saima Bibi², Sidra Bano¹, Aneeta Riasat², Sibgha hakeem¹, Zohra Iqbal¹, Asad Ali^{1*}

¹Department of Biology, The University of Haripur, KP Pakistan

²Department of Zoology Government Post Graduate College Haripur KP Pakistan

*asadali15307@gmail.com

DOI: <https://doi.org/10.5281/zenodo.21884295>

Keywords

Vitamin D₃, Hypervitaminosis D, Hypercalcemia, Hypercholesterolemia, Alanine aminotransferase, Creatinine, Rabbit model, *Oryctolagus cuniculus*.

Article History

Received: 01 January 2026

Accepted: 17 March 2026

Published: 30 March 2026

Copyright @Author

Corresponding Author: *

Asad Ali

Abstract

Vitamin D is a fat soluble secosteroid hormone that regulates calcium and phosphate homeostasis. Although therapeutic supplementation is generally safe, excessive intake can cause hypervitaminosis D and associated metabolic disturbances. This study evaluated the effects of high dose vitamin D₃ on serum calcium, total cholesterol, alanine aminotransferase (ALT), and creatinine in *Oryctolagus cuniculus*. Sixteen healthy adult female New Zealand White rabbits were randomly assigned to either a control group or a vitamin D₃ treated group (n = 8 per group). The treatment group received 25,000 IU of vitamin D₃ orally once daily for nine consecutive days. Blood samples were collected at the end of the experiment for biochemical analysis. High dose vitamin D₃ significantly increased serum calcium and total cholesterol concentrations, indicating hypercalcemia and hypercholesterolemia. Conversely, serum ALT activity and creatinine concentrations were reduced compared with controls. These findings demonstrate that short-term administration of excessive vitamin D₃ disrupts mineral homeostasis and alters hepatic and renal biochemical markers, highlighting the potential metabolic consequences of vitamin D toxicity.

INTRODUCTION

Vitamin D is a fat soluble secosteroid hormone that plays a fundamental role in calcium and phosphate homeostasis, skeletal mineralization, and numerous extra skeletal physiological processes. Beyond its classical role in bone metabolism, vitamin D regulates cellular proliferation, differentiation, immune function, cardiovascular health, and metabolic homeostasis (Adams & Hewison, 2010). Vitamin D exerts its

biological effects primarily through its active metabolite, 1,25-dihydroxyvitamin D, which binds to the vitamin D receptor (VDR) expressed in a wide variety of tissues. Although physiological supplementation is considered safe, prolonged administration of excessive doses can result in hypervitaminosis D, a potentially life threatening condition characterized by persistent hypercalcemia and widespread organ dysfunction (Płudowski et al., 2016).

Vitamin D toxicity (VDT) is almost exclusively associated with excessive intake of vitamin D supplements rather than dietary sources or sunlight exposure, as endogenous vitamin D synthesis is tightly regulated by photochemical mechanisms. Hypervitaminosis D is generally characterized by serum 25 hydroxyvitamin D [25(OH)D] concentrations exceeding 150 ng/mL, accompanied by hypercalcemia, hypercalciuria, and marked suppression of parathyroid hormone (PTH) secretion (Vieth, 2007). The resulting hypercalcemia arises from increased intestinal calcium absorption, enhanced bone resorption, reduced renal calcium excretion, or a combination of these mechanisms (Guerra, Vieira et al., 2016). Persistent elevations in serum calcium can promote metastatic calcification, disrupt normal cellular function, and contribute to multisystem toxicity involving the kidneys, cardiovascular system, gastrointestinal tract, and other organs.

Among the organs affected, the kidneys are particularly vulnerable to vitamin D intoxication. Sustained hypercalcemia may induce nephrocalcinosis, renal tubular injury, impaired glomerular filtration, and progressive renal insufficiency, frequently reflected by elevated serum creatinine concentrations (Naik, Banday et al., 2008). Clinically, vitamin D intoxication has been associated with renal disorders (65%), renal insufficiency (51%), arterial hypertension (52%), and gastrointestinal disturbances (23%), emphasizing the systemic consequences of prolonged vitamin D excess (Naik, Banday et al., 2008). Consequently, biochemical markers of renal and hepatic function are important indicators for assessing the toxicological effects of excessive vitamin D exposure.

