Research Article

Histopathological and Cytogenetic Effects of Hyperdoses of Vitamin D in Female Rats

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Abstract | The Highlights of the present study was undertaken to investigate the toxic effects of the higher dose group (HG) (549.640 IU, i.e., 1.3741 mg/kg body weight), which represent 1/10 LD50, and the lower dose group (LG) (274.820 IU, i.e., 0.6875 mg/kg body weight), which represents 1/20 LD50 of vitamin D3. So, the objectives of this rapid review were to assess the median lethal dose (LD50) of VD in female albino rats and to assess the effects of vitamin D (VD) on the severity of acute upper or chronic vitamin D intoxication (VDI) through the study of cytogenetic effects in addition to histopathological changes in the tissues of female rats. The methods included in the first experiment, the female rats were orally administered the following lethal doses: 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, and 16 mg/kg b.w. of vitamin D. Survival and mortality numbers were observed following 24 hours of intoxication. In the second experiment, the selected groups were divided according to the doses they received. In a cross-sectional study, 30 female rats were divided into three groups: a control group (CG: 0 mg/kg VD), a high VD group (HG: 1/10 LD50), and a low VD group (LG: 1/20 LD50). While the Results included at the dose of 1/10 LD50, the lesions were more severe than the lesions at 1/20 LD50. The characteristic histological finding in the liver is severe and generalized hepatocellular necrosis, which revealed congestion, hemorrhage, and degeneration of hepatic cells. Some sections of the kidneys show vacuolation of mesangial cells in the glomerular tuft. Muscles of treated rats revealed severe histopathological changes under stress from both doses of toxic cholecalciferol, also showing the widespread mineralization of muscle fibers. From this study concluded that both doses of vitamin D can be toxic to organisms at higher levels, so they need to be used carefully and should not be taken excessively.

Keywords | Hypervitaminosis D, Chromosome gap, Histopathological, Cytogenetic effects, Vitamin D, Median lethal dose, Mitotic index, Rats, Female rats.

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INTRODUCTION

The vitamins are a chemically disparate group of compounds whose only common feature is that they are dietary essentials that are required in small amounts for the normal functioning of the body and maintenance of metabolic integrity (Bender., 2009; Walsh and Tang., 2019). Most vitamins are provided by food, so they are classed as 'essential' and are divided into two groups: fat-soluble and water-soluble (Broom and Dominiczak, 2014; Godswill *et al*., 2020; Rafeeq *et al*., 2020). Vitamin D is fat-soluble and is stored in fatty tissue (Khalfa *et al*., 2019; Stevens, 2021; Chen *et al*., 2022). The most important compound of vitamin D is cholecalciferol (vitamin D3, or VD3), which is produced in the skin following exposure to sunshine or ultraviolet light or obtained from

nutritional sources, especially fatty fishes such as salmon, sardines, herring and mackerel (Kadhim and Abdul-hassan., 2017; Balwan and Saba., 2021; Javed *et al*., 2021), That plays a central role in metabolism and also in muscle, cardiac, immune, and neurological functions, as well as in the regulation of inflammation (Dominguez *et al*., 2021; Marazziti *et al*., 2021). Tooth mineralization and bone remodeling depend on the quantity of serum vitamin D (VD) (Khamees *et al*.,2023). Numerous of vitamin D compounds have been created as medications to treat a range of illnesses (Yasuda *et al*., 2021). Vitamin D (VD) status is a modifiable factor that may reduce risk of breast cancer BC through modulation of cell proliferation, apoptosis, invasion and metastasis via binding to a specific receptor, the vitamin D receptor (VDR) (Abboud *et al*., 2022). In addition, higher levels of serum vitamin D cause breast cancer women to survive twice more than those with low levels, Also, up to 78% of breast cancers cells contain vitamin D receptors which might prevent tumor growth and eradicate carcinogenic cells due to an increase in blood supply (Ahmed *et al*., 2020). Hypervitaminosis D, another name for vitamin D toxicity, is an uncommon but potentially dangerous illness that arises from having too much vitamin D in the body (Janoušek *et al*., 2022). Vitamin D consumption increases tissues concentration of vitamin D (Lim and Thadhani., 2020). High vitamin D levels inhibit cell growth and division and can lead to cell death (Karagul *et al*., 2020). Usually, high dosages of vitamin D supplements—rather than food or sun exposure—cause vitamin D toxicity (Marcinowska-Suchowierska *et al*., 2018; Galior *et al*., 2018). This is due to the fact that even fortified foods don't contain significant levels of vitamin D, and the body controls how much vitamin D is created by sun exposure (Gillie, 2006; Zareef and Jackson, 2021). Supplementation may infrequently cause vitamin D intoxication (VDI), however reports of it have increased recently (Hu *et al*., 2023). The toxicity of non-target species was reduced, and the chance of secondary poisoning was decreased when cholecalciferol was used as a rodenticide, so, cholecalciferol, or vitamin D3, is thought to be a safe option as rodenticides (Noh *et al*., 2023).

