Supraphysiological Doses of Vitamin D Changes Brainwave Activity Patterns in Rats


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Abstract:
Low plasma levels of vitamin D causes bone mineral change that can precipitate osteopenia and osteoporosis, and could aggravate autoimmune diseases, hypertension and diabetes. The demand for vitamin D supplementation becomes necessary; however, the consumption of vitamin D is not without risks, which its toxicity could have potentially serious consequences related to hypervitaminosis D, such as hypercalcemia and cerebral alterations. Thus, the present study describes the electroencephalographic changes caused by supraphysiological doses of vitamin D in the brain electrical dynamics, and the electrocardiographic changes. After 4 days of treatment with vitamin D at dose of 25,000 IU/Kg, the serum calcium levels found was increased in comparison with control group. The ECoG analysis found a reduction in wave activity in the delta, theta, alpha and beta frequency bands. For ECG was observed changes with shortened QT follow-up, which could be related to serum calcium concentration. This study presented important evidence about the cerebral and cardiac alterations caused by high doses of vitamin D, indicating valuable parameters in the screening and decision-making process for diagnosing patients with symptoms suggestive of intoxication.
Introduction

Vitamin D is an important pro-hormone that plays a key role in preserving the skeletal system, serum calcium levels and playing important roles cardiovascular and central nervous systems and cancer prevention (1). According to international guidelines, recommended doses of vitamin D range from 400-800 IU/day and that the choice of dosage is influenced by age (2–4). These levels are sufficient to maintain the homeostasis of physiological functions and, according to the Food and Nutrition Board, doses above 2000 IU/day induces an increase in serum calcium concentrations above normal values and controlling cerebral development and function in adulthood and the immune-mediated response (5–7).

The lack of this vitamin leads to symptoms of hypocalcemia and problems in bone mineralization. In addition, this reduction can be associated with neuroinflammatory, neurodegenerative and neuropsychological diseases, suggesting the hypothesis that vitamin D may play an important role in its pathogenesis (8,9). The increasing information about vitamin D deficiency and the consequences associated have been increased the demand for supplementation. However, the increased consumption of vitamin D is not without risks, and vitamin D toxicity has potentially serious consequences (10,11). An increase intake of vitamin D by the population, especially the elderly, associated with medical prescriptions with high doses without laboratory monitoring can result in a set of consequences related to hypervitaminosis D, with symptoms may be related hypercalcemia, which, although rare, has been reported more frequently in recent years (8,12–14).

The symptoms of vitamin D toxicity (VDT) can be like other hypercalcemic conditions and include neuropsychiatric alterations, such as attention deficit, apathy,
confusion, drowsiness and, in severe cases, coma (8,15). Although these symptoms are nonspecific and belong to other endocrine clinical conditions, effects of excess vitamin D in the brain can lead to important changes, since there is a strong presence of vitamin D receptors mediating responses such as neuronal plasticity, as well as neural circuits connectivity (16,17). Thus, few studies have been dedicated to characterizing the electrical alterations caused by excess vitamin D. On the cardiovascular system could be observed changes in ventricular repolarization and cardiac rhythm conduction resultant of the excess of calcium (18). Other organs are also affected, such as the eye and the auditory system, but with a lower degree of clinical repercussion (19).

There are increasing reports in the literature about VDT in all age groups. Some studies has reported cases of poisoning by vitamin D in babies who evolved with lethargy, vomiting and weight loss, and biochemical evaluation showing serum calcium >18 mg/dL, suggesting severe hypercalcemia (20,21). In addition, it was also reported cases of overdose of vitamin D in young individuals, evolving with anorexia, neurological symptoms, and vomiting, and calcium levels >14 mg/dL (22).

Cardiovascular symptoms together with neurological symptoms are most likely the findings clinically more important, because they present instability that can immediately put the patient's life at risk and deserve more attention. In this regard, the aim of the study is describe the electroencephalographic changes caused by an acute high-dose vitamin D for understand of brain electrical changes, as well as the electrocardiographic changes that could be caused by high levels of vitamin D.

