Advance Publication

The Journal of Veterinary Medical Science

Accepted Date: 17 Apr 2013
J-STAGE Advance Published Date: 1 May 2013

Category: Physiology

Type of paper: Full paper

Title: Static Magnetic Field Induced Hypovitaminosis D in Rat

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Running head: HYPOVITAMINOSIS D AND STATIC MAGNETIC FIELD

ABSTRACT

In the following study, we mainly investigate the effects of static magnetic field (SMF) (128 mT, 1 hr/day during 5 consecutive days) on 25-hydroxyvitamin D_3 and calcium homeostasis. Wistar male rats, weighing 50-70 g, were randomly divided into four experimental groups: control, SMF-exposed rat, co-exposed rats (the last day and after exposure rats received a single dose of vitamin D *per os*) and supplemented with vitamin D group (without exposure to SMF). Exposure to SMF induced a decrease of plasmatic 25-hydroxyvitamin D_3 level ($P<0.001$). While, calcium and phosphorus levels were not affected ($P>0.05$). The same treatment failed also to alter body, relative liver and kidney weights. Interestingly, oral supplementation with vitamin D corrected hypovitaminosis D induced by SMF. Likewise, the same treatment failed to alter calcium homeostasis. More studies are needed to evaluate how SMF induces hypovitaminosis D.

KEY WORDS: calcium, phosphorus, static magnetic field, rats, 25-hydroxyvitamin D_3

INTRODUCTION

The natural static magnetic field of the Earth is ~ 50 μT and, depending on the geographic location, varies from ~ 30 to 70 μT. Over recent years, there has been rapid increase in the use of technologies employing electromagnetic fields and radiations covering all parts of the electromagnetic spectrum. Magnetic flux densities of the order of 20 μT are produced under high direct current (DC) transmission lines [14]. Magnetic flux densities up to about 50 mT may be encountered by workers that use DC equipment for electrolytic processes or in high energy physics research facilities. Static magnetic fields (SMF) up to 50 mT could be also encountered by the general public at floor level in magnetically levitated trains. Magnetic resonance imaging (MRI) systems used for medical diagnosis expose patients to flux densities as high as 2.5 T. MRI operators are occupationally exposed to fields up to about 5 mT [17, 20].

Vitamin D_3 (cholecalciferol) is taken in the diet (from fortified dairy products and fish oils) or is synthesized in the skin from 7-dehydrocholesterol by ultraviolet irradiation (290 –315 nm) [12, 13]. Vitamin D_3 produced in the skin, or taken by the diet and absorbed through the intestine, is accumulated in the liver, where it undergoes a first hydroxylation on C-25 by the action of CYP450-dependent microsomal hydroxylases to form the 25-(OH)D_3. 25-hydroxyvitamin D_3 is further hydroxylated in the kidney on C-1 to form the 1α,25-$(OH)_2D_3$ by the action of 25-hydroxyvitamin D_3 1-α-hydroxylase. [8, 9, 12, 13].

The aim of the present work is to investigate the effects of subacute exposure to SMF on the plasmatic 25-hydroxyvitamin D_3 level in young rats.

MATERIAL AND METHODS

Animals : Wistar male rats (SIPHAT, Tunisia), weighing 50–70 g were randomly divided into the following groups: control (n = 6), SMF-exposed rat (128 mT; 1 hr/day for 5 days) (n = 6), vitamin D treated rats (Dedrogyl®, 1600 UI/100g, received by gavage (oral route) for 5 consecutive days) (n = 6) and co-exposed rats (the last day and after exposure rats received a single dose of vitamin D (1600UI) *per os* (oral route) (n = 6). Animals were housed in group of six in cages at 25° C with the relative humidity of 80% under a 12 : 12 hr light/dark cycle, with free access to water and commercial wash (Company Almes, Tunisia). Animals were cared, under the Tunisian code of practice for the Care and Use of Animals for Scientific Purposes. The experimental protocols were approved by the Faculty Ethics Committee (Sciences Faculty of Bizerta, Tunisia).

Exposure system : We used an electromagnet (Model EM4-HVA, Lake Shore Cryotronic. Inc., Westerville, OH, U.S.A) and a magnet power supply (Model 647, Lake Shore Cryotronic. Inc.) with an air gap of 11 cm (Fig 1). This apparatus incorporates water-cooled coils and precision yokes that assure precise cap alignment and excellent field stability and uniformity when high power is required to achieve the maximum field capability for the electromagnet. SMF intensity was measured and standardized over the total floor area of the Plexiglas cage at 128 mT. SMF uniformity in the active exposure volume was 0.2% over 1 cm^3. The cage measured 20 × 10 × 20 cm. The two bobbins of the Lake Shore electromagnet were separated by a 12.1 cm. Exposed and sham control rats (*n* = 2 /each time) were placed in the cage at the center of the uniform field area and exposed, or not, to 128 mT SMF.