MATERIALS AND METHODS

The current investigation was carried out between December 1, 2022, and March 25, 2023. 75 healthy female albino rats, weighing 180 ± 30 grams and aged 10–14 weeks, were used in the present investigation. For two weeks, the experimental rats were given conventional rat food and water and were allowed to become well-acclimated to the laboratory environment. These habituated albino rats were then employed in two experiments. In the first experiment, 45 rats were utilized in accordance with Dixon's procedure (Dixon., 1965) to determine the lethal dose. A solution of cholecalciferol (vitamin D) was used to estimate the median lethal dose (LD50) of the experimental compounds under study. In the second experiment, thirty animals were given two different vitamin D doses over two distinct time periods. The selected groups were divided according to the doses they received. Initially, they were split into three major groups, each with ten rats. Within each group, there were two subgroups, each with five rats, representing varying dosages of vitamin D (10 individuals per group and five rats each cage). Cholecalciferol was used as a source of VD, and rats were fed a basic diet with different levels of VD in each group.

Ethics Approval

The project was approved by the Research Ethics Committee, College of Veterinary Medicine, Baghdad, Iraq. (P-G/2467, 6/11/2023). All experiments with rats were conducted in the animal house located in the College of Veterinary Medicine at the University of Baghdad.

Determination Of LD_{50} For Vitamin D By Dixon's (Up And Down) Method

Finding the median lethal dosage (LD50) of a substance is a crucial first step in determining its potential for toxicity (Morris-Schaffer and McCoy, 2020). The experimental test compound, a stock solution of vitamin D3, was a prepared solution (0.25 mg/ml). The solution was brought to room temperature before use. 45 rats were utilized in accordance with Dixon's (Up and Down) method in order to determine the Median Lethal Dose (LD50). Different doses quantitatively 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16 mg/kg b.w. of Vitamin D were administered to animals through a stainless-steel gavage tube to female albino rats. After 24 hours, the mortality and survival number of rats were recorded for each dose. Dixon's (up and down) method was used (Dixon., 1965) for LD_{50} determination (Tables 1, 8, 9). The dose that was started depends on Özkan (Özkan *et al*., 2012).

Determination Of Residual Amount Of Cholecalciferol

Tissue residues were quantified using the protocol outlined by Kennedy (Kennedy, 1986).

Cytogenetic Study

The metrics used in the cytogenetic analysis followed Dutta (2022) methodology.

Histopathology

At the end of the exposure period of 45 and 90 days, rats were taken from each plastic cage. Tissues like the liver, kidney, and muscles were isolated from normal and experimental rats. The samples were initially fixed in a 10% formalin buffer for 24 hours and then processed and embedded in paraffin for block (56–58 °C) preparation. The

sections were cut at 5–6 microns and stained with hematoxylins and eosin (Nagpal *et al*., 2023). The slides were examined under a light microscope and photographed for histopathological study.

Table 1: Pilot study shows the dead and survived animals after 24 hours from treating with Vitamin D, by using different doses for LD50 determination.

Dose (mg/kg)	Animals' number	Survived animals	Dead animals
0.5	2	0	2
1	2	0	2
1.5	2	0	2
2	2	0	2
2.5	2	0	2
3	2	0	2
4	2	0	2
5	2	0	2
6	2	0	2
7	2	0	2
8	2	0	2
9	2	0	2
10	2	1	1
11	2	1	1
12	2	1	1
13	2	1	1
14	2	2	0
15	2	2	0
16	2	2	0

Statistical Analysis

The results were subjected to statistical analysis in order to identify the differences between the different results. Using the Windows SPSS (Version 19) statistical program, the sample methods were evaluated using the ANOVA test, and the mean (mean ± S.D.) was found. P ≤0.05 values were considered significant (Kim, 2017).

RESULTS

Clinical Signs

In this study, both groups (HG and LG) of rats were observed to have vitamin D toxicity symptoms. Clinically, vitamin D3-treated rats were dull, off-feed, and showed anorexia, severe diarrhea, severe dehydration, excessive thirst, weakness, rigidity of limbs, and significant progressive emaciation. There were also changes in body weight.

Determination Of Ld $_{50}$

The LD 50 for VD was measured according to Dixon's calculation.