In addition to its effects on mineral metabolism, vitamin D has been implicated in lipid metabolism; however, the nature of this relationship remains controversial. Several observational studies have reported positive associations between vitamin D status and high-density lipoprotein cholesterol (HDL-C), along with reductions in low-density lipoprotein cholesterol (LDL-C) and the LDL-C/HDL-C ratio. Conversely, other investigations have

demonstrated inconsistent or contradictory relationships between circulating 25(OH)D concentrations and serum lipid profiles, including total cholesterol and triglycerides. Moreover, most intervention studies evaluating vitamin D supplementation were not specifically designed to investigate lipid metabolism, did not recruit hyperlipidemic participants, and lacked sufficient statistical power. Among these studies, only one reported a significant increase in LDL-C (8%; 0.28 mmol/L) and a decrease in triglycerides (16%; 0.22 mmol/L) following vitamin D supplementation, indicating that the influence of vitamin D on lipid metabolism remains inconclusive (Jorde and Grimnes et al., 2011).

Animal models provide a controlled experimental platform for investigating the physiological and toxicological effects of vitamin D. Among laboratory species, the New Zealand White rabbit (*Oryctolagus cuniculus*) is widely used in biomedical research because of its manageable size, ease of handling, and suitability for metabolic, toxicological, and pharmacological investigations. Although the macro and microarchitecture of rabbit bone differs from that of humans, rabbits remain valuable preclinical models for evaluating physiological responses and screening therapeutic interventions before translation to larger animal models (Thomas et al., 2012).

Despite increasing clinical use of high-dose vitamin D supplementation, comprehensive evaluation of its systemic biochemical effects remains limited. In particular, the combined effects of excessive vitamin D administration on calcium homeostasis, lipid metabolism, hepatic integrity, and renal function have not been fully elucidated. Therefore, the present study was designed to investigate the biochemical consequences of high-dose vitamin D₃ administration by assessing serum calcium, total cholesterol, alanine aminotransferase (ALT), and creatinine concentrations in New Zealand White rabbits.

Materials and method

Acclimatization:

Sixteen healthy adult female rabbits were procured from the local animal market in Haripur, Pakistan, and transported to the Zoology Laboratory, Government Postgraduate College, Haripur, in well ventilated transport boxes to minimize handling stress. Upon arrival, the animals were acclimatized to the laboratory environment for 2–3 days before the initiation of the experimental procedures. During the acclimatization period, the rabbits were housed in a well-ventilated animal room (approximately 1200 m²) under standard husbandry conditions. They were provided with a daily diet consisting of fresh cucumber, tomato, mixed grasses, and green leafy vegetables, with free access to clean drinking water throughout the study. Vitamin D₃ (cholecalciferol) supplementation was administered using a commercially available preparation (Sunny D Insta Ampoule; obtained from a licensed pharmacy in Haripur, Pakistan). Each 1 mL ampoule contained 5 mg of cholecalciferol, equivalent to 200,000 IU of vitamin D₃, and was used as the experimental treatment.

Experimental Design:

The sixteen female rabbits were randomly allocated into two experimental groups (n = 8 per group): a control group and a vitamin D₃-treated group. Rabbits in the control group received no vitamin D₃ supplementation and were maintained under identical husbandry conditions throughout the experimental period. The treatment group received oral vitamin D₃ (cholecalciferol) supplementation for nine consecutive days. To facilitate uniform administration, a dosing solution was prepared by mixing 1 mL of vitamin D₃ solution (200,000 IU) with 4 mL of milk, yielding a total volume of 5 mL. Each rabbit in the treatment group received 0.625 mL of the prepared mixture once daily by oral administration, corresponding to 0.125 mL of vitamin D₃ (25,000 IU) and 0.5 mL of milk per animal per day.

