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Original Article

In vivo electrophysiological study of vitamin D3 protective effects on PTZ-induced seizures in rats

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Abstract

Background and purpose: The purpose of the current study was to investigate the protective effects of acute and chronic administration of different doses of vitamin D3 on pentylenetetrazol (PTZ)-induced epileptiform activities in rats.
Experimental approach: Sixty Wistar rats in chronic and acute groups were used in this study. In the chronic groups, animals received vitamin D3 at 50, 100, and 150 μg/kg; vitamin D3 (50 μg/kg, i.p.) + diazepam (0.1 mg/kg, i.p.), and almond oil (i.p.) daily for two weeks whereas, in the acute groups the animal received a single dose of chemicals just 30 min before PTZ administration. The electrophysiological recording was performed by implanting a unilateral bipolar electrode in the pyramidal cell layer of the CA1 region of the hippocampus. Epileptic activities were induced by intraperitoneal injection of PTZ (80 mg/kg, i.p.). The spike count and amplitude were analyzed using the eTrace software.
Finding/Results: Chronic administration of all doses of vitamin D3 and its combination with diazepam significantly reduced both spike counts and amplitudes following PTZ administration. While the acute doses were ineffective.
Conclusion and implication: The results of the study indicated that chronic but not acute administration of vitamin D3 has a protective effect on PTZ-induced epileptiform activity in rats.

Keywords: Epileptiform activity; PTZ; Vitamin D3.

INTRODUCTION

It is well known that the initial role of vitamin D3 was related to the dynamic balance of calcium and phosphate homeostasis, forming, and maintaining bone structures (1). However, the wide distribution of vitamin D3 receptors in various organs and tissues suggest that it can have several physiological effects (2). Vitamin D3 plays an essential role in supporting bone health, muscle strength, the efficiency of the immune system function, and neurotransmission in the central nervous system. Additionally, the neurological role of vitamin D3 is supported by the fact that specific vitamin D3 receptors and enzymes are present in neurons and glial cells throughout the central, and peripheral nervous systems (3). Vitamin D3 deficiency affects all age groups (4) and has been considered in the pathogenesis of cerebrovascular, psychological, and neurological diseases as well as epilepsy (2). Epilepsy affects more than 50 million people worldwide, making it the third most common neurological disorder (5).

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Over the past decade, significant efforts have been made to develop new antiepileptic medications, but only 30 to 40 percent of epilepsy patients respond to drugs (6). Moreover, vitamin D3 deficiency can lead to hypocalcemia, which increases the neural membranes' irritability and seizures. It has been shown that vitamin D3 receptor mRNA expression in the hippocampus significantly increases following the pilocarpine-induced epilepsy model in rats (7). Also, the severity of pentylenetetrazol (PTZ)-induced seizures is increased in mice lacking the vitamin D3 receptor gene (8).

Dynamics of seizures fall into a much lower dimensional space as suggested by electroencephalography, electrocorticography, and local field potential measurements. Motivated by this fact neuroscientists have developed models that are composed of fewer system variables and parameters. These macroscopic models are the mean-field models and are typically extensions of the pioneering Wilson-Cowan equations (9). Furthermore, studies examining electroshock-induced seizures have reported anticonvulsant effects of calcitriol, which can increase hippocampal seizure threshold levels in rats (10) and reduce the severity of PTZ-induced attacks in mice. The precise mechanism of the anticonvulsant effect of vitamin D3 is unknown, but its protective effects may be associated with a decrease in the cytoplasmic concentration of calcium ions (Ca^{2+}) in brain cells and an increase in the synthesis of antioxidants such as glutathione. Also, other mechanisms, including oxidative stress pathways, certain enzymes, and neurotransmitters, can mediate the role of vitamin D3 in various seizure models (11). Considering these factors, further experimental studies are required to investigate the potential role of vitamin D3 in epilepsy and the possible direct anticonvulsant properties of this neurosteroid hormone, focusing on the effects of vitamin D3 on seizure management. Previous studies have more behaviorally investigated the effects of vitamin D3 on seizure activity, but in the present study, its effects were investigated by recording seizure activity in the hippocampus and changes in the number, amplitude, and time of onset of seizure activity. In addition, in this study, a combination of ketamine-xylazine that has not been previously reported was used to anesthetize the animals.