LD_{50} = xf + kd
= 13 + (0.741) × 1
= 13 + 0.741
= 13.741 mg/kg

Applying this calculation, LD_{50} came out to be 13.741 mg/kg b. wt. for Vitamin D

The Residual Concentration

The residual concentration showed insignificant increases in kidney and liver during both periods (45 days and 90 days) Tables (2) and Table (3). For the muscle, there was a significant increasing which reach to (4.051±0.436 in group 2, and 4.191±0.52 in group 3 after 45 days. In addition to (4.854±0.867 in group 2, and 4.949±0.848 in group 3 after 90 days) Table (4).

Cytogenetic Study

The result of cytogenetic study showed presence of cytogenetic effects for vitamin D, especially in the high VD group (1.3741 mg/kg. bw VD) at 1/10LD50 as compared with low VD group (0.6875 mg/kg. bw VD) 1/20LD_{50}, that showed a significant increase in Mitotic Index (MI) reaching (6.891±1.093 in group 2 (1/10 LD_{50}) after 45 days., 6.832±0.635 in group 3 (1/20 LD_{50}) after 45 days) with insignificant increase reaching 7.364±1.237 in group 2 (1/10 LD_{50}) after 90 days., 7.304±1.202 in group 3 (1/20 LD_{50}) after 90 days), in addition to significant increase in Micronuclei (MN) in both groups and after both periods as compared with the control group Table (5). (Fig. 1) and (Fig. 2).

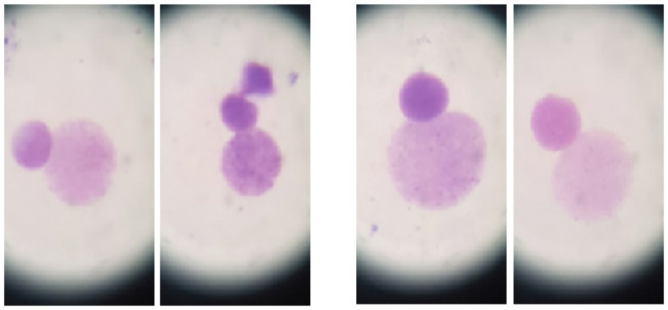

Figure 1: Shows the Micronucleus after 45 days from the beginning of experience

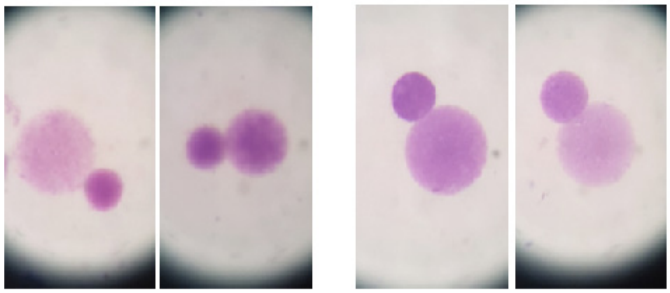

Figure 2: Shows the Micronucleus after 90 days from the beginning of experience

Table 2: Shows the residual concentration of vitamin D in kidneys after 45 days and after 90 days of experiment

Kidney/Groups	Before 45 days	After 45 days	P Value	Sign.	Before 90 days	After 90 days	P Value	Sign.
Control Group I	A,a 4.803±0.628	B,a 4.357±0.559	0.468	Non Sign.	A,a 5.025±0.543	AB,a 5.21±0.324	0.546	Non Sign.
Group II Vit.D 1/10	A,a 4.803±0.628	A,a 5.178±0.383	0.436	Non Sign.	A,a 5.025±0.543	AB,a 5.197±0.597	0.705	Non Sign.
Group III Vit.D 1/20	A,b 4.803±0.628	A,a 5.459±0.303	0.0011	Sign.	A,a 5.025±0.543	A,a 5.503±0.583	0.541	Non Sign.
P-Value		0.0062				0.0036		
LSD		0.530865				0.619874		

Table 3: Shows the residual concentration of vitamin D in livers after 45 days and after 90 days of experiment.