Blood sampling:

At the end of the 9-day treatment period, all rabbits were transported to Noor Surgical Laboratory, Haripur, Pakistan, for blood collection. Blood samples were obtained aseptically from the marginal ear vein of each rabbit using sterile disposable syringes under standard handling procedures. The collected blood was immediately transferred into EDTA and plain (clot activator) vacutainer tubes, as appropriate for subsequent hematological and biochemical analyses.

Determination of calcium, cholesterol, ALT and creatinine Levels Statistical tests:

Blood samples collected from both the control and vitamin D₃-treated rabbits were processed for serum separation, and the resulting serum was analyzed for biochemical parameters, including serum calcium, total cholesterol, alanine aminotransferase (ALT), and creatinine, using an automated biochemical analyzer according to the manufacturer's instructions.

Statistical Analysis

Statistical analyses were performed using an appropriate statistical software package. Data are presented as mean $\pm$ standard deviation (SD). Differences in serum calcium, total cholesterol, alanine aminotransferase (ALT), and creatinine concentrations between the control and vitamin D₃-treated groups were evaluated using an independent-samples t-test. A P-value of < 0.05 was considered statistically significant.

Results

Weight of rabbits

Rabbits of both control and experimental group were placed in basket individually and that basket was placed on triple beam balance. Weight of the basket: 107gram, Weight of rabbits of control group increased after experiment, by mean weight of experimental group, it is concluded that weight of rabbits of experimental group decreased after dose. Mean values of weight of 8 rabbits in experimental group showed the value 44.5 (P=0.000) which is < 0.05 which shows the significant decrease in weight. The results showed

the reduction of body weight of experimental group in rabbits.

Table.1 statistically summarized weight of both control and experimental groups in rabbits

		t-test for Equality of Means						
		t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
Weight	Equal variances assumed	-7.890	14	.000	-103.12500	13.07115	-131.15982	-75.09018
	Equal variances not assumed	-7.890	9.093	.000	-103.12500	13.07115	-132.64774	-73.60226

