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# Molecular docking to investigate the inhibitory activity and the role of nitric oxide in anticonvulsant effects of vitamin D on pentylenetetrazole-induced epileptic seizures in rats

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## Highlights

- The combination of vitamin D PTZ-induced epileptogenesis in rats.
- Vitamin D inhibited cognitive impairment in PTZ-kindled rats.
- Vitamin D has a role in cognitive function and learning, including neuroprotection and antioxidative mechanisms.
- The combination of vitamin D reduced oxidative stress in PTZ-kindled rats.
- Vitamin D decreased the levels of iNOS, nNOS, and NO in the brain in PTZ-kindled rats.
- The combination of vitaminD decreased hippocampal neuronal damage.
- The *in silico* molecular docking results suggest that vitamin D has a great potential to be developed as a supplement against epileptics.
- The chemical reactivity descriptors analysis revealed that vitamin D is more reactive than most of the NO inhibitors and moderately stable.

## Abstract

Despite the great breakthroughs in the field of epilepsy studies, the present drugs are ineffective in one-third of the patients, as well as without providing a definite treatment. There is accumulating evidence suggesting the role of vitamin D in epilepsy. The aim of this study was to investigate the effect of vitamin D on seizure formation, post-seizure cognitive functions, and the possible role of nitric oxide and oxidative stress in this effect, in the PTZ-induced seizure rat model. Sixty male Wistar albino rats were used in this study. The rats were randomly divided into 11 groups each containing 6 animals: Group 1: Control; Group 2: Saline+PTZ (45 mg/kg); Group 3: Vitamin D (1.5 mg/kg)+PTZ; Group 4: L-Arginine (500 mg/kg)+PTZ; Group 5: L-NAME (60 mg/kg)+PTZ; Group 6: 7-Nitroindazole (40 mg/kg); Group 7: Aminoguanidine (100 mg/kg)+PTZ; Group 8: Vitamin D+L- Arginine+PTZ; Group 9: Vitamin D+L-NAME+PTZ; Group 10: Vitamin D+7-Nitroindazole+PTZ; Group 11: Vitamin D+Aminoguanidine+PTZ. Animal behavior was evaluated with open field and passive avoidance tests. Nitric oxide (NO), total oxidative status (TOS), and total antioxidant status (TAS) levels in the cortex and hippocampus brain regions were determined by ELISA method. The treatment of vitamin D in combination with NO inhibitors has a positive effect on the PTZ-induced seizures and ameliorate post-seizure learning deficits. Moreover, the combination of vitamin D with NO inhibitors reduced oxidative stress and NO levels after seizures in the cortex and hippocampus. Also, we employed molecular docking and density functional theory analysis to investigate the inhibitory activity, reactivity, and stability of Vitamin D with other NO inhibitors. Vitamin D supplementation could be effective as a supportive treatment drug in epileptic patients, according to the findings.

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## Keywords

Epilepsy; Nitric oxide; Pentylenetetrazole; Rat; Vitamin D; DFT; Molecular docking

## 1. Introduction

Epilepsy is one of the most common neurological diseases affecting more than 0.5% of the world's population [1]. The disease is characterized by recurrent seizures that occur spontaneously. An excitation/inhibition imbalance in the brain (increased excitation, decreased inhibition, or both) promotes a hyperexcitable state and an increased propensity for seizure formation [2]. The current treatment is limited for seizures and antiepileptic drugs (AED) have no impact on the progression of the disease. Moreover, the current AEDs are approximately 1/3 of the patients' ineffective. Surgery is an alternative option for these patients identified as a drug-resistant group. However, these therapeutic approaches (existing AEDs and epilepsy surgery) are associated with cognitive and behavioral disorders [3]. This implies that there is a need to develop innovative and effective therapies for epilepsy.