Liver /Groups	Before 45 days	After 45 days	P Value	Sign.	Before 90 days	After 90 days	P Value	Sign.
Control Group I	A,a 4.803±0.628	A,a 4.713±0.525	0.355	Non Sign.	A,a 5.019±0.685	A,a 5.153±0.343	0.308	Non Sign.
Group II Vit.D 1/10	A,a 4.599±0.584	A,a 5.045±0.38	0.438	Non Sign.	A,a 5.019±0.685	A,a 4.847±0.608	0.709	Non Sign.
Group III Vit.D 1/20	A,a 4.599±0.584	A,a 4.962±0.299	0.216	Non Sign.	A,a 5.019±0.685	A,a 5.108±0.666	0.430	Non Sign.
P-Value		0.00046				0.008		
LSD		0.473691				0.58208		

Table 4: Shows the residual concentration of vitamin D in muscles after 45 days and after 90 days of experiment

Muscles/Groups	Before 45 days	After 45 days	P Value	Sign.	Before 90 days	After 90 days	P Value	Sign.
Control Group I	A,a 3.166±0.77	AB,a 3.561±0.571	0.522	Non Sign.	A,a 5.025±0.543	AB,a 5.21±0.324	0.546	Non Sign.
Group II Vit.D 1/10	A,a 3.166±0.77	A,b 4.051±0.436	0.0006	Sign.	A,a 5.025±0.543	AB,a 5.197±0.597	0.705	Non Sign.
Group III Vit.D 1/20	A,a 3.166±0.77	A,b 4.191±0.52	0.0014	Sign.	A,a 5.025±0.543	A,a 5.503±0.583	0.541	Non Sign.
P-Value		0.00062				0.0036		
LSD		0.681356				0.619874		

Table 5: Shows the Micronuclei (MN), and Mitotic Index (MI) after 45 days and after 90 days of experiment.

Groups	MN % After 45 days	MN % After 90 days	MI % After 45 days	MI % After 90 days
Group Control (CG)	**C 5.483±0.625**	C 6.391±0.923	B 5.723±0.778	C 6.413±0.775
Group 2 (HG) 1/10 LD_{50}	AB 7.983±0.799	A 8.46±1.09	A 6.891±1.093	BC 7.364±1.237
Group 3 (LG) 1/20 LD_{50}	AB 7.537±0.704	A 8.238±1.403	A 6.832±0.635	BC 7.304±1.202
LSD	0.907049	1.064587	1.017477	1.342605

In addition to the changes in chromosomal aberration (which appear 0.439±0.2026 Deletion %., 0.344±0.1319 Dicentric %., 0.348±0.1475 Acentric %., 0.3944±0.1359 Ring %.,0.3417±0.1241 Chromatid Gap %.,0.3961±0.1438 Chromosome Gap %., 0.3961±0.1438 Chromatid break %., 0.4464±0.1233 Chromosome break % in high dose group (HG) after 45 days., while in low dose group (LD) the result was 0.3497±0.1453 Deletion %., 0.5005±0.1955 Dicentric %., 0.251±0.252 Acentric %., 0.2957±0.1013 Ring %., 0.3454±0.1292 Chromatid Gap %., 0.2952±0.2029 Chromosome Gap %., 0.401±0.298 Chromatid break %., 0.3454±0.1292 Chromosome break % after 45 days) Table (6). (Fig. 3) and (Fig. 4), in the period of 90 days the results for the Group 2 (HG) 1/10LD50, the results were 0.4382±0.183 Deletion %., 0.3924±0.1239 Dicentric %., 0.3468±0.1381 Acentric %., 0.3468±0.1381 Ring %., 0.3924±0.1239 Chromatid Gap %., 0.4936±0.1688 Chromosome Gap %., 0.4981±0.1854 Chromatid break %.,

Table 6: Shows the Chromosomal aberration after 45 days of experiment.

Groups	Deletion %	Dicentric %	Acentric %	Ring %	Chromatid Gap %	Chromosome Gap %	Chromatid break %	Chromosome break %	Total %
Group Control (CG)	A 0.2181± 0.1524	A 0.261± 0.1762	A 0.2208± 0.162	A 0.338± 0.198	A 0.1778± 0.1144	B 0.1439± 0.1463	B 0.1853± 0.1399	B 0.2144± 0.0743	B 1.759± 0.606
Group 2 (HG) 1/10LD$_{50}$	A 0.439± 0.2026	A 0.344± 0.1319	A 0.348± 0.1475	A 0.3944± 0.1359	A 0.3417± 0.1241	A 0.3961± 0.1438	AB 0.3961± 0.1438	A 0.4464± 0.1233	A 3.106± 0.422
Group 3 (LG) 1/20LD$_{50}$	A 0.3497± 0.1453	A 0.5005± 0.1955	A 0.251± 0.252	A 0.2957± 0.1013	A 0.3454± 0.1292	AB 0.2952± 0.2029	AB 0.401± 0.298	AB 0.3454± 0.1292	A 2.784± 0.7
LSD					0.197235	0.215699	0.224377	0.179413	0.920742

Table 7: Shows the Chromosomal aberration after 90 days of experiment.