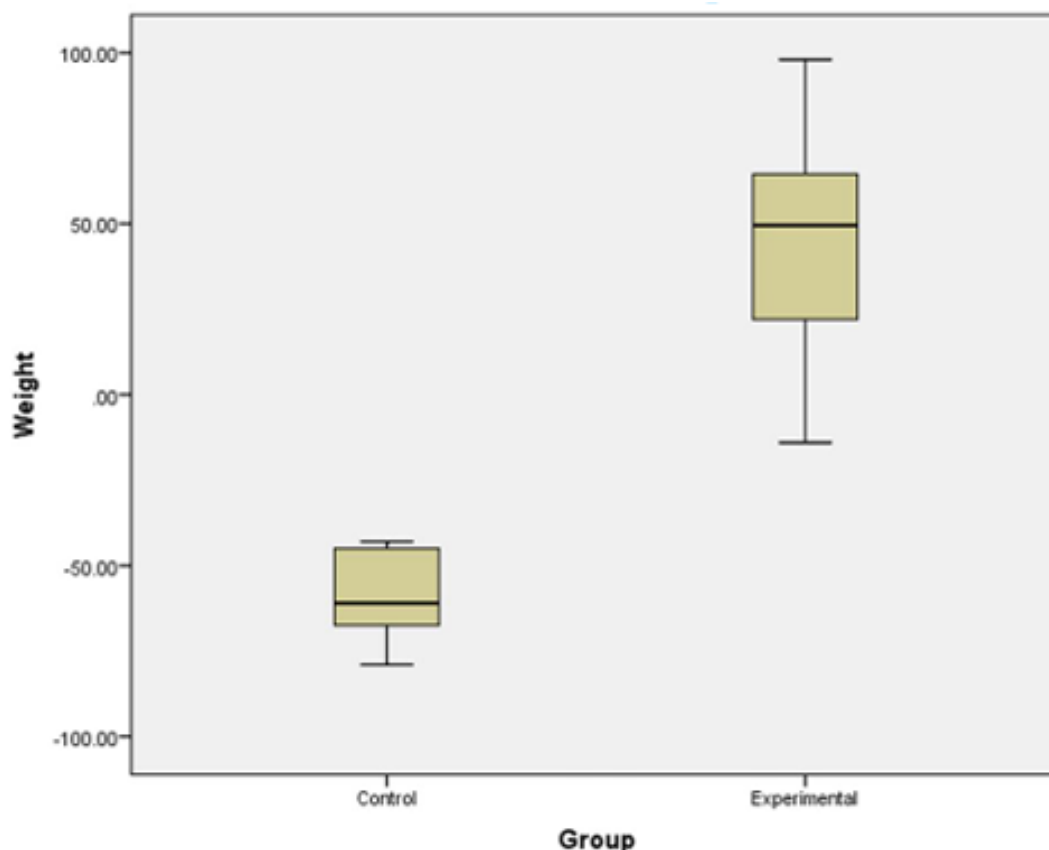


Figure.1 weight of both control and treated groups of rabbit

Serum Calcium

Mean values of serum calcium for 8 rabbits in experimental group showed the value 15.2 (P=0.021) which is < 0.05 which shows the

significant increase in serum calcium concentration. The results showed the increase of serum calcium in rabbits.

Table.2 statistically summarized serum calcium of both control and experimental groups in rabbits

		t-test for Equality of Means						
		t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
Serum Calcium	Equal variances assumed	-2.903	14	.012	-1.36250	.46940	-2.36926	-.35574
	Equal variances not assumed	-2.903	7.639	.021	-1.36250	.46940	-2.45389	-.27111

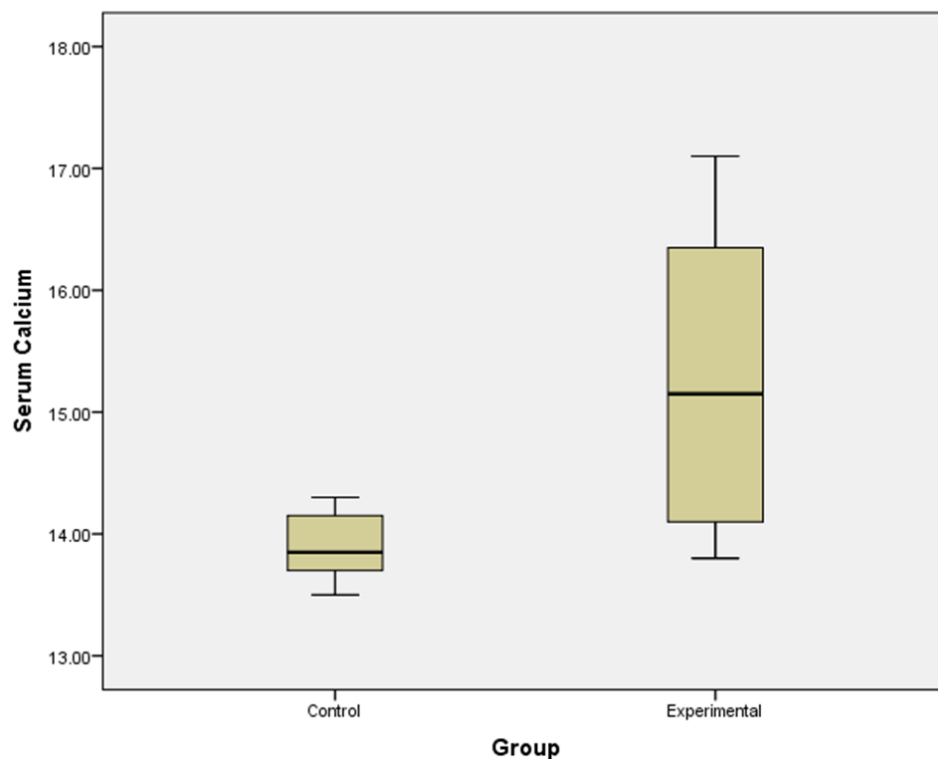


Figure.2 serum calcium of both control and experimental groups in rabbits

Serum Cholesterol

Mean values of serum cholesterol for 8 rabbits in experimental group showed the value 65.3 (P=0.003) which is < 0.05 which shows the highly

significant increase in serum cholesterol concentration. The results showed the increase of serum cholesterol in rabbits.

