



Effect of Alkaline Drinking Water on Vitamin D3 Toxicity in Female Rats



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THIS investigation focused on how consuming alkaline water affected vitamin D3 toxicity and how it related to rats' motor and neurobehavioral activity as well as some biochemical factors. 15 rats were employed, each allocated into three groups. Tap water was provided to the first group (the negative control), alkaline water to the second group, and alkaline water combined with a weekly intramuscular injection of vitamin D3 in a dose of 100,000 unit/animal to the third group for a period of 4 weeks. Changes in the neurobehavioural and motor activity of the rats were noted in the open field. The weights of the rats in the vitamin D3 group increased. The number of squares and times they stand on their hind legs, and the number of pocking were all significantly higher in the group of vitamin D3 also vitamin D3 levels increased noticeably and considerably lower levels of total cholesterol, Low density lipoprotein levels were also reduced. This investigation has led us to the conclusion that drinking alkaline water can help people and animals who are taking large amounts of vitamin D3, in addition to its effect in reducing the levels of total cholesterol and Low-density lipoprotein with improving body weight and oxidative stress status.

Keywords: Alkaline water, Vitamin D3, Toxicity, Female rats.

Introduction

1,25-dihydroxycholecalciferol, the active metabolite of vitamin D, influences target cell function via controlling gene expression as well as by non-genetic activity. The intracellular vitamin D receptor (VDR), a member of the nuclear receptor family that regulates gene expression, acts as a transcription factor and controls the expression of several genes involved in metabolism. Rapid binding of the cell membrane and the vitamin D steroid-binding receptor (MARRS) found in the plasma membrane is another 1,25-(OH)D impact that is not hereditary [1,2].

By releasing calcium ions (Ca) from intracellular reserves and allowing calcium ions

to enter the cell through calcium channels, the hormonal form of vitamin D controls calcium concentration in the cytosol. Additionally, it influences the activity of the enzymes adenylate and phospholipase C (PLC) [3,4].

Vitamin D receptors are found throughout the body's cells, not just those immediately involved in calcium metabolism. It consists of brain cells, parathyroid tissue, ovaries, testicles, thymus, stomach cells, pancreatic cells, and progenitors of white blood cells [5]. These findings highlight the multifaceted impacts of vitamin D by pointing to a significant function for these receptors as well as for vitamin D itself in regulating many metabolic pathways.

Through its involvement in the operation of the central nervous system, vitamin D has been linked to evidence supporting its role in the regulation, development, and function of neurons. The existence of vitamin D receptors in the brain, particularly in the hypothalamus and dopaminergic neurons in the substantia nigra, as well as the enzyme 25(OH)D-1-hydroxylase, which is responsible for the synthesis of the active form of vitamin D, support the central nervous system [6].

Due to its interaction with MARRS receptors, vitamin D is thought to function similarly to neurosteroids in the nuclear 1-hydroxylase VDRs and other intracellular metabolic pathways [1, 2]. They are also present in the central nervous systems (CNS) non-neuronal cells known as microglia [7].

We chose to carry out this study since there haven't been many investigations into how alkaline water affects vitamin D3 toxicity.

Material and Methods

Animals

The University of Mosul's College of Veterinary Medicine's animal house provided the white female rats used in this investigation. The rats were between 200 and 250 g in weight. The rats were housed in cages designed especially for this purpose, given access to plenty of water and food, and given ventilation, temperature, lighting, and bedding requirements.

Chemicals and medicines

Italy's Sirton Pharmaceuticals S.P.A. produces vitamin D3 and sodium bicarbonate.

Ethical approvals

The ethical approvals were obtained from the University of Mosul, College of Veterinary Medicine, its number is UM.VET.2021.35.

Animal Weighing

Animals were weighed weekly, and their weights and rates were noted both before and after the tests.

Blood sample collection

To extract the blood serum, blood was drawn from a vein in the inner corner of the eye and deposited in specialized tubes. The serum samples centrifuged at 3000 rpm for 15 minutes, then stored in special plastic tubes and frozen at -20°C for the completion of laboratory biochemical tests.

Preparing Alkaline water

Using a pH meter, the pH of drinking water, which

is pH>8, was determined. The pH was then fixed using sodium bicarbonate.