Procedure: After 2 hours, rats were sacrificed. Blood samples were immediately collected in heparinized chilled tubes and centrifuged. Aliquots of plasma were frozen and stored at –80°C until use.

Body, liver and kidney weights: Each rat was weighed with a triple beam balance with 0.1-gram readability on a daily basis between 9 and 10 a.m. During the weight measurement, the cages were cleaned, the straw renewed and sufficient amounts of water and rat food were replenished every day. Animals were sacrificed, and the liver and kidney were immediately removed and weighed and then then the organs weight ratio was calculated. The relative weight was calculated as g/100 g body weight.

25-hydroxyvitamin D_3 determination: Briefly, 3ml of plasma and 6 ml of acetonitrile (ACN) were pipetted into test tubes, and the mixture was shaken vigorously for 10 sec and then centrifuged for 15 min. The supernatant was filtered through a HLB column. The column was washed with 1.75 ml of water and 500 µl of ACN-H_2O (1:2, v/v). 25-hydroxyvitamin D3 was eluted with 500 µl of ACN. All samples were injected in a 100 µl volume into an HPLC system. Merck-Hitachi chromatograph was used, consisting of a Lachrom 7100 pump, Lachrom 7200 autosimpler and a Lachrom 7455 UV detector. A R18 chromatographic column, 250 x 4.6 mm, were used. For chromatographic separation, a phase LiClO4 (0.1 M) /Methanol (3:97, v/v) was used at a flow rate of 1.25 ml / min. UV detection was performed at 265 nm.

Calcium and phosphorus concentration: Plasmatic calcium was determinate by o-cresolphtaleine (Reactif Kit Modular P), and phosphorus levels were determined by phosphomolybdate **(**Reactif Kit Modular P, Roche, Belgium)

Data presentation and statistical analysis: Data were reported as the mean ± SEM. Differences between means were evaluated by one-way analysis of variance (ANOVA). Statistical significance of the differences between means was assessed by Student's *t*-test. The level of significance was set at $p < 0.05$.

RESULTS

Exposure to SMF (128 mT) during 1 hour/day for 5 consecutive days failed to alter calcium level (2.32 ± 0.13 vs. 2.16 ± 0.05 nmol/l, $p>0.05$) (Fig 2), phosphorus concentration (63.00 ± 5.60 vs. 78.50 ± 4.91 mg/l, $p>0.05$) (Fig 3), body weight (Fig 4) and relative liver and kidney weights in young rats (table 1). By contrast, the same treatment decreased plasmatic 25-hydroxyvitamin D_3 level (14.4 ± 3.91 vs. 21.84 ± 2.21 ng/ml, $^{**}p<0.001$) (Fig 5). Rats treated with vitamin D (1,600 IU for 5 consecutive days) showed a normal blood 25-hydroxyvitamin D_3 level (19.83 ± 0.67 vs. 21.84 ± 0.90 ng/ml, $p>0.05$) (Fig 5), calcium (2.05 ± 0.05 vs. 2.16 ± 0.05 nmol/l, $p>0.05$) (Fig 2) and phosphorus (88.66 ± 11.63 vs. 78.50 ± 0.15 mg/l, $p>0.05$) (Fig 3) concentration. Interestingly, supplementation with vitamin D corrected the decrease in 25-hydroxyvitamin D_3 level induced by SMF-exposure (Fig 5), and calcium and phosphorus levels remained unchanged.

DISCUSSION

This study is the first to report that exposure to SMF (128 mT, 1 hr/day during 5 consecutive days) reduced 25-hydroxyvitamin D_3 level. The same treatment failed to alter body, relative liver and kidney weights, plasmatic calcium and phosphorus concentrations. Interestingly, after vitamin D supplementation, we found that disruption of plasmatic 25-hydroxyvitamin D_3 concentration, induced by SMF, was corrected, and the others parameters remained unchanged.

Vitamin D is transported in the blood by the vitamin D binding protein (DBP) to the liver. In the liver, vitamin D is hydroxylated at C-25 by one or more cytochrome P450 vitamin D 25 hydroxylase [6]. The decrease of vitamin D level could be the result of decrease in vitamin D binding protein (DBP).

