

Acute hepatologic and nephrologic effects of calcitriol in Syrian golden hamster (*Mesocricetus auratus*)*

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Although vitamin D is included in the group of fat-soluble vitamins, it must be considered as a prohormone. Its active forms, including calcitriol, have pleiotropic effects and play an important role in the regulation of cell proliferation, differentiation and apoptosis, as well as in hormone secretion, and they demonstrate anti-cancer properties. Since calcitriol delivery can be beneficial for the organism, and Syrian golden hamsters represent a unique experimental model, we decided to investigate its toxicity in this species. In this study, we injected calcitriol intraperitoneally at doses 0 (control), 0.180 ± 0.009 $\mu\text{g}/\text{kg}$ and 0.717 ± 0.032 $\mu\text{g}/\text{kg}$. Animal behavior was observed for 72 hrs after injection, and afterwards blood, liver and kidneys were collected for post-mortem examination, electron microscopy, and hematology analyses. The highest dose of calcitriol induced a change in animal behavior from calm to aggressive, and the liver surface showed morphological signs of damage. Following injection of calcitriol, ultrastructural changes were also observed in the liver and kidneys, e.g. vacuolization and increased number of mitochondria. There was also a trend for increased serum levels of aspartate aminotransferase (AST), but not of alanine aminotransferase (ALT) or GGTP

(gamma-glutamyl transpeptidase). There was no change in Ca, Mg and P levels, as well as in blood morphology between experimental and control groups. These results indicate that calcitriol at 0.717, but not at 0.180 $\mu\text{g}/\text{kg}$, may induce acute damage to the liver and kidneys, without inducing calcemia. We propose that the hepatotoxic effect of calcitriol in hamster constitutes the primary cause of behavioral changes.

Key words: calcitriol, Syrian golden hamster, hepatologic toxicity, nephrologic toxicity

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Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ES, Endocrine Society; GGTP, gamma-glutamyl transpeptidase; UVB, ultraviolet B; VDBP, Vitamin D Binding Protein; VDT, Vitamin D Toxicity

Figure S1

Detailed composition of the Labofeed B standard (Wytwórnia Pasz "Morawski", Poland).

[<https://www.sukces.info.pl/labofeed-b>]

Raw protein	g	175	Vitamin B1	mg	8
Crude fat	g	35	Vitamin B2	mg	7
Crude fiber	g	70	Vitamin B6	mg	11
Starch	g	330	Vitamin B12	µg	42
Ash	g	32	Pantothenic acid	mg	25
Calcium	g	9.5	Folic acid	mg	2
General phosphorus	g	6.5	Biotin	mg	0.3
Magnesium	g	3	Nicotinic acid	mg	94
Potassium	g	7.5	Choline	mg	1900
Sodium	g	1.9	Lysine	g	9
Sulfur	g	1.9	Methionine + Cysteine	g	6.3
Iron	mg	144	Tryptophan	g	2
Manganese	mg	50	Threonine	g	6
Zinc	mg	50	Isoleucine	g	6
Copper	mg	11	Leucine	g	12
Iodine	mg	0.2	Valine	g	8
Selenium	mg	0.4	Histidine	g	4
Vitamin A	IU	12000	Arginine	g	10
Vitamin D3	IU	800	Phenylalanine	g	7
Vitamin E	mg	78	Tyrosine	g	5.5
Vitamin K3	mg	2.4	Betaine		17

Figure S2

Liver mass of the hamsters treated with calcitriol for 72 hours. The data labels indicate the animal code.