Table.3 statistically summarized serum cholesterol of both control and experimental Groups in rabbits

		t-test for Equality of Means						
		t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Interval of the Difference	Confidence of the
							Lower	Upper
Serum Cholesterol	Equal variances assumed	-4.402	14	.001	-25.25000	5.73640	-37.55335	12.94665
	Equal variances not assumed	-4.402	7.333	.003	-25.25000	5.73640	-38.69044	11.80956

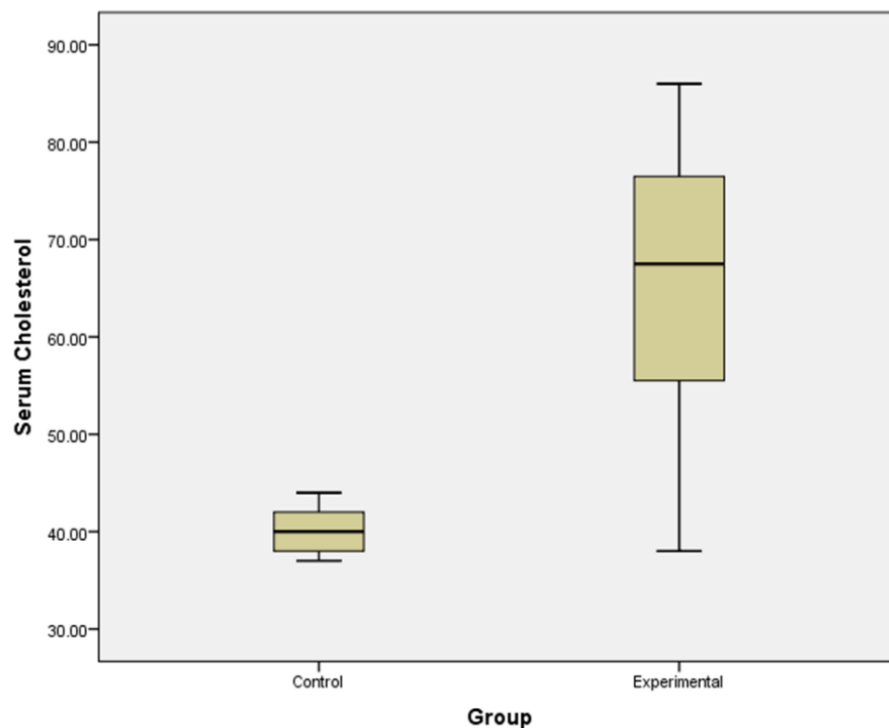


Figure.3 serum cholesterol of both control and treated groups of rabbits

Serum ALT

Mean values of serum ALT for 8 rabbits in experimental group showed the value 71.3 (P=0.001) which is <0.05 which shows the highly

significant decrease in serum ALT concentration. The results showed the reduction in serum ALT in rabbits

Table.4 statistically summarized serum ALT of both control and experimental groups in rabbits

		t-test for Equality of Means						
		t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
ALT	Equal variances assumed	5.019	14	.000	18.50000	3.68576	10.59483	26.40517
	Equal variances not assumed	5.019	8.423	.001	18.50000	3.68576	10.07440	26.92560