There is abundant evidence that the small fractions of unbound or free vitamin D compounds are biologically active [5]. The carrier protein DBP is impermeable to the cell and functions as a reservoir for the systemic delivery of the ligand. Entry of 25-hydroxyvitamin D and 1,25dihydroxyvitamin D into cultured keratinocytes [3)] and monocytes [7] is decreased in the presence of DBP or serum. These and other findings demonstrate two roles of DBP in vitamin D physiology: prolonging the circulating half-lives of vitamin D metabolites and limiting their access to target tissues. DBP-ablated mice have very low levels of total 25-hydroxyvitamin D and 1,25dihydroxyvitamin D [17].

Likewise, both calcium and phosphorus levels were not modified after exposure to SMF and following vitamin D supplementation. Calcium homoeostasis that results from the interactions of three processes (bone resorption, tubular reabsorption and intestinal absorption) was rapidly reached [4, 18]. Therefore, we cannot conclude on the effect of SMF exposure on plasma calcium concentration. The effects of magnetic fields on biological systems have

yielded compelling data for the involvement of the calcium signalling pathway as the primary target of magnetic fields [11]. Recently, Belton *et al.,* [1] showed that the application of 1, 10 or 100 mT SMF during 800 sec did not affect the cytosolic free calcium response to ATP in HL-60 cells. Sert *et al.,* [19] show that intracellular Ca^{2+} accumulation in cardiac ventricles can increase in rats exposed to extremely low frequency (ELF) magnetic field (0.25 mT, 3 hr/day during 14 consecutive days).

Stability of phosphorus level can be due to the absence of correlation with 25-hydroxyvitamin D_3. Indeed, Bhan *et al.,* [2] found no relationship of bioavailable vitamin D levels to serum phosphorus echoing the results in the original ArMORR report that total 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels did not correlate with serum phosphorus, which is more tightly regulated by fibroblast growth factor-23 [21].

Also, our finding is in accordance with the study of Safadi *et al.,* [18]. They showed that despite low vitamin concentrations and when mice are provided with a steady source of dietary vitamin D, serum calcium and phosphorus remained unchanged [18].

On the other hand, Liu *et al.,* [15] reported that deficiency of phosphate stimulates CYP27B1 to produce more calcitriol (1,25-dihydroxyvitamin D_3), which in turn stimulates phosphate absorption in the small intestine; and calcitriol can also induce the secretion of FGF23 by osteocytes in bone, which results in phosphate excretion in the kidney, as well as feedback on vitamin D metabolism.

Preliminary studies prove that static magnetic field exposure produced a loss of plasma 25-hydroxyvitamin D_3 level, and this damage was corrected by vitamin D supplementation. However, more studies are needed to evaluate how SMF disrupts plasmatic 25-hydroxyvitamin D_3 concentration and induces hypovitaminosis D

ACKNOWLEDGEMENTS

We express our gratitude toward *Laboratory of Analytical Chemistry*, *Liège, Belgium* staff, because this couldn't be accomplished without their development of method for vitamin D measurement. Also, we thank laboratory of clinical biochemistry *Liège, Belgium* staff for their contribution to this study.

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Figure legends

Fig 1. Electromagnet (Model EM4-HVA) dimensions (front view, A) and magnetic field propagation (B)

Fig 2: Calcium level in rats exposed to static magnetic field and/or treated with vitamin D

Fig 3: Phosphorus level in rats exposed to static magnetic field and/or treated with vitamin D

Fig 4: Effects of vitamin D administration and /or SMF-exposed on the body weight.

Fig 5: 25-hydroxyvitamin D3 level in rats exposed to static magnetic field and/or treated with vitamin D