MATERIALS AND METHODS

Animals

Sixty male Wistar rats weighing 200 to 250 g were housed in a room with a controlled temperature (22 ± 1 °C) and under a 12/12-h light-dark cycle (lights on from 7 a.m.). All rats had *ad libitum* access to standard food and water. All animal procedures in the present study conformed to the mandates of the Helsinki Declaration (4305969678).

The animals were randomly allocated into the acute and chronic groups. In both acute and chronic groups, animals are divided into five subgroups which contain one control group receiving almond oil, and four other subgroups of animals receiving vitamin D3 at 50, 100, and 150 (μg/kg, i.p.) and vitamin D3 (50 μg/kg, i.p.) + diazepam (0.1 mg/kg, i.p.).

The animals in the acute groups received only a single dose of chemicals just 30 min before the seizure induction whereas, in the chronic groups, daily administration of chemicals was performed until 14 days (n = 6 animals in each subgroup). for these doses and the chronic period of vitamin D intake, no toxicity has been reported in other studies (1). Almond oil has been used to prepare different concentrations of vitamin D3.

Surgical procedure

The animals were anesthetized using a combination of ketamine (70 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.), and then subjects were placed in a stereotaxic apparatus (Stoelting, Wood Lane, IL, USA) (12). The scalp was incised, and the bregma was identified. The CA1 region of the right hippocampus was the target area for the implantation of a bipolar tungsten electrode. The tip of the electrode was aimed at the following coordinates: -2.76 mm posterior to the bregma, -1.4 mm left and right sides of the

midline, and 3 mm below the top of the skull (13). Because in the previous similar studies we verified the correct location of the electrode and verification cases were performed, in the current study, this was not carried out (12).

Electrophysiological recordings

Local field potential recording from hippocampal CA1 pyramidal neurons was performed. The eTrace Analyzer software (Science Beam, Tehran, Iran) was used to digitize the data, storing it on a computer hard drive for further offline analysis. Single-site recordings of seizure-like events were obtained from the CA1 pyramidal cell layer. 10-Hz low-pass and 100-Hz high-pass filters were utilized. The epileptiform activity was induced by injection of PTZ (80 mg/kg, i.p.).

The baseline field potentials in the control and treatment groups were recorded for 10 min in anesthetized rats, then vitamin D3 was injected, the effect was recorded for 25 min, then PTZ was injected and epileptic activity was recorded for another 10 min. Epileptiform activity began 1-2 min after the PTZ administration. Finally, diazepam (10 mg/kg) was injected to terminate PTZ-induced epileptiform activity (12).

Data analysis

Statistical differences were determined by one-way analysis of variance (ANOVA) followed by Tukey post hoc test using IBM® SPSS® software version 27 (IBM company, USA). In the figures, all values are expressed as mean ± SEM. *P*-values < 0.05 were considered statistically significant.

RESULTS

In all acute and chronic groups, the onset time of epileptiform activities induced by PTZ did not change ($P > 0.05$) in comparison with the control group and the onset time of seizure activity after injection of PTZ was less than 100 s. As shown in Fig. 1 A and B the acute administration of all doses of vitamin D3 and its combination with diazepam did not alter the mean spike counts and amplitude in comparison with the control group which received only almond oil. Representative sample tracings for acute treatment groups are displayed in Fig. 2. As shown in Fig. 3 A and B the chronic administration of all doses of vitamin D3 and its combination with diazepam significantly reduced the mean spike counts as well as mean spike amplitude in comparison with the control group which received only almond oil. Representative sample tracings for chronic treatment groups are displayed in Fig. 4.