Methodology

Animals
Seventy two young adult males Wistar rats (220 ± 20 g, and seven weeks old) were obtained from the Central Animal Facility of the Federal University of Pará. The animals were housed in standard white cages (48 cm × 38 cm × 21 cm) and at a controlled temperature, of 23 ± 2 °C and 12/12 h light–dark cycle, with food and water available ad libitum. All experimental procedures were conducted in accordance with the principles of laboratory animal care and the guidelines of the Brazilian National Council for the Control of Animal Experimentation (CONCEA), with the approval of the Ethics Committee on Experiments in Animals of the Federal University of Pará (CEUA no. 2252220321). All necessary precautions were taken to prevent animal suffering and distress.

### Drugs

The anesthetic ketamine hydrochloride was obtained from the Köing Laboratory (Santana de Parnaíba, SP, Brazil), while xylazine hydrochloride was obtained from the Valéé laboratory (Montes Claros, MG, Brazil). The lidocaine, local anesthetic, was obtained by Hipolabor laboratory (Sabará, MG, Brazil) for electrode implantation. The diazepam 10mg/2ml was obtained from União Química (Embu-Guaçu, SP, Brazil). The vitamin D3 (ADDERA D3® 3.300 UI/ml) was purchased from the FARMASA (São Paulo, SP, Brazil) and peanut oil (Katiguá, MG, Brazil) as vehicle control. Calcium gluconate (100mg/ml) purchased from Veafarm laboratory (São Paulo, SP, Brazil).

### Experimental design

The study was had two phases. Phase 1 consisted of the analyses of electrocorticogram (ECoG), and phase 2 consisted of the analyses of electrocardiogram (ECG) and biochemical analyses. In the both phases, the rats were divided randomly (n...
= 9 animals per group) into: (a) control group (animals that received anything); (b) vehicle-treated group (animals that received peanut oil as a vehicle); (c) calcium gluconate group and (d) vitamin D group.

First, the vitamin D3 was diluted in peanut oil for the concentration of 2.500 IU/mL, followed by the treatment with vitamin D3 at dosage of 25,000 IU/kg, via gavage, once a day for four successive days (23). For the group treated with calcium gluconate the dose used was 100 mg/kg (intravenously) (24) in the lateral vein of the tail, once a day for four days. Finally, on fifth day, the ECoG and ECG recordings were performed, and blood was collected for biochemical parameters (serum creatinine, calcium and vitamin D).

Electrocorticographic recordings and data analyses

The ECoG recordings were obtained using the procedures described by Ferreira et al. (25). For this, two days before treatment, the animals were anesthetized with ketamine hydrochloride (80 mg/kg, i.p.) and xylazine hydrochloride (10 mg/kg, i.p.), and after the abolishment of their interdigital reflex, they were placed in a stereotaxic apparatus. The skull was exposed, and stainless-steel electrodes (tip exposure, 1.0 mm in diameter) were placed on the dura mater above the frontal cortex at the coordinates of the bregma -0.96 ± 1.0 mm lateral. A screw was fixed in the occipital skull and the electrodes were fixed with self-curing acrylic cement.

Seven days after surgery, the electrodes were connected to a digital data acquisition system consisting of a high-impedance amplifier (Grass technologies, P511), an oscilloscope (Protek, 6510), and a board for data acquisition and digitization (National Instruments, Austin, TX). Data were sampled continuously at 1 kHz at a low pass of 3 kHz and a high pass of 0.3 Hz. During recording, animals were confined in
tightly spaced acrylic boxes (20 x 45 x 15 cm). For all treatments, the ECoG recordings followed a standard protocol: 10 minutes of accommodation, followed by recording lasting 3 minutes.