Experiment Design

In this study, 15 rats were employed, each allocated into three groups. Tap water was provided to the first group (negative control), alkaline water to the second group (positive group), and alkaline water combined with a weekly intramuscular injection of vitamin D3 in a dose of 100.000 unit/animal to the third group for a period of 4 weeks.

Neurobehavioral and motor tests

First, examine the rat's neurological and motor behaviour in an open field. Used was a rectangular wooden box with the following measurements: 90 x 60 x 30 cm. Its floor was divided into 24 identical squares, each with a 15 cm side. According to the quantity of cut squares, and how many times the animal must stand on its hind legs after being placed in the box's middle. Each animal underwent the test for three minutes. After each animal has been measured, the floor is cleaned by wiping it down with cotton and alcohol. This measurement examines the rats' general motion inside the box [8].

Second, examine is poking test A plastic surface with 10 circular holes and a 20 cm height was used for the test. It has a 30 cm radius. The experiment involved watching the animal and counting how many times its head was placed into the perforations. Each animal will undergo the test for 3 minutes. This test evaluates the animal's interest and level of acculturation to its environment [8].

Biochemical markers

- *Measuring the serum level of vitamin D*
Utilizing a unique VD3 (Vitamin D3) ELISA Kit from Elabscinsce, USA.

- *Measuring the serum calcium level*
A detection for the quantitative assessment of was developed using a testing kit from the French company Biolabo.

Five minutes at room temperature and a wavelength of 570 nm were used to measure the total serum calcium response (550-590).

- Monitoring malondialdehyde and glutathione levels in the serum to examine the level of oxidative stress.

- Measuring the serum glutathione concentration: The modified Elman method [9] was employed

- to gauge the serum glutathione concentration.
- Measuring the serum's MDA concentration: Malondialdehyde and thiobarbituric acid interact to generate the MDA-TBA2 complex, which is absorbed at a wavelength of 352 nm. This interaction constituted the basis for the approach [10].
 - Researching the impact on the serum's cholinesterase activity.

The following procedures were followed in order to measure the modified cholinesterase using the modified electrometric method, [11].

- The blood lipid profile

A unique tool was used to assess triglycerides, high-density lipoprotein, and very low-density lipoprotein. Following is a list of measurements for these variables:

- Calculation of the serum total cholesterol concentration

Using the German company Roche's 311 Cobas C equipment and the enzymatic approach with the CHO21 measurement kit, the concentration of cholesterol in the serum was estimated as shown in the following equations:

- Measuring the serum's triglycerides

Using the 311 Cobas C instrument from the German company Roche and the enzymatic approach by the TRIGL.

- Very low density lipoproteins in serum (VLDL-c)
VLDL-c (100 ml\mg)=Triglycerides/5
- Measurement of serum high-density lipoprotein (HDL-c)

Using the German company Roche's 311 Cobas C equipment and the enzymatic method described in the measurement kit

Analytical statistics

The one-way analysis of variance test (ANOVA) was used to statistically examine the parametric data before the LSD test was run on them and nonparametric data analysis by the Man Whitney test.

Results

After four weeks of therapy, the weights of the rats in the vitamin D3 and alkaline water groups significantly increased as compared to the positive and negative control groups (Table 1).

Rats were tested for neurobehavioral and motor activity in an open field, and the results revealed that, when compared to the positive control group, the vitamin D3 with alkaline water group significantly increased the number of squares cut by the rats, the number of times they stood on their hind legs, and the number of times they put their heads into the holes (Table 2).

The group of vitamin D3 exhibited a substantial rise in their concentration in the serum

TABLE 1. effect of the Alkaline water on the weights of rats treated vitamin D3

Groups	Weight of rats/g
Negative control	216± 12
Positive control (Alkaline water).	218± 12
Vit. D3+ Alkaline water	225 ±23*#

Each group consisted of 5 animals,

* Represents a difference from the negative control group,

represents a difference from the positive control group

TABLE 2. Effect of alkaline water and vitamin D3 on the motor neuro- behavioral response

Groups	Number of Squares	Rearing	Number of Pocking
Negative control	82 ±4	15± 2	8± 1
Positive control (Alkaline water).	70± 5	16 ±4	5 ±1.2
Vit. D3+ Alkaline water	80± 4#	252± *#	7 ± 1 #

Each group consisted of 5 animals,

* Represents a difference from the negative control group,

represents a difference from the positive control group

when compared to the positive control and the negative control (Table 3).