Groups	Deletion %	Dicentric %	Acentric %	Ring %	Chromatid Gap %	Chromosome Gap %	Chromatid break %	Chromosome break %	Total %
Group Control (CG)	A 0.2587± 0.1093	A 0.2617± 0.2098	A 0.3307± 0.1644	A 0.3307± 0.1644	B 0.2189± 0.1515	A 0.2953± 0.1082	C 0.2277± 0.1633	A 0.2216± 0.1578	B 2.218± 0.579
Group 2 (HG) 1/10LD$_{50}$	A 0.4382± 0.183	A 0.3924± 0.1239	A 0.3468± 0.1381	A 0.3468± 0.1381	AB 0.3924± 0.1239	A 0.4936± 0.1688	AB 0.4981± 0.1854	A 0.4975± 0.1821	A 3.46± 0.501
Group 3 (LG) 1/20LD$_{50}$	A 0.408± 0.228	A 0.361± 0.231	A 0.2546± 0.1787	A 0.2546± 0.1787	AB 0.355± 0.227	A 0.405± 0.29	ABC 0.4076± 0.1365	A 0.4605± 0.1171	AB 3.058± 0.568
LSD					0.220606		0.208468		0.916095

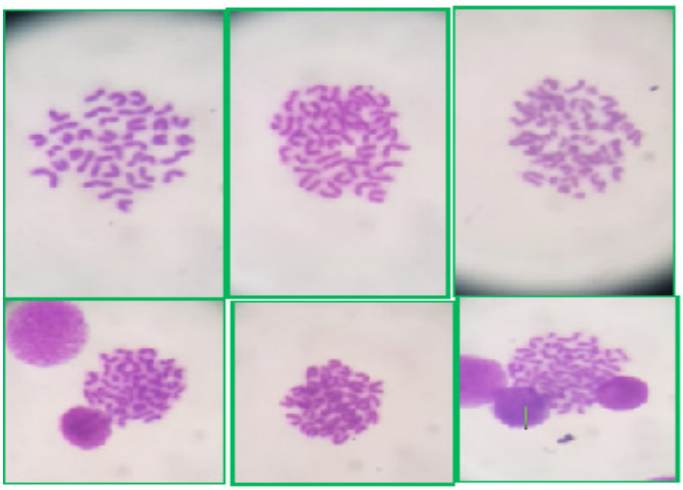

Figure 3: Shows the Chromosomal Aberration in high dose group (HG) After 45 days of experiment

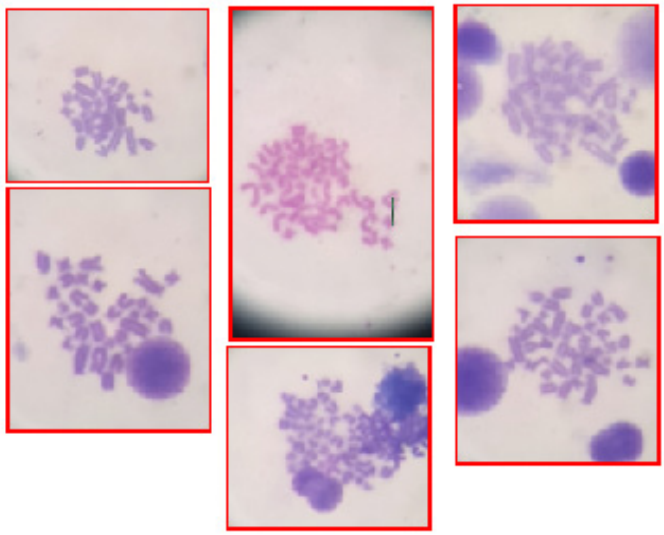

Figure 4: Shows Chromosomal Aberration in the Low dose group (LG) After 45 days of experiment

0.4975±0.1821 Chromosome break %., in the Group 3 (LG) 1/20LD50, the results were 0.408±0.228 Deletion %., 0.361±0.231 Dicentric %., 0.2546±0.1787 Acentric %., 0.2546±0.1787 Ring %., 0.355±0.227 Chromatid Gap %., 0.405±0.29 Chromosome Gap %., 0.4076±0.1365 Chromatid break %., 0.4605±0.1171Chromosome break %. Table (7). (Fig. 5) and (Fig. 6).

Gross Pathological Examination

All samples were collected after 45 days and after 90 days of experimental periods from anesthetized rats. Post-mortem examination of rats poisoned with cholecalciferol has shown congestion and hemorrhages in all visceral organs.