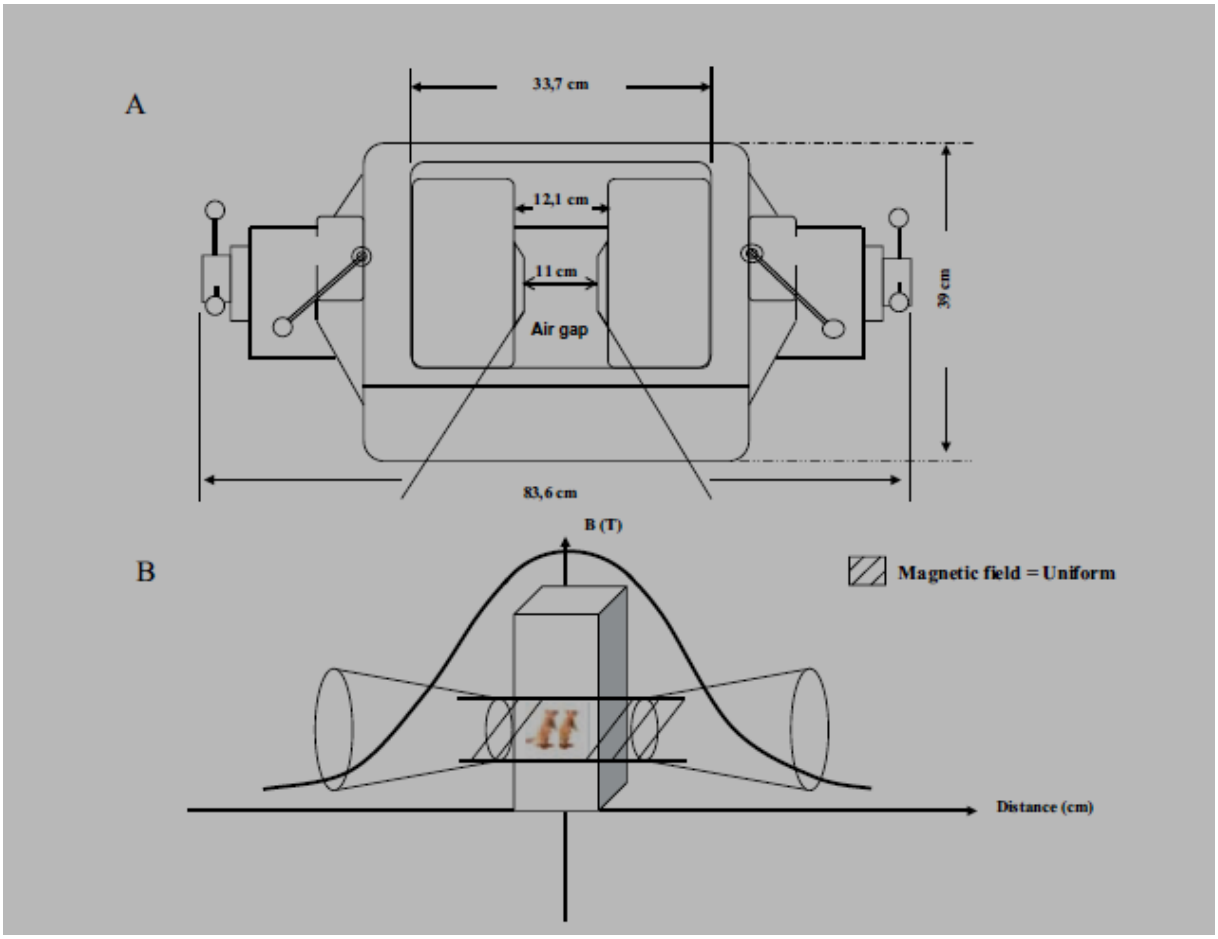

Fig 1. Electromagnet (Model EM4-HVA) dimensions (front view, A) and magnetic field propagation (B).