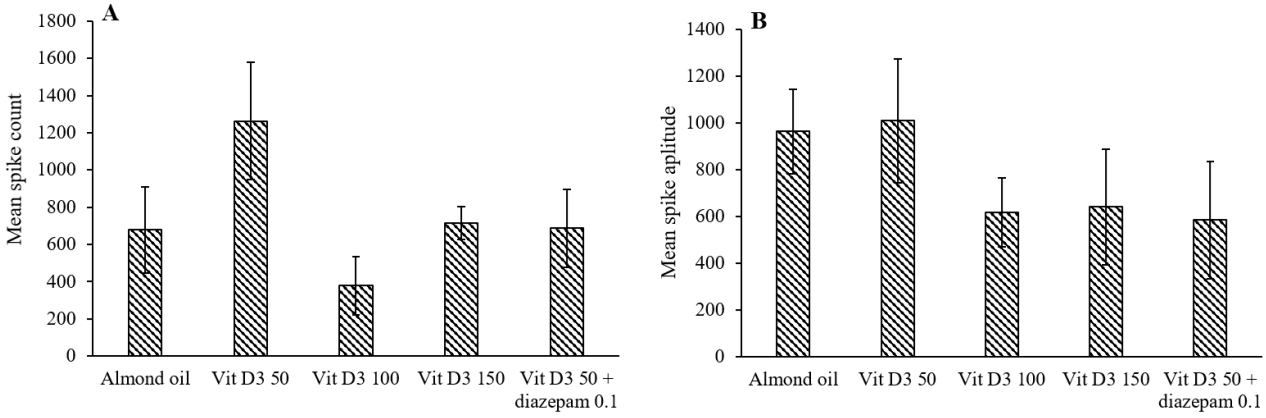

Fig. 1. Effect of acute administration of vitamin D3 (50, 100, and 150 µg/kg) and its combination at 50 µg/kg with diazepam at 0.1 mg/kg on (A) spike counts and (B) amplitudes of pentylenetetrazol-induced epileptiform activities. Each column represents the mean ± SEM, n = 6 for each group.