Malondialdehyde, glutathione, and cholinesterase concentrations did not change significantly when vitamin D3 was combined with alkaline water compared to the negative and positive controls (Table 4).

In comparison to the negative and positive control groups, the vitamin D3 group treated with alkaline water had considerably lower levels of total cholesterol, and the same group also had lower levels of very low density lipoprotein (Table 5).

Discussion

Our findings suggest that Alkaline water has a role in demonstrating the benefits of vitamin D3 even at high dosages when compared to the control, as evidenced by our study's findings.

Rats given alkaline water with vitamin D3 gained weight when compared to the control group, which clarifies how vitamin D3 affects insulin levels [12].

Furthermore, studies indicate that vitamin D may directly alter appetite, presumably through recently identified vitamin D receptors in the brain [13]. Rats' motor activity and neurobehavioral both significantly increased as compared to the control group, as seen by an increase in the number of squares they pass, the number of times they stood

on their back legs, and the number of times they put their heads into the holes. Given that vitamin D3 reaches all bodily cells, including neurons and glia, and has an impact on them, it may play a part in this. In a study on neurobehavior and cognition in rats, it was revealed that enhanced cognition may be the cause of the rats' increased exploratory behaviour during the curiosity test.

As shown by prior research, vitamin D can have neuroprotective benefits and ameliorate cognitive impairment in a variety of animal models. Additionally, the substance 1,25-(OH)D has a significant role in altering the synthesis of several neurotransmitters, including acetylcholine, by boosting the gene expression of the CAT enzyme [14].

Additionally, it has been discovered that vitamin D affects the expression of genes involved in GABA-ergic neurotransmission and that it promotes the production of the enzyme tyrosine hydroxylase (TH), which is in charge of catecholamine synthesis [15].

The GABA neurotransmitter, which is the primary "brake" in the motor cortex and influences the brain on muscular relaxation via cortical neurons [16], is one neurotransmitter that VDRs in glial cells are engaged in absorbing and releasing. Due to vitamin D's role in enhancing the transcription of the tryptophan hydroxylase-2 gene, which results in an increase in the conversion of tryptophan to serotonin in the brain, one study

TABLE 3. effect of Vitamin D3 with alkaline water in level D3 and Ca in rat serum

Groups	Vit. D3 (ng/L)	Ca (mmol/L)
Negative control	25.1±3.01	2.1±0.01
Positive control (Alkaline water).	42.4±3.01 *	2.7±0.01
Vit D3+ Alkaline water	117.6±8.01 *#	2.2±0.01

Each group consisted of 5 animals,

* Represents a difference from the negative control group

represents a difference from the positive control group,

TABLE 4. shows the effect of alkaline water on oxidative stress and acetylcholinesterase activity.

Groups	MDA (nmol/L)	GSH (mmol/L)	Ache activity
Negative control	2.52±0.03	0.71± 0.02	0.28 ±0.01
Positive control (Alkaline water).	2.72± 0.008	0.59 ±0.02	0.37 ±0.009
Vit D3+ Alkaline water	2.70±0.009	0.76 ±0.009	0.44 ±0.02

Each group consisted of 5 animals and the values represented the mean ± standard error

* Represents a difference from the negative control group,

represents a difference from the positive control group,

TABLE 5. vitamin D3-related lipid profile in blood using alkaline water.

Parameters/ Groups	TG (mmol/L)	TC(mmol/L)	VLDL(mmol/L)	c-HDL(mmol/L)
Negative control	0.6±0.01	2.2±0.02	1.6±0.01	0.4±0.01
Positive control (Alkaline water).	0.7±0.1	2.8±0.1	1.4 ± 0.3	1.6±0.1
Vit D3+ Alkaline water	0.6±0.2	0.8±0.2*#	2.0±0.3	0.2 ± 0.01*#

The values, which reflected the mean and standard error for each group of five animals.

*Represent a difference from the negative control group,

represents a difference from the positive control group.

found that vitamin D-treated rats had significantly higher serum serotonin levels than the control group [17].

The creation of calcium-binding proteins, such as parvalbumin, as part of vitamin D's protective action, helps to maintain cellular calcium homeostasis, which is crucial for brain cell function [18].