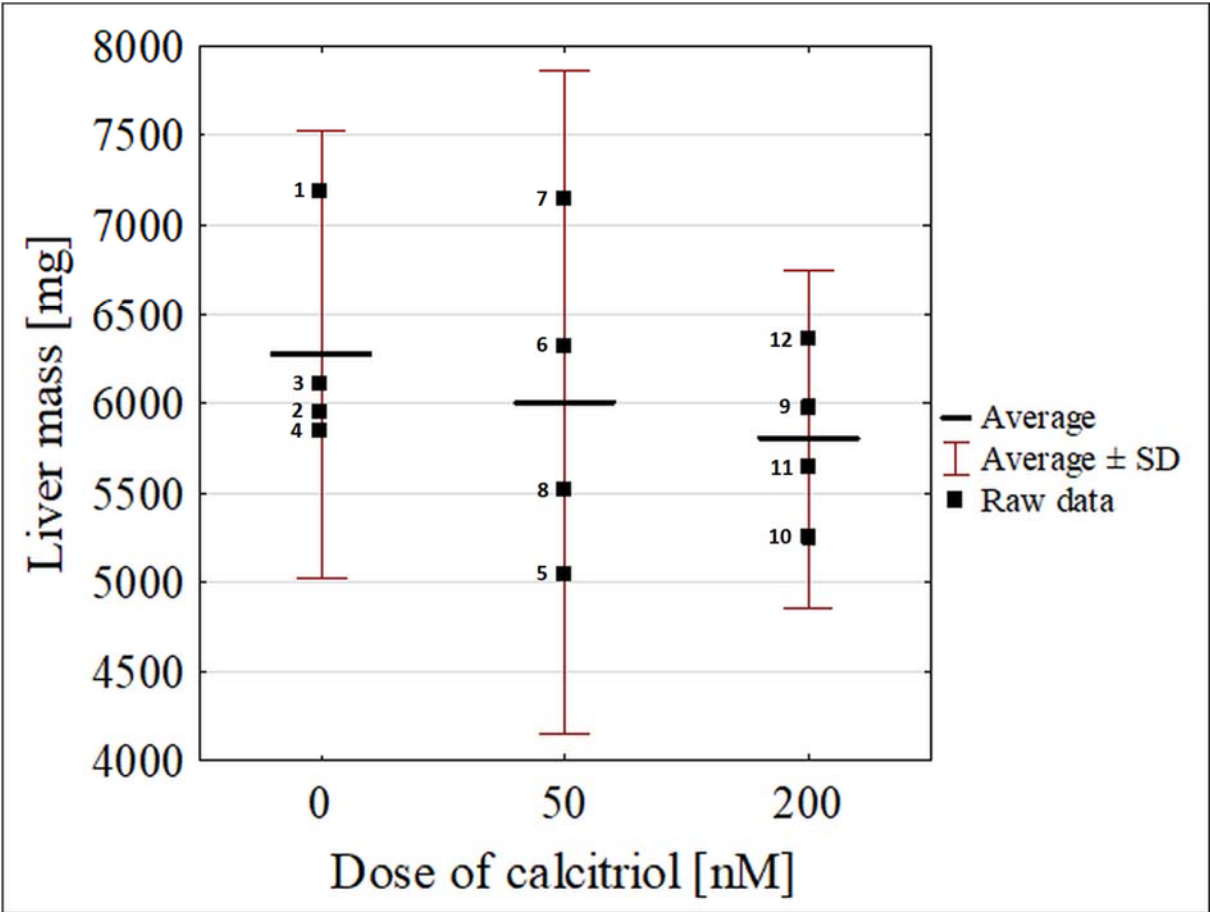


Figure S3

Mean values for hematological parameters of the blood from hamsters treated with calcitriol. Levels of Mg, P and Ca (Table S1), leukocyte parameters (Table S2) and red blood cell parameters (Table S3).

Dose of calcitriol	Parameters		
	Mg (mg/dl)	P (mg/dl)	Ca (mg/dl)
	Mean ± SD	Mean ± SD	Mean ± SD
0 nM	4,38 ± 0,31	9,60 ± 1,44	13,97 ± 0,97
50 nM	4,57 ± 0,13	10,55 ± 0,98	13,80 ± 0,85
200 nM	4,72 ± 0,21	8,71 ± 1,25	14,53 ± 1,23

Table 1.

Dose of calcitriol	Parameters				
	WBC (x10 ³ /ul)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Monocytes (%)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
0 nM	3,53 ± 1,38	27,50 ± 5,07	70,25 ± 4,92	2,25 ± 0,50	0,00 ± 0,00
50 nM	1,90 ± 0,22	25,75 ± 2,89	73,25 ± 2,62	1,08 ± 0,91	0,00 ± 0,00
200 nM	3,35 ± 2,47	9,94 ± 1,04	88,72 ± 1,16	1,33 ± 0,58	0,00 ± 0,00

Table 2.

Dose of calcitriol	Parameters					
	RBC (x10 ⁶ /ul)	PCV (%)	Hb (g/dl)	MCV (fl)	MCH (pg)	MCHC (%)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
0 nM	6,16 ± 0,10	36,15 ± 0,76	12,83 ± 0,17	58,65 ± 0,64	20,83 ± 0,17	35,48 ± 0,65
50 nM	6,57 ± 0,34	38,45 ± 1,87	13,28 ± 0,62	58,58 ± 0,72	20,23 ± 0,45	34,53 ± 0,43
200 nM	6,70 ± 0,28	38,60 ± 0,99	13,70 ± 0,28	57,65 ± 0,92	20,45 ± 0,49	35,50 ± 0,14

Table 3.

Figure S4

Scatter plot of ALT vs AST for a calcitriol dose of 200 nM. ALT = 26.8489+0.1653x. AST:
ALT r= 0.9845; p= 0.0155; r²= 0.9692