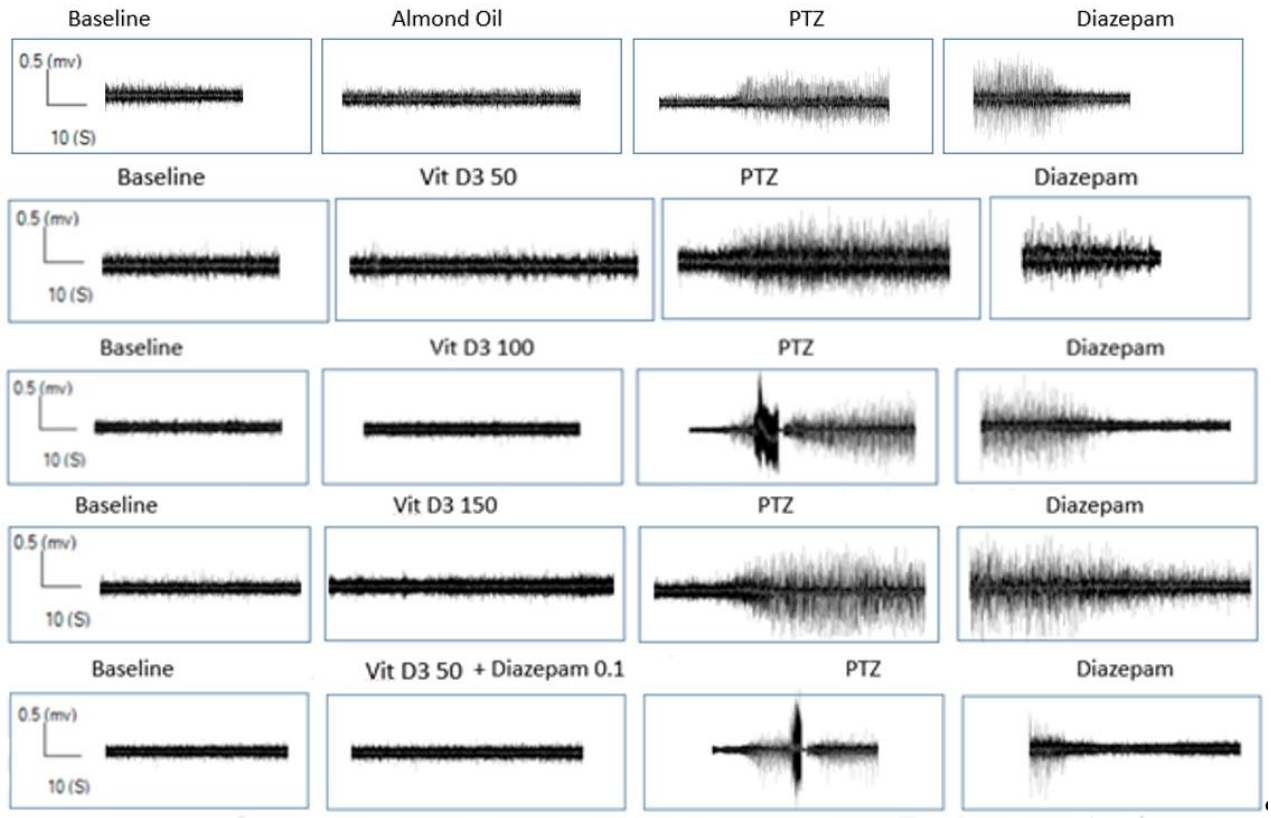

Fig. 2. Trace samples were recorded from the CA1 region of the hippocampus after acute administration of almond oil, vitamin D3 (50, 100, and 150 µg/kg), and vitamin D3 (50 µg/kg) + diazepam (0.1 mg/kg) obtained from pentylenetetrazol-treated rats.

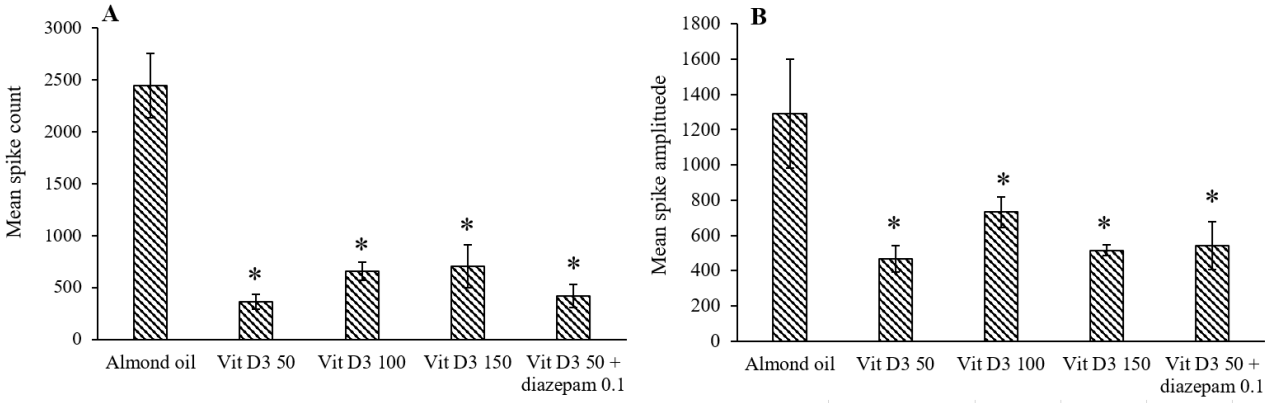

Fig. 3. Effect of chronic administration of vitamin D3 (50, 100, and 150 µg/kg) and vitamin D3 at 50 µg/mL + diazepam at 0.1 mg/kg on (A) spike counts and (B) amplitudes of pentylenetetrazol-induced epileptiform activities. Each column represents the mean ± SEM, n = 6 per group. **P* < 0.05 Indicates significant differences compared to the control group (administered almond oil).