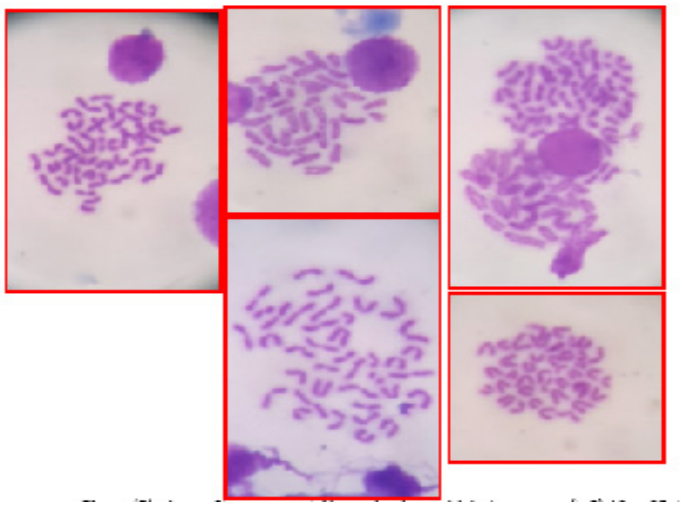

Figure 5: shows Chromosomal Aberration in the high dose group (HG) After 90 days of experiment

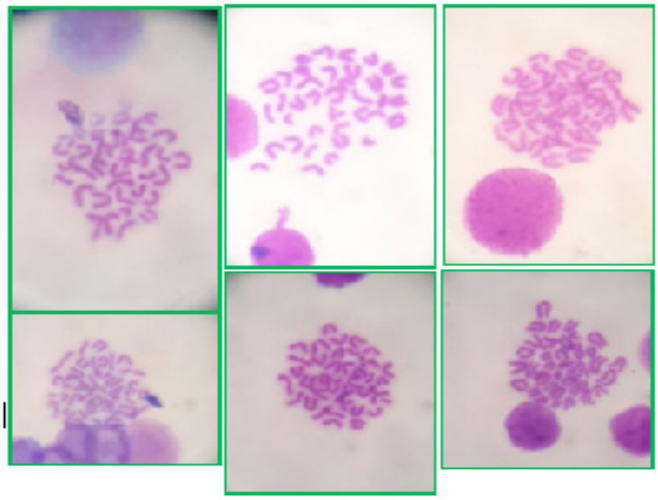

Figure 6: shows the Chromosomal Aberration in low dose group (LG) After 90 days of experiment

Histopathological Study

The histopathological study of tissues affected by toxic cholecalciferol exhibited varying stages of mild to severe degenerative changes and necrosis of the tissues.

LIVER

The lesions after 45 days showed simple histopathological changes in the liver, which were represented by congestion of the hepatic sinusoids and central vein (Fig. 7: A). In addition to cellular swelling and congestion of blood vessels (Fig. 7: B). At dose 1/20LD50 (274.820 IU, i.e., 0.6875 mg/kg body weight), lesions appear milder than at dose 1/10LD50 (549.640 IU, i.e., 1.3741 mg/kg body weight). but it can progress more severely in the period of 90 days, that shows severe inflammation as represented by pericentral necrosis and marked infiltration of inflammatory cells (Fig. 7: C). In the lower toxic dose (1/20LD50), besides congestion of the central vein, there was slight fibrosis in pericentral necrotic areas due to cholecalciferol toxicity (Fig. 7: D).