The ECoG was recorded using a digital data acquisition system and the tracing was registered in mV (millivolts), and the offline analysis was run as described by Estumano et al. (26). Thus, offline analyses were run using a tool built in the Python programming language (version 2.7), with “Numpy” and “Scipy” libraries being used for the mathematical processing and a “matplotlib” library to obtain the data. A graphic interface was developed using the PyQt4 library. The spectrograms were calculated using the Hamming window with 256 points (256/1000 s). For power spectral density (PSD), each frame was generated with an overlap of 128 points per window. For each frame, the PSD was calculated by the Welch mean periodogram method. The frequency histograms were obtained by calculating the PSD of the signal using the Hamming window with 256 points without overlap, producing a resolution of 1 Hz per box. Each waveform displayed in PSD is an average of a set of experiences. PSDs were calculated for each group and averages are shown in individual boxes. The analyzes were performed at frequency up to 50 Hz and divided into bands according to Santos et al. (27). In delta (1-4 Hz), theta (4-8 Hz), alpha (8-12 Hz) and beta (12-8 Hz) for interpretation of the brain waves dynamics during the development of intoxication.

**Electrocardiogram analysis**

The electrocardiographic activity was obtained by the vector in lead D2, the electrodes were made in a non-conjugated way, the reference electrode was placed under the right axilla (0.5 cm) and the recording electrode was fixed in the tenth intercostal space, 3.5 cm below the left armpit, obeying the registration
vector. All animals received 2.5 mg/kg intraperitoneal of diazepam to prevent movement and noise in the registers. Each record was made at lasted 3 minutes for each animal and the analyzed data were heart rate (bpm), amplitude (mV), RR interval (ms), PQ interval (ms), QRS duration (ms) and QT interval (ms) (28).

**Biochemical Analyses**

For the biochemical procedures, the rats were anesthetized intraperitoneally with ketamine and xylazine, and the serum was collected by cardiac puncture for the analysis of the levels of creatinine and calcium. The serum creatinine level was assayed using the kinetic absorbance method (Labtest, MG, Brazil) and the serum calcium was measured by quantitative, end point colorimetric assay (Calcium Liquiform, Labtest, MG, Brazil). The 25-hydroxyvitamin D (25-OHD) was determined by immunoenzymatic and chemiluminescence assays (Abbott Lab, Chicago, Illinois, USA). After this procedure, the animals were euthanized with overdose of anesthetics.

**Statistical analysis**

The normality and homogeneity of variations were verified using the Kolmogorov-Smirnov and Levene's tests, respectively. The residuals were normally distributed and the variations are equal, all comparisons between groups were made using one-way ANOVA, followed by Tukey's test. All data are presented as the mean and standard deviation (SD), and the F and p-values are included, when pertinent. A \( p < 0.05 \) significance level was considered for all analyses. The GraphPad® Prism 9 software (Graph-Pad Software Inc., San Diego, CA, USA) was used for statistical tests.

**Results**
High-dose vitamin D induced hypercalcemia and increased serum creatinine level

To evaluate if the high-dose vitamin D was capable to induce hypercalcemia, we analyzed the levels of serum calcium. Thus, animals that received calcium gluconate and vitamin D presented serum calcium level increased comparison with control and vehicle groups, and the vitamin D group was higher than calcium gluconate (F (3, 32) = 40.38, p < 0.0001; control: 8.55 ± 0.96 mg/dL; vehicle: 8.99 ± 0.88 mg/dL; calcium gluconate: 16.54 ± 2.44 mg/dL; vitamin D: 13.58 ± 2.29 mg/dL; p < 0.01 for all comparisons; Figure 1A).

To assess renal function after administration of high-dose vitamin D was measured serum creatinine level (F (3, 32) = 5.933; p = 0.0025). Here, we showed that vitamin D (1.17 ± 0.50 mg/dL) increased serum creatinine level in comparison with control and vehicle groups (control: 0.69 ± 0.13 mg/dL; vehicle: 0.70 ± 0.09 mg/dL; p < 0.01 for all comparisons), but not in relation to calcium gluconate (0.24 ± 0.16 mg/dL; p = 0.0547; Figure 1B).