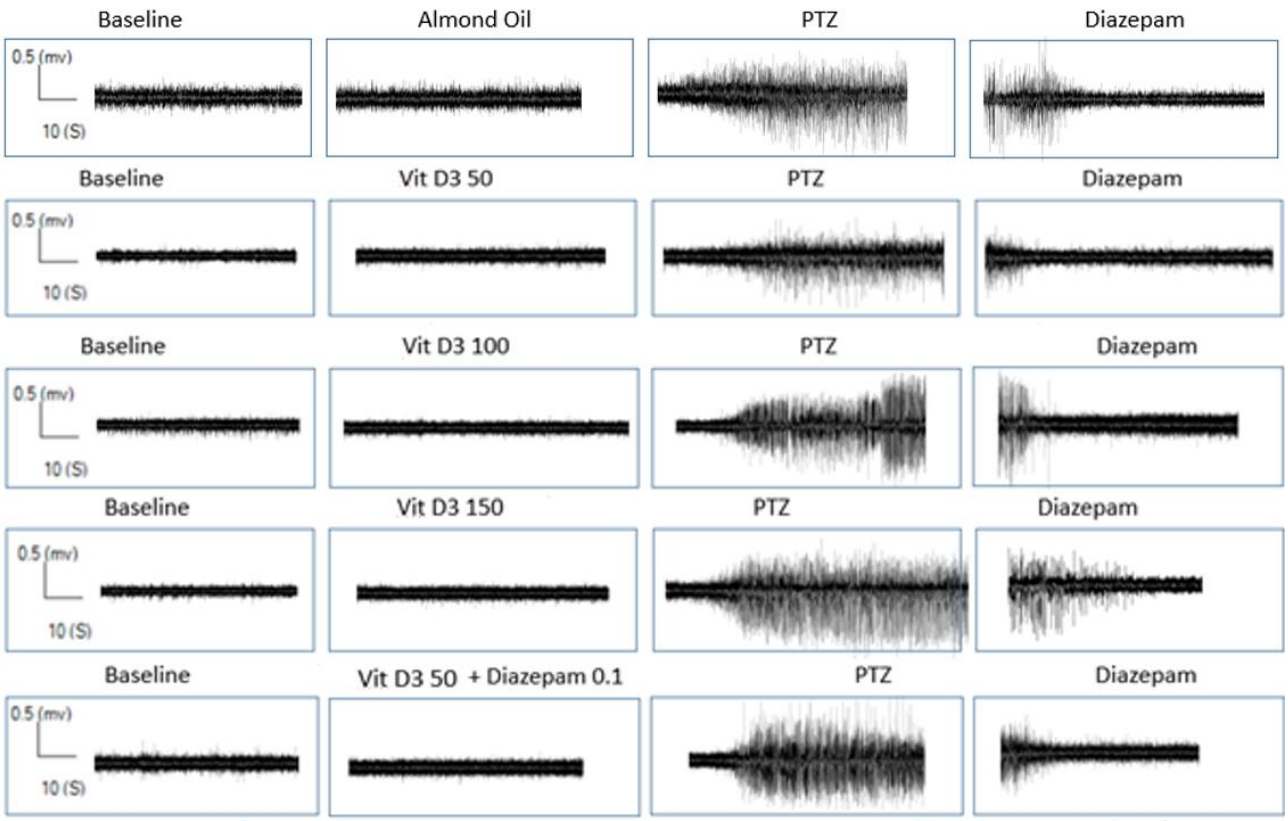

Fig. 4. Trace samples were recorded from the CA1 region of the hippocampus after chronic administration of almond oil, vitamin D3 (50, 100, and 150 µg/kg), and vitamin D3 (50 µg/kg) + diazepam (0.1 mg/kg) obtained from pentylenetetrazol-treated rats.

DISCUSSION

According to our findings, chronic administration of all doses of vitamin D3 and its combination with diazepam significantly reduced both spike counts and amplitudes following PTZ administration. While the acute doses were ineffective. There have been many studies on the effects of different forms of vitamin D on seizure activity in the past that confirm the results of the present study, for example, the effects of calcitriol on seizures have been described for more than 30 years and subcutaneous injection of it at a dose of 33 mg/kg has been shown to reduce the severity of PTZ-induced seizures (14). Vitamin D2 supplementation reduced the frequency of seizures (14). Siegel *et al.* showed that the injection of vitamin D3 into the hippocampus of rodent animal models elevated the chemically induced seizure threshold level. Also, the susceptibility to seizures was increased in vitamin D3 receptor knockout mice. It has been reported that intra-hippocampal and intravenous administrations of vitamin D3 at 50 and 100 mg/kg increase the seizure threshold (10). Although the exact mechanism of vitamin D3 in epilepsy has not been fully elucidated (2). It has been proven that vitamin D3 exerts its effects *via* the nuclear and specific membrane receptors (15). Nuclear receptors are important pathways in the brain that mediate the genomic effects of vitamin D3. In addition, various non-genomic ways, such as calcium channel regulation, decreased interleukin-6 activity (11), inhibited inducible nitric oxide synthesis (16), increase in glutathione levels, the protein kinase C activation, and the rapid modulation of GnRH neuron activity acting as mediators of vitamin D3 (11). According to some reports, there is a significant association between vitamin D3 status and gamma-aminobutyric acid (GABA) levels in the body, and vitamin D3 deficiency for ten weeks in adult mice reduces GABA levels in the brain (11). Furthermore, vitamin D3 (50 or 100 ng/kg) supplementation for six weeks increased GABA levels in the hippocampus of mice (18). Since GABA-A receptors play an essential role in the non-