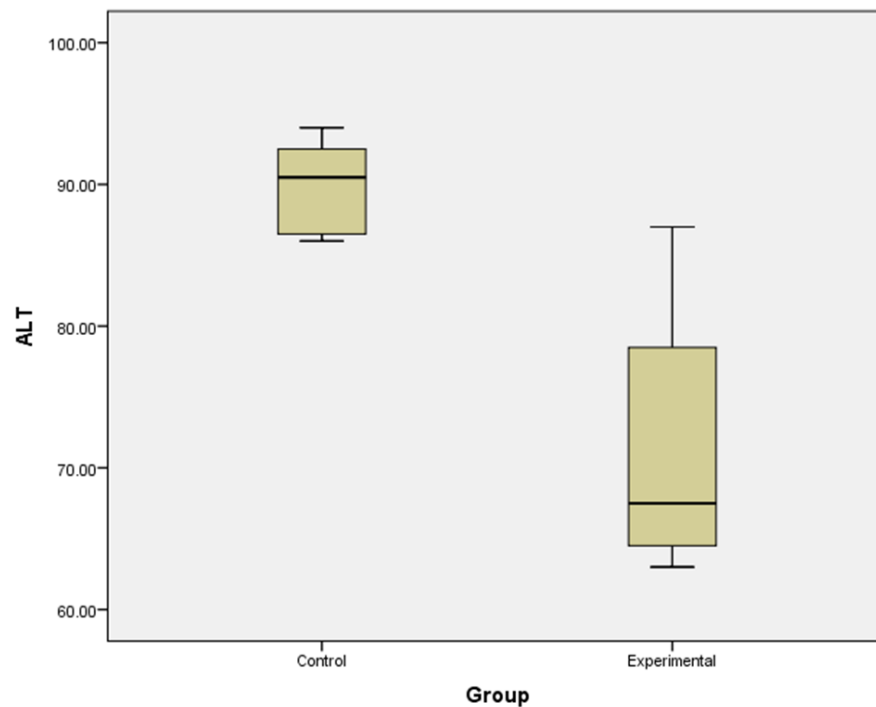


Figure.4 serum ALT of both control and treated groups of rabbits

Serum Creatinine

Mean values of serum creatinine for 8 rabbits in experimental group shows the value 1.92 (P =0.244) which is > 0.05 which shows the non-

significant decrease in serum creatinine concentration. The results showed the reduction in serum creatinine in rabbits.

Table.5 Statistically Summarized serum creatinine of both control and experimental groups in rabbits

		t-test for Equality of Means						
		t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper

Creatinine	Equal variances assumed	1.267	14	.226	.26250	.20718	-.18186	.70686
	Equal variances not assumed	1.267	7.290	.244	.26250	.20718	-.22349	.74849