The plasma levels of 25-OHD on the groups was received high-dose vitamin D showed an increase compared with the control, vehicle and calcium gluconate groups (F (3, 32) = 143.1; p < 0.0001; control: 18.32 ± 4.48 ng/mL; vehicle: 18.29 ± 5.93 ng/mL; calcium gluconate: 21.15 ± 8.15 ng/mL; vitamin D: 175.9 ± 37.71 ng/mL; p < 0.0001 for all comparisons; Figure 1C).

High-dose vitamin D reduced all brainwaves bandpower

During the work, the vitamin D-treated animals did not show behavioral changes and maintained their food and water intake similar to control and vehicle groups (data not shown).
The ECoG recordings of the control group presented amplitude below 0.1 mV (typically low amplitude, Figure 2A), as demonstrated on the 1-second magnification (Figure 2A, center) with spectrogram showed the highest energy intensity below 10 Hz (Figure 2A, right). The ECoG recordings to the vehicle group (Figure 2B) and calcium gluconate group (Figure 2C) were similar to the control group; on the vitamin D group, by contrast, ECoG changes can be observed with a decrease on the energy intensity below to 10 Hz (Figure 2D, right); however, the tracing amplitude was kept below 0.1 mV (Figure 2D, center).

The decomposition of the distribution of the total spectral power revealed a subtle shift in the amplitude tracing of the vitamin D animals in comparison with the control and vehicle groups (Figure 3). Differences between vitamin D and others groups was found in the analysis of the distribution of the linear frequencies of up to 40 Hz (F (3, 32) = 15.69, p < 0.0001). In this case, the vitamin D group had lower total spectral power than the control, vehicle and calcium gluconate groups (control: 0.76 ± 0.04 mV²/Hz x 10⁻³; vehicle: 0.74 ± 0.07 mV²/Hz x 10⁻³; calcium gluconate: 0.80 ± 0.09 mV²/Hz x 10⁻³; vitamin D: 0.60 ± 0.06 mV²/Hz x 10⁻³; p < 0.001 for all comparisons; Figure 3).

The decomposition of the brainwaves was also analyzed for the distribution of power levels in the delta (1-4 Hz), theta (4-8 Hz), alpha (8-12 Hz) and beta (12-28 Hz).

There was observed difference for the delta wave frequencies (F (3, 32) = 6.804; p = 0.0011), with a significant reduction observed for animals treated with vitamin D (0.25 ± 0.03 mV²/Hz x 10⁻³) in comparison with the control (0.31 ± 0.02 mV²/Hz x 10⁻³, p = 0.0029), vehicle (0.30 ± 0.03 mV²/Hz x 10⁻³; p = 0.0205) and calcium gluconate (0.31 ± 0.05 mV²/Hz x 10⁻³; p = 0.0025) groups. No difference was observed between control, vehicle and calcium gluconate groups (p > 0.05; Figure 4A).
For the theta waves, it was also observed differences between groups (F (3, 32) = 16.16; p < 0.0001). Animals from vitamin D group had a lower mean theta power (0.20 ± 0.02 mV²/Hz x 10⁻³) than that of the control (0.27 ± 0.015 mV²/Hz x 10⁻³; p < 0.0001), vehicle group (0.27± 0.027 mV²/Hz x 10⁻³; p < 0.0001) and calcium gluconate group (0.28 ± 0.04 mV²/Hz x 10⁻³; p < 0.0001) and no difference was observed between control, vehicle and calcium gluconate groups (p > 0.05; Figure 4B).