genomic action of numerous neurosteroids (14) and vitamin D3 potentiates the effect of diazepam administered concurrently with vitamin D3, Neveu *et al*. (18) suggested that vitamin D3 modulates nerve excitability through GABA-A receptors (15). Although the role of the GABAergic system has been acknowledged in seizures. Therefore, it can be drawn that GABA receptors are probably one of the pathways through which the protective effects of vitamin D3 are manifested. In addition to the GABAergic system, the N-methyl-D-aspartate (NMDA) pathway is involved in the pathogenesis of epilepsy along with the GABAergic system, so that seizure activity can occur due to the imbalance between the GABAergic inhibitory system and the NMDA excitatory system (1). In some reports using the patch-clamp technique, it has been shown that the acute administration of vitamin D3 decreases NMDA and kainate-induced inward currents. This highlights the protective role of vitamin D3 (11). However, since the ketamine/xylazine combination was used to induce anesthesia in the animals of the present study and ketamine, as an NMDA-receptor antagonist, had no inhibitory effect on seizure induction, the possible influence of the NMDA pathway was reduced. Considering the effect of the chronic administration of vitamin D3 on seizure activity (14), genomic or non-genomic pathways that take time to be activated, get more attention. But, since 3-24-h pretreatment with vitamin D3 did not show any anticonvulsant properties, the effect of the non-genomic mechanisms diminishes somewhat (19). Although further studies are needed to rule out the effects of this vitamin since vitamin D3 plays an essential role in calcium homeostasis, and its anticonvulsant action likely arises from changes in calcium metabolism because it has an immediate effect on intestinal calcium absorption (1). Moreover, according to a study in ten-day-old rats, calcitriol induces its non-genomic effects through membrane receptors by modulating filament phosphorylation and calcium uptake through voltage-gated channels in the cerebral cortex (20). There are also reports that vitamin D3 down-regulates voltage-gated L-type calcium channels in the hippocampus. This is related to its neuroprotective effect. With this in mind, vitamin D3 could alter the seizure threshold by changing the level of extracellular and intracellular calcium, since vitamin D3 deficiency causes hypocalcemia, which leads to seizures due to increased excitability of the neural membranes (21). In the present study, however, the calcium concentration of the subgroups was not measured to test this hypothesis, since according to an earlier study (11) the measurement of the calcium concentration of the study groups did not show any significant changes, and also it is not the only determinant of seizures, and some seizures do not respond to calcium at the start of treatment, but rather effectively respond to vitamin D3. This suggests that the chemical effects of vitamin D3 may not be related to its anticonvulsant effects, as the administration of 4000-16000 units of vitamin D3 per day has already been observed to produce significant antiepileptic effects unrelated to calcium concentration levels of plasma (22). But about measuring the concentration of vitamin D3, since a previous study (1) similar to this one did not measure the amount of vitamin D3, we did not cite it. However, it is recommended that if this vitamin is used to help treat the disease better, its amount in the blood should be measured to check the toxicity of the drug at specific times. Overall, these findings could contribute to studies attempting to develop new anti-epileptic drugs based on synthetic, non-toxic, selective, vitamin-D3-related ligands in association with pathways involved in epilepsy.

CONCLUSION

According to the results of the present study, chronic administration of vitamin D3 reduced PTZ-induced seizure activity recorded from the CA1 region of the hippocampus. Acute administration, however, did not alter electrophysiological seizure responses. Although the mechanism of action of long-term administration of vitamin D3 on the reduction of seizure activity has not been precisely determined, based on what was mentioned in the discussion, it seems that chronic administration of vitamin D3 by creating normal amounts of intracellular and

extracellular calcium and the use and strengthening of the biochemical and cellular protective system of the brain could reduce seizures activity in this model which has needs for further investigation in future studies.

Conflict of interest statement

The authors declared no conflicts of interest in this study.

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Author's contribution

Y. Panahi proposed the topic and designed the study; E. Khalilzadeh contributed to the design of the study and statistical analysis of the data and final editing; S. Hosseinchi conducted the laboratory work and took care of animals, and G. Vafaei provided the laboratory equipment and helped in the design of the study. The final version of the study was approved by all authors.

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