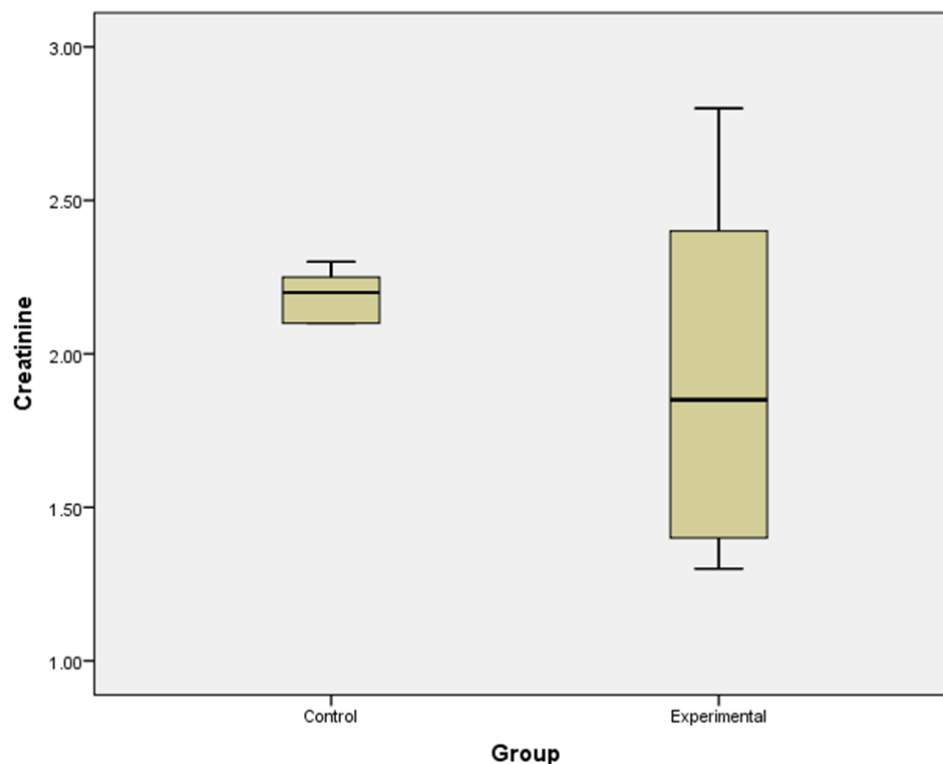


Figure.5 serum creatinine of both control and treated groups of rabbits

Discussion

Rabbits are the most commonly used for toxicity testing of substances such as drugs and chemicals. Rabbits are cheap to maintain and have a short gestation period of 28-35 days. High dose of vitamin D can be toxic and cause hypervitaminosis D which leads to hypercalcemia and hypercholesterolemia which may result in nephrotoxicity, but nor normal or excessive dose of vitamin D cause hepatotoxicity. The aim of study was figure out the toxicity of vitamin D3 on hematological, nephrological and hepatological parameters of *O.cuniculus*. No death occurred during the 9 days of experiment. The intoxication of *O.cuniculus* with high doses of vitamin D3 showed various signs of behavioral distress like polyuria, polydipsia and loss of weight. In the present study, serum calcium

increased by high dose of vitamin D3 caused hypercalcemia in rabbits which is quite similar to the findings of (Tebben, 2016) who suggested that vitamin D-associated hypercalcemia occurs only when extremely large doses of vitamin D (often several hundred-fold the recommended intake) are ingested. As vitamin D is used to increase bone and serum calcium, so the results which indicated hypercalcemia by high dosage of vitamin D were supported by previous studies. In the present study, serum cholesterol increased by high dose of vitamin D3 caused hypercholesterolemia in rabbits, which is supported by the results of (Tavakoli, 2016) showed that vitamin D supplements seem to have a positive impact on serum HDL-C levels. It is not sure that by increasing vitamin D supplementations, hypercholesterolemia occur

because in many studies cholesterol level also decreased by hypervitaminosis D. But the current study showed that hypervitaminosis D results in hypercholesterolemia. In the present study, serum ALT decreased by high dose of vitamin D3 due to which hepatotoxicity was not observed in rabbits. The results of also showed (Yaghchiyan, 2019) that vitamin D administration significantly reduced AST and ALT concentrations in the treatment groups ($p < 0.05$). In most of the researches, serum ALT was reduced by hypervitaminosis D which showed that vitamin D supplementations are not harmful for liver. In the present study, serum creatinine was reduced by high dose of vitamin D3 due to which nephrotoxicity was not observed in rabbits which is opposite to the results of (Agarwal, 2011) in which serum creatinine was increased by high dose of vitamin D. Most of the studies showed the increase in serum creatinine level by high dose of vitamin D supplementations which indicated that hypervitaminosis D cause kidney injury. But the current results were opposite to the previous literature. The reason behind reduction of serum creatinine level may be polyuria due to which more creatinine was excreted from body.

Conclusion

Based on the results achieved in this study we concluded that Level of serum calcium, serum cholesterol, serum ALT and serum creatinine altered by high dose of vitamin D. High dose of vitamin D induced hypercalcemia and hypercholesterolemia. A significant decrease in serum level of ALT, and creatinine shows that chronic hypervitaminosis D affects liver and kidney.

Recommendations:

Our present research is only limited to the hematological parameters. Further investigations on histological toxicity can be correlated with physiological parameters. Effect of high dose of vitamin D on bone, muscles and arteries can be observed.