Similar to delta and theta waves, it was showed significant variation between groups for the alpha waves (F (3, 32) = 11.64; p < 0.0001). Once again, animals treated with vitamin D had the lowest alpha power of all the groups, with a mean of 0.053 ± 0.005 mV²/Hz x 10⁻³ (p < 0.001 for all comparisons). The control, vehicle and calcium gluconate groups not were different each other (control: 0.069 ± 0.006 mV²/Hz x 10⁻³; vehicle: 0.066 ± 0.005 mV²/Hz x 10⁻³; calcium glutamate: 0.066 ± 0.008 mV²/Hz x 10⁻³; p > 0.05; Figure 4C).

As in the other cases, animals that received vitamin D presented beta wave power lower than control and vehicle groups (F (3, 32) = 5.509, p = 0.0036; control: 0.064 ± 0.002 mV²/Hz x 10⁻³; vehicle: 0.063 ± 0.003 mV²/Hz x 10⁻³; calcium gluconate 0.062 ± 0.004 mV²/Hz x 10⁻³; and vitamin D: 0.058 ± 0.004 mV²/Hz x 10⁻³; p < 0.05 for all comparisons; Figure 4D).

**High-dose vitamin D induced electrocardiographic changes**

The Figure 5 showed the cardiac activity of the control group, presenting the amplitude of the recordings and the sinus rhythm (Figure 5A). When amplified, it could be observed all cardiac deflagrations, with atrial activity represented by the P wave, ventricular activity represented by the QRS complex, and ventricular repolarization T wave (Figure 5B).
Animals that received peanuts oil (vehicle) group presented similar characteristics to the control group, demonstrating cardiac activity in sinus rhythm with all the triggers of cardiac functioning (Figure 5C and D). For the group treated with calcium gluconate was observed sinus bradycardia (Figure 5E and F). For the vitamin D group, the electrocardiographic characteristics and the sinus rhythm are similar comparison with control group (Figure 5G); however, on the amplification of 1-second amplification, it possible observed the sinus bradycardia (Figure 5H).

The cardiac activity and intervals was performed. For the heart rate was observed statistical difference between groups (F (3, 32) = 106.9; \( p < 0.0001 \)). Animals that received vitamin D showed increasing of heart rate average of 375.8 ± 12.8 bpm in comparison with control (321.6 ± 9.0 bpm; \( p < 0.0001 \)) and vehicle (316.9 ± 8.2 bpm; \( p < 0.0001 \)) groups. Animals that received calcium gluconate showed decrease of heart rate average in comparison with all groups (269.1 ± 18.1 bpm; \( p < 0.0001 \) for all comparisons). No difference was observed between control and vehicle groups (\( p = 0.8619 \); Figure 6A).

In relation of fire amplitude was observed difference between groups (F (3, 32) = 6.771; \( p = 0.012 \)), and only the calcium gluconate group showed an increase in fire amplitude (1.31 ± 0.19 mV) when compared to the control group (1.05 ± 0.13 mV; \( p = 0.0014 \)) and vehicle group (1.08 ± 0.1 mV; \( p = 0.0050 \)). There was no difference between calcium gluconate and vitamin D group (1.14 ± 0.09 mV; \( p = 0.0638 \)) (Figure 6B).

The RR interval varied significantly among treatments (F (3, 32) = 79.69; \( p < 0.0001 \)), with a significant decrease being found in the vitamin D group (157.7 ± 6.5 ms) in comparison with the other groups (control: 186.3 ± 5.566 ms; vehicle: 188.9 ± 5.5 ms; and calcium glutamate: 223.9 ± 15.1 ms; \( p < 0.0001 \), for all comparisons).
The calcium gluconate group had an increase in the RR interval in relation to control and vehicle groups ($p < 0.0001$). No difference was observed between control and vehicle groups ($p = 0.9337$; Figure 6C).

For PQ interval ($F(3, 32) = 1.583; p = 0.2126$) and QRS duration ($F(3, 32) = 2.555; p = 0.0727$) no difference was observed between groups (Figure 6D and E).