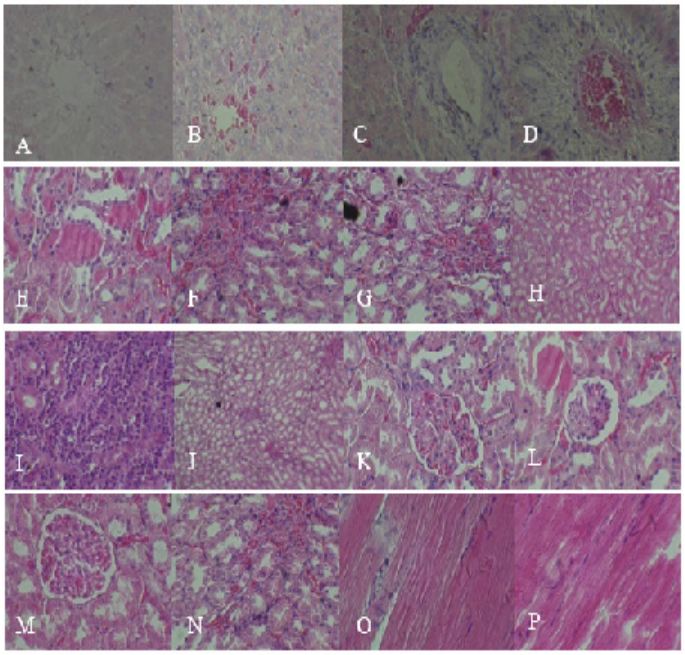

Figure 7: Histopathological section for different animals showing congestion of hepatic sinusoids and central vein (A), and cellular swelling (B), pericentral necrosis with infiltration of inflammatory cells (C), with fibrosis and congestion of central vein (D). The kidney sections show the presence of hyaline cast with sloughing of epithelial lining urinary tubules (E), congestion with infiltration of inflammatory cells (F, G, H and J), besides areas of necrosis (I), besides sloughing of epithelium lining urinary tubules (K), with dilatation of Bowman space (L) and congestion of the glomerular tuft with vacuolation in renal tubules (M), with degenerative changes (swelling) in the renal tubular epithelium (N). Muscle section shows infiltration of inflammatory cells besides edema causes separation of muscle's fibers in addition to the presence of fragmentation and necrosis (O and P).

KIDNEY

Histological descriptions of kidneys reveal renal injury of variable intensity, congestion with inflammation, and toxic tubular necrosis. The severity of the lesion was noted in the 45th day, which is less severe than the lesion in the 90th days, which was characterized by the infiltration of inflammatory cells along with the presence of hyaline cast with sloughing in the epithelial lining of urinary tubules (Fig. 7: E), In the period of 90 days, in group II (HG), severe infiltration of inflammatory cells in interstitial tissue (Fig. 7: F, G, H, and J), in addition to necrosis, was also observed (7: I). In group III (LG), there is sloughing of the epithelium lining urinary tubules (Fig. 7: K) and hyaline casts were also present with dilation of Bowman space (Fig. 7: L), and glomerular congestion with vacuolation was observed in renal tubules (Fig. 7: M). Along with the degenerative changes (swelling) of varying severity in the renal tubular

epithelium and infiltration of inflammatory cells (Fig. 7: N).

MUSCLES

In the muscle, the lesions were very staggering and became more severe as the dose increased or the period increased. In the 45th day (1/10 LD50) (549.640 IU, i.e. 1.3741 mg/kg body weight), There was fragmentation in addition to the presence of necrosis (Fig. 7: O), The severity of inflammation increased with the period of 90 days. At the 90th day (1/20LD50), edema causes separation of the muscle's fibers besides infiltration of inflammatory cells, in addition to the presence of fragmentation and necrosis (Fig. 7: P). All these changes are clearly evident, especially at the dose (1/10 LD50) (549.640 IU, i.e., 1.3741 mg/kg body weight), where they lead to lesions in the muscle, in addition to lesions varying in doses or periods.

Table 8: Shows the doses of Vitamin D by using Dixon's method for LD50 determination. Survived (O) and dead (X) Result was (OOOXOXO)

Dose (mg/kg)	Survived and dead animals
11	O
12	O
13	O
14	X
13	O
14	X
13	O

Table 9: value of K for estimating LD_{50} from Up and Down. If the table is entered from the foot, the sign of K is to be reversed (Dixon, 1965).

N	Second Part of Series	k for Test Series Whose First Part is O	OO	OOO	OOOO		Standard Error of LD_{50}
2	X	−.500	−.388	−.378	−.377	O	.88σ
3	XO	.842	.890	.894	.894	OX	.76σ
	XX	−.178	.000	.026	.028	OO	
4	XOO	.299	.314	.315	.315	OXX	.67σ
	XOX	−.500	−.439	−.432	−.432	OXO	
	XXO	1.000	1.122	1.139	1.140	OOX	
	XXX	.194	.449	.500	.506	OOO	
5	XOOO	−.157	−.154	−.154	−.154	OXXX	.61σ
	XOOX	−.878	−.861	−.860	−.860	OXXO	
	XOXO	.701	.737	.741	.741	OXOX	
	XOXX	.084	.169	.181	.182	OXOO	
	XXOO	.305	.372	.380	.381	OOXX	
	XXOX	−.305	−.169	−.144	−.142	OOXO	
	XXXO	1.288	1.500	1.544	1.549	OOOX	
	XXXX	.555	.897	.985	1.000+1	OOOO	
6	XOOOO	−.547	−.547	−.547	−.547	OXXXX	.56σ
	XOOOX	−1.250	−1.247	−1.246	−1.246	OXXXO	
	XOOXO	.372	.380	.381	.381	OXXOX	
	XOOXX	−.169	−.144	−.142	−.142	OXXOO	
	XOXOO	.022	.039	.040	.040	OXOXX	
	XOXOX	−.500	−.458	−.453	−.453	OXOXO	
	XOXXO	1.169	1.237	1.247	1.248	OXOOX	
	XOXXX	.611	.732	.756	.758	OXOOO	
	XXOOO	−.296	−.266	−.263	−.263	OOXXX	
	XXOOX	−.831	−.763	−.753	−.752	OOXXO	
	XXOXO	.831	.935	.952	.954	OOXOX	
	XXOXX	.296	.463	.500	.504+1	OOXOO	
	XXXOO	.500	.648	.678	.681	OOOXX	
	XXXOX	−.043	.187	.244	.252+1	OOOXO	
	XXXXO	1.603	1.917	2.000	2.014+1	OOOOX	
	XXXXX	.893	1.329	1.465	1.496+1	OOOOO	
		X	XX	XXX	XXXX	Second Part of Series	
		−k for Series Whose First Part is					