Cholecalciferol (vitamin D3) is produced on the skin from 7-dehydrocholesterol under the influence of ultraviolet radiation with a wavelength of 290–315 nm, which is the main source of endogenous vitamin D production [4]. The relationship between epilepsy and vitamin D has been the subject of numerous studies, both clinically and experimentally. It has been reported that fluctuations in vitamin D levels that occur with seasonal changes affect the frequency of seizures and epileptic seizures peak in January [5]. Many clinical and experimental studies have reported that vitamin D has anticonvulsant effects. In a pilot study, vitamin D supplementation with antiepileptic drugs reduced seizures in patients with drug-resistant epilepsy [6]. In addition, the anticonvulsant activity of vitamin D has also been demonstrated in different animal models of epilepsy, such as lithium-pilocarpine-induced status epilepticus in rats [7], and PTZ-induced acute and chronic models [8,9]. In previous studies, it has been suggested that vitamin D has a role in cognitive function and learning, including neuroprotection and antioxidative mechanisms [10]. In addition, some clinical studies have shown that low serum 25-hydroxyvitamin D3 levels are associated with dementia and impaired cognitive function [11,12]. Nitric oxide (NO-), a potential neurotransmitter in the central nervous system, is formed from L-arginine by the activity of the nitric oxide synthetase (NOS) enzyme in the brain. It has three different isoforms: endothelial (eNOS), inducible (iNOS) and neuronal (nNOS) [13]. In addition, recent studies have shown that especially nNOS and iNOS have an important place in epileptogenesis [14]. Furthermore, Djeraba et al. showed that vitamin D reduces NO production in patients with Behçet's disease and supported the immunomodulatory effect of vitamin D [15]. In this study, our aim was to investigate the effect of vitamin D on pentylenetetrazol-induced epileptic seizures in rats and the role of the nitric oxide pathway in this effect. Also, to investigate the inhibitory potential, reactivity, and stability of the vitamin D and other NO inhibitors utilizing molecular docking and density functional theory analysis.

## 2. Materials and methods

### 2.1. Animals

This study was carried out with 66 adult (2–3 months old) male Wistar albino rats weighing 230–250 g obtained from Cumhuriyet University Animal Laboratory. Animals were housed in the standard conditions, including the controlled temperature (23±2°C) and humidity (35% - 60%), a 12:12-h light–dark cycle. The rats were randomly divided into 11 groups each containing 6 animals: Group 1: Control; Group 2: Saline+PTZ (45 mg/kg); Group 3: Vitamin D (1.5 mg/kg)+PTZ; Group 4: L-Arginine (500 mg/kg)+PTZ; Group 5: L-NAME (60 mg/kg)+PTZ; Group 6: 7-Nitroindazole (40 mg/kg); Group 7: Aminoguanidine (100 mg/kg)+PTZ; Group 8: Vitamin D+L- Arginine+PTZ; Group 9: Vitamin D+L-NAME+PTZ; Group 10: Vitamin D+7-Nitroindazole+PTZ; Group 11: Vitamin D+Aminoguanidine+PTZ. All procedures were performed in accordance with the Local Ethics Committee guidelines (01.03.2018-No:106). All arrangements were made to minimize the number of animals used and their suffering.

### 2.2. Drugs

Vitamin D, L-NAME, L-Arginine, 7-Nitroindazole, Aminoguanidine and PTZ were purchased from Sigma-Aldrich (USA). Commercial kits for the measurement of Total Anti-Oxidant (TAS) and Total Oxidant (TOS), Nitric Oxide (NO) levels were procured from Rel Assay Diagnostics (Gaziantep, Turkey) and YL Biont (Shangai, China). Vitamin D was dissolved in olive oil. and PTZ was dissolved in physiological saline. All treatments were administered intraperitoneally (i.p.).

### 2.3. Experimental protocol

To induce seizures, a convulsive dose of PTZ (45 mg/kg) was injected into rats. After each injection, the rats were placed separately into plexiglass cages for observation lasting 30 min and the first myoclonic jerk (FMJ) latencies was recorded. The development of seizure a behavioral test (Racine's Convulsion Scale (RS)) as follows: 0-no changes; 0.5-abnormal behavior (e.g., automatisms, increased orienting