Once, it was observed difference significantly between groups in QT interval ($F(3, 32) = 13.15; p < 0.0001$), with the animals treated with vitamin D (10.6 ± 0.8 ms;) and calcium gluconate group (10.1 ± 0.8 ms) presented a significant reduction in comparison with the controls groups (control: 12.2 ± 1.3 ms; vehicle: 12.6 ± 1.0 ms; $p < 0.05$). No difference was observed between control and vehicle groups ($p = 0.8549$); and calcium gluconate and vitamin D groups ($p = 0.6620$; Figure 6F).

**Discussion**

Vitamin D and its metabolites regulate hormonal flow and play an important role in calcium homeostasis, such as calcium absorption by intestinal villi, calcium mobilization from bones and calcium reabsorption by renal tubules (29). There are numerous reports of high doses of vitamin D causing hypercalcemia by hyperactivation of calcium regulatory functions (30,31).

The benefits of correct supplementation of vitamin D in health promotion and disease prevention are steadily increasing. This has led to an unrestrained increase in supplementation, which can often lead to hypercalcemic crisis and induce ionic disturbances with cardiac and brain repercussions (32). In our work, we showed that acute supplementation (high dose) of vitamin D caused secondary hypercalcemia, which induced significant alterations on the cardiac function, although the increase in calcium independent of vitamin D did not reveal this effect.
Furthermore, important evidence has shown that vitamin D receptors in the brain may be responsible for alterations in neuronal circuitry and that they are independent of calcium homeostasis (16). Vitamin D is a potent neurosteroid that crosses the blood-brain barrier and acts directly on cells that contain its nuclear and surface receptors, such as PDIA3 (protein disulfide isomerase family member 3) or also known as ER57, in neurons and glial cells, to carry out genomic and non-genomic actions (16,33).

In our work, we found alterations in brain waves that were not dependent on calcium levels induced by supraphysiological doses of vitamin D. A possible explanation for this phenomenon is that these alterations may be caused by non-genomic rapid signaling pathways (16). Studies have shown that PDIA3 is one of the main regulators of vitamin D actions in the brain and that it may be involved in the connectivity of neural circuits (34,35). However, further studies are needed to confirm its regulatory actions.

Furthermore, it is important to note that there are 3 possible primary outcomes in acute supplementation of vitamin D: hypercalcemia, hypercalciuria and nephrolithiasis. Perez-Barrios et al. (36) showed in a series of cases that vitamin D supplementation without medical follow-up resulted in critical serum calcium levels greater than 13 mg/dL. Our group in this work found similar data, which was found serum calcium level greater than 13 mg/dL in both groups (VitD and CaG).

Kidney changes were also reported by Malihi et al (31) and Khadgawat et al. (37), in which hypercalcemia can lead to calcium oxalate stones and this develop post-renal acute kidney injury, with an increase or not in nitrogenous metabolites, but this outcome depends on other factors, such as water intake. Here, creatinine levels were increased for the group that received high doses of vitamin D. Furthermore, acutely, use of vitamin D in supraphysiological doses also is associate hypercalcemia and can lead to
acute kidney injury by direct renal vasoconstriction and a reduction in extracellular fluid volume (38,39). This may explain the elevated creatinine values only in the group supplemented with vitamin D.

In addition, among the clinical manifestations, changes in the level of consciousness are one of the most common symptoms of vitamin D in high-doses associate hypercalcemia, especially when serum levels are greater than 13 mg/dL. In this case, the individual usually progresses to lethargy, mental confusion with probable outcome to the state of coma or seizures (40,41). There are numerous reports of encephalopathy/coma in hypercalcemic patients and this is closely related to a delta-theta activity pattern (42). Studies show that patients with encephalopathy have a decrease in the collective activity of these two frequency bands; this is a serious condition, which can progress to hospitalization and critical care (43). In this sense, our work suggests same wave pattern, in which the reduced activity in low frequency waves in the presence of hypervitaminosis D may indicate an encephalopathy, if persistent, with possible progression to coma.