Table 10: List of the abbreviations

Abbreviations	Full name
bw	body weight
°C	centigrade
CG	CG control group
HG	High VD group
i.e.	That is
IU	International Units
LD_{50}	Median Lethal Dose
LG	Low VD group
mg/kg	milligram/kilogram
MI	Mitotic Index
MN	Micronuclei
SD	Standard Deviation
VDI	Vitamin D Intoxication
VDR	Vitamin D Receptors
VD	Vitamin D
VD3	Vitamin D3

DISCUSSION

Median Lethal Dose (LD50) came out to be 13.741 mg/kg b. wt. for Vitamin D; this value disagrees with previous studies such as (Özkan *et al.*, 2012; Singla and Kaur., 2015). This difference may be due to the uneven pharmacological purity among suppliers' origins, the individual variation among numbers of rats, or perhaps the altered surroundings. The results show a significant decline in daily change in body weight, in both dosed groups during the dosing period, which concurs with the research that Marcinowska conducted (Marcinowska-Suchowierska *et al.*, 2018). Higher oxidative stress can promote the acceleration of cell death, while lower oxidant levels may promote slower cell death. That is why higher vitamin D toxicity (VDT) is severer than lower vitamin D toxicity (VDT), which also agrees with Nicotera (Nicotera and Orrenius., 1994). Since the target tissue organs of vitamin D are the kidney, liver, and muscle, that is why vitamin D toxicity (VDT) killed cells of these tissues, which also agrees with the other researchers (Wimalawansa, 2019). Microscopic lesions were seen in the kidneys of both dosed rats killed at the end of the first and second periods, which agrees with the study conducted by Abbas and Al-Shaha (2021).A hyperdose of vitamin D led to congestion of blood vessels with degeneration in hepatocytes that is showing fibrosis; this agrees with previous studies (Chavhan *et al.*, 2011; Lim and Thadhani., 2020). The groups II and III were characterized by highly aggregation of inflammatory cells, which concurs with the research that Qaisi conducted (Al-Qaisi, 2000). Histopathological examination showed infiltration of the nuclear cell, which agrees with the findings of Ahmed and

Mohammed's research (Ahmed and Mohammed.,2022). The histopathological examination of the kidney shows congestion of blood vessels and infiltration with neutrophils in the lumen, which concurs with Saffar's research findings (AL-Saffar, 2012). Glomerular lesions in groups II and III were found, characterized by dilation of glomeruli, which showed lipid accumulation, which is consistent with Ibrahim Mustafa's and Elkhadragy's research (Ibrahim Mustafa., 1996; Elkhadragy *et al*., 2022). The results indicated that cholecalciferol significantly increased the micronucleus in groups II and III, which concurs with the research that Qaisi conducted (Al-Qaisi, 2000).

CONCLUSION

Vitamin D consumption increases tissue levels of vitamin D supplements (1/10 LD50 and 1/20 LD50) compared with a control group. From the results of this study, it can be concluded that oral intake of lethal dose of cholecalciferol (vitamin D3) by rats led to their toxicity in the form of mineralization in the vital organs and resulted in the mortality of rats. This preliminary study shows that both doses of vitamin D can be toxic to organisms at higher levels, so they need to be used carefully and should not be taken excessively.

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RECOMMENDATIONS

Study of vitamin D intoxication influence in the brain and bones. More study is needed to know the effect of different doses of vitamins on human and animal health.

NOVELTY STATEMENT

In recent years, there has been a renaissance in the study of vitamin D actions as evidence continues to accumulate about its role in the etiology of chronic diseases such as infection response, autoimmune disease, cardiovascular disease, diabetes mellitus, and cancer. The novelty of the study is focusing on cytogenetic, histopathological, and residual concentrations of vitamin D hyperdoses that influence tissues (livers, kidneys, and muscles) due to their pathological toxic effects.

AUTHORS CONTRIBUTION

Both of these authors contributed equally.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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