REFERENCES:

- Agarwal, R., et al. (2011). "Short-term vitamin D receptor activation increases serum creatinine due to increased production with no effect on the glomerular filtration rate." *80*(10): 1073-1079.
- Buffenstein, R., et al. (1995). "Vitamin D3 intoxication in naked mole-rats (*Heterocephalus glaber*) leads to hypercalcaemia and increased calcium deposition in teeth with evidence of abnormal skin calcification." *99*(1): 35-40.
- Crowell-Davis, S. L. J. J. o. e. p. m. (2007). "Behavior problems in pet rabbits." *16*(1): 38-44.
- Flux, J. E. and P. J. J. M. R. FULLAGAR (1992). "World distribution of the Rabbit *Oryctolagus cuniculus* on islands." *22*(3-4): 151-205.
- Guerra, V., et al. (2016). "Hypercalcemia and renal function impairment associated with vitamin D toxicity: case report." *38*: 466-469.
- Irungbam, K., et al. (2013). "Hypervitaminosis/vitamin poisoning in animals." *13*(2): 3-8.
- Jorde, R. and G. J. P. i. l. r. Grimnes (2011). "Vitamin D and metabolic health with special reference to the effect of vitamin D on serum lipids." *50*(4): 303-312.
- Lombardi, L., et al. (2003). "Habitat-related differences in rabbit (*Oryctolagus cuniculus*) abundance, distribution, and activity." *84*(1): 26-36.
- Mäkitaipale, J., et al. (2019). "Diet is a main source of vitamin D in Finnish pet rabbits (*Oryctolagus cuniculus*)." *103*(5): 1564-1570.
- Malek, H., et al. (2014). "Effect of a high dose of vitamin D on a rabbit model of atherosclerosis" *27*(2): 195-201.
- Mapara, M., et al. (2012). "Rabbit as an animal model for experimental research." *9*(1): 111.
- Marcinowska-Suchowierska, E., et al. (2016). "Vitamin D toxicity." *29*(10): 756-759.

- Naik, M., et al. (2008). "Vitamin D intoxication presenting as acute renal failure." **18**(3): 125
- Nwosu, B. U. and L. J. P. O. Maranda (2014). "The effects of vitamin D supplementation on hepatic dysfunction, vitamin D status, and glycemic control in children and adolescents with vitamin D deficiency and either type 1 or type 2 diabetes mellitus." **9**(6): e99646.
- Radagdam, S., et al. (2019). "Calcitriol modulates Cholesteryl ester transfer protein (CETP) levels and lipid profile in hypercholesterolemic male rabbits: A pilot study."
- Raymond, C. J. L. and E. P. J. V. J. Branch (1996). "PATHOLOGY OF THE RABBIT." **37**: 303-305.
- Shehata, E. and R. J. J. o. c. g. Qayyum (2016). "The effect of serum vitamin D on serum ALT levels in healthy individuals." **50**(9): e81-e84.
- Takeo, S., et al. (1991). "Alterations in cardiac function and subcellular membrane activities after hypervitaminosis D 3." **107**(2): 169-183.
- Tavakoli, F., et al. (2016). "Vitamin D supplementation and high-density lipoprotein cholesterol: a study in healthy school children." **26**(4).
- Tavakoli, H., et al. (2019). "High dose vitamin D supplementation is associated with an improvement in serum markers of liver function." **45**(3): 335-342.
- Tebben, P. J., et al. (2016). "Vitamin D-mediated hypercalcemia: mechanisms, diagnosis, and treatment." **37**(5): 521-547.
- Yaghchiyan, M., et al. (2019). "Histologic, metabolic, and inflammatory changes in the liver of high-fat diet-induced obese rats before and after vitamin D administration."
- Zimmerman, H. J. (1999). "Hepatotoxicity: the adverse effects of drugs and other chemicals on the liver."
- Norbury, G., & Reddiex, B. (2005). European rabbit. *The handbook of New Zealand mammals*. Oxford University Press, Melbourne, 131-150.
- Adams, J. S., & Hewison, M. (2010). Update in vitamin D. *The Journal of Clinical Endocrinology & Metabolism*, **95**(2), 471-478.
- Vieth, R. (2007). Vitamin D toxicity, policy, and science. *Journal of Bone and Mineral Research*, **22**(S2), V64-V68.