This illustrates how preventing calcium ions from flooding neurons can protect the brain from the hormonal form of vitamin D [19].

Since 1,25-dihydroxyvitamin D can lower the activity of reactive oxygen species (ROS) and may even stimulate the phagocytosis of amyloid beta (A) in Alzheimer's patients, the high amount of vitamin D3 in alkaline water also preserved the natural antioxidant status [6]. And what supports this is the absence of oxidative stress, where the levels of glutathione and malondialdehyde remained roughly the same in the experimental groups while not rising, suggesting that vitamin D3 is efficient in preventing lipid oxidation. The findings are in line with those of an earlier study that discovered that vitamin D inhibits iron-induced lipid peroxidation by stabilising the membrane against lipid peroxidation and by the interaction between its hydrophobic rings of the molecule structure and that the hydrophobic portions of 25(OH)D interfere with fatty acid residues, which weakens the effect of lipid peroxidation. As a result of its lipophilic characteristics, the cell membrane's viscosity shields it from lipid peroxidation [20].

By promoting and inducing the expression of the calcium-stored proteins lipidin-D28K and calbindin-d9K, which lower malonaldehyde (MDA) levels during injury to the central nervous system, vitamin D can also regulate lipid peroxidation as a form of neuroprotection [21].

1,25-(OH)D has been demonstrated to raise glutathione levels in neurons in studies using mice. The antioxidant glutathione (GSH), which is produced by neurons and astrocytes, is crucial for defending cells against ROS and the oxidative stress that causes apoptosis. In order to prevent oxidative damage to the central nervous system, the active form of vitamin D is essential for neuroprotection [22].

As shown in one study, vitamin D supplementation improved endothelial function in both the non-diabetic population and in patients with type 2 diabetes mellitus. As a result, cholesterol levels were low in vitamin D3 with alkaline water. Alternative cardiovascular disease markers, such as atherosclerosis, improved more in response to higher vitamin D levels [23].

This investigation has led us to the conclusion that the level of acidity in drinking water directly affects the development of vitamin D3 toxicity. Our research demonstrated that acidic water plays a significant role in This investigation has led us to the conclusion that drinking alkaline water can help people who are taking large amounts of vitamin D3, in addition to its effect in reducing the levels of total cholesterol and Low density lipoprotein with improving the body weight and the oxidative stress status

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Conflict of interest

There are no competing interests.

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تأثير ماء الشرب القلوي في سمية فيتامين D3 في اناث الجرذان

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ركز هذا البحث على كيفية تأثير استهلاك المياه القلوية على سمية فيتامين D3 وكيفية ارتباطه بالنشاط السلوكي العصبي والحركي للجرذان فضلا عن بعض العوامل البيوكيميائية. تم استخدام ١٥ جرذاً ، قسمت إلى ثلاث مجاميع. تم توفير ماء الصنبور للمجموعة الأولى (السيطرة السالبة) ، والماء القلوي للمجموعة الثانية ، والمياه القلوية مع الزرق العضلي الاسبوعي من فيتامين D3 بجرعة ١٠٠,٠٠٠ وحدة / حيوان للمجموعة الثالثة لمدة ٤ أسابيع. لوحظت تغييرات في السلوك العصبي والنشاط الحركي للفئران في الميدان المفتوح. حيث ازدادت أوزان الفئران في مجموعة فيتامين D3. كما ان عدد المربعات المقطوعة وعدد مرات الوقوف على القوائم الخلفية، وعدد مرات الفضول قد ازداد بشكل ملحوظ في مجموعة فيتامين D3 مقارنة بالسيطرة السالبة والموجبة، كما زادت مستويات فيتامين D3 ايضا وانخفض مستوى الكوليسترول الكلي ومستويات البروتين الدهني قليل الكثافة أيضاً. قادنا هذا التحقيق إلى استنتاج مفاده أن شرب المياه القلوية يمكن أن يساعد الأشخاص الذين يتناولون كميات كبيرة من فيتامين D3 إذ انه يقلل من مستوى الكوليسترول الكلي والبروتين الدهني قليل الكثافة ويحسن من وزن الجسم وحالة الاجهاد التأكسدي.

كلمات المرور: الماء القلوي , السمية , فيتامين D3 , اناث الجرذان.