Another important data is the significant decrease in alpha and beta bandpower, compared to control group. Interestingly, this pattern is observed in patients with attention deficit, in which reduced values of these waves were present in brain regions responsible for the processing and planning of actions (44). This supports the hypothesis that patients with hypervitaminosis D could develop lethargy and reasoning difficulty, it may be due to a reduction in these waves (1). This suggests that specific brain areas could be strongly influenced by serum vitamin D levels and by the activation of ER57 receptors in these regions.

In the heart, hypercalcemia causes ECG changes and can mimic myocardial infarction (45). It is essential to know how to differentiate these two changes, as it can
generate suffering and unnecessary intervention measures for the patient. The short QT interval is one of the most frequent alterations found, and this is due to the shortening of the ST segment (46,47). Similar data were found in this study, in which animals supplemented with vitamin D and calcium gluconate presented hypercalcemia, had a significantly shorter QT interval, with a slight shortening in the ST segment (not significant).

Furthermore, other studies report that in hypercalcemic states there may be a widening of the PQ interval, due to a widening of the QRS complex (48). These alterations were not observed in this study; however, it is important to clarify that such alterations can vary according to the serum calcium levels (48). These can also induce atrial and ventricular arrhythmias, such as atrial fibrillation and ventricular premature contractions (49), although they were not observed in this study.

In conclusion, this presented important evidence about brain and cardiac alterations caused by high-dose vitamin D. However, further studies are needed to better understand the changes caused in neurons and cardiomyocytes. In addition, the data from this study can provide valuable diagnostic parameters, thus improving screening and decision-making in patients with symptoms suggestive of vitamin D intoxication.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Data Availability Statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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**Figure 1.** Results of the biochemical analyses of the animals treated or not with vitamin D. (A) Renal function based on creatinine levels. (B) Serum calcium levels. (C) Serum 25-hydroxyvitamin D levels. The test used was one-way ANOVA. The data are expressed as means ±SD (n = 9 animals per group, **p < 0.01 and ***p < 0.001). Ctrl: Control. Veh: Vehicle. CaG: Calcium gluconate. VitD: Vitamin D.

**Figure 2.** Electrocorticographic recordings of animals performed for 3 minutes. (A) Control group, (B) vehicle group, and (C) Calcium Gluconate and (D) vitamin D group. Record of linear tracing performed by electrocorticographic (ECoG, left), representative fragment of 1 s from ECoG tracing (center), and frequency
Figure 3. Quantitative distribution of the total linear power of the brainwaves recorded by electrocorticography. The test used was one-way ANOVA. The data are expressed as the mean ± SD (n = 9 animals per group; ***p < 0.001). Ctrl: Control. Veh: Vehicle. CaG: Calcium gluconate. VitD: Vitamin D.

Figure 4. Quantitative linear frequency distribution of the brainwaves: (a) delta waves; (b) theta waves; (c) alpha waves; (d) beta waves. The test used was one-way ANOVA. The data are expressed as the mean ± SD (n = 9 animals per group; *p < 0.05, **p < 0.01 and ***p < 0.001). Ctrl: Control. Veh: Vehicle. CaG: Calcium gluconate. VitD: Vitamin D.

Figure 5. Electrocardiogram recorded of the control, vehicle, calcium gluconate and vitamin D groups. (A, C, E, G) Amplification of 10-second showing the sinus rhythm of control, vehicle and vitamin D groups, respectively. (B, D, F, H) Amplification of 1-second showing cardiac intervals of control, vehicle and vitamin D groups, respectively.

Figure 6. Electrocardiogram parameters recorded of the control animals for 3 minutes: (A) heart rate (bpm); (B) amplitude (mV); (C) RR intervals; (D) PQ interval; (E) QRS duration; (F) QT interval. The test used was one-way ANOVA. The data are expressed as the mean ± SD (n = 9 animals per group; **p < 0.01 and ***p < 0.001). Ctrl: Control. Veh: Vehicle. CaG: Calcium gluconate. VitD: Vitamin D.
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