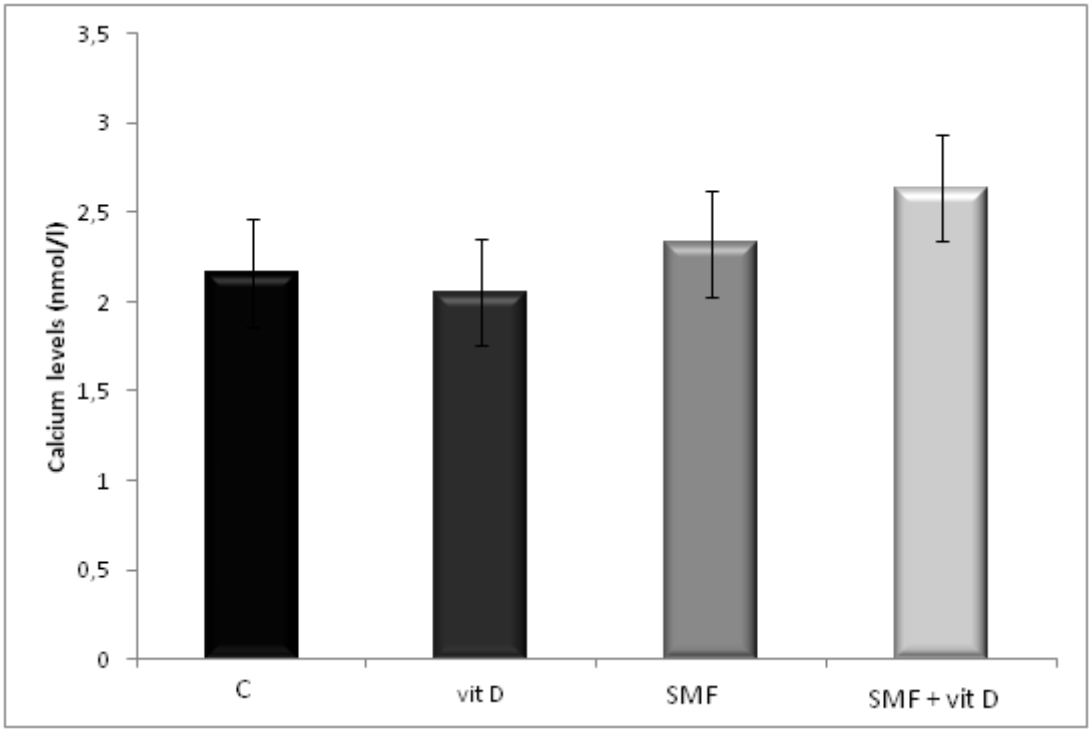

Fig 2: Data represent the means ± SEM of six animals per group. SMF: static magnetic field and vit D: vitamin D. $p > 0.05$, compared to control (C).

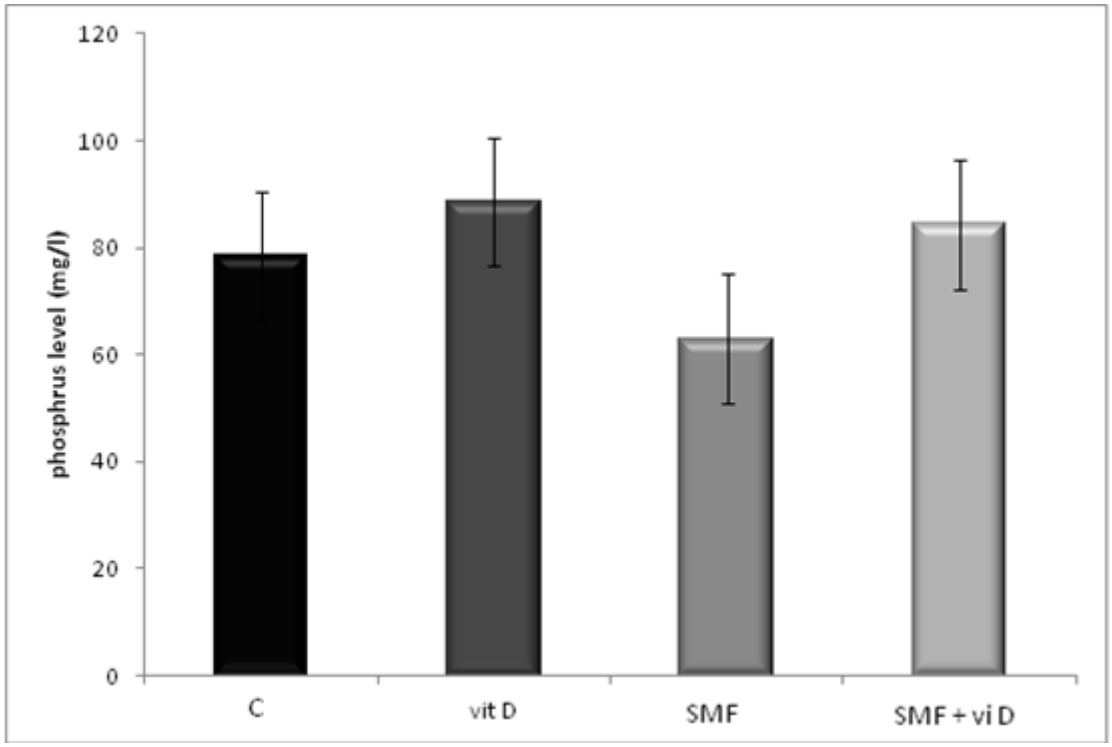

Fig 3: Data represent the means ± SEM of six animals per group. SMF: static magnetic field and vit D: vitamin D. $p > 0.05$, compared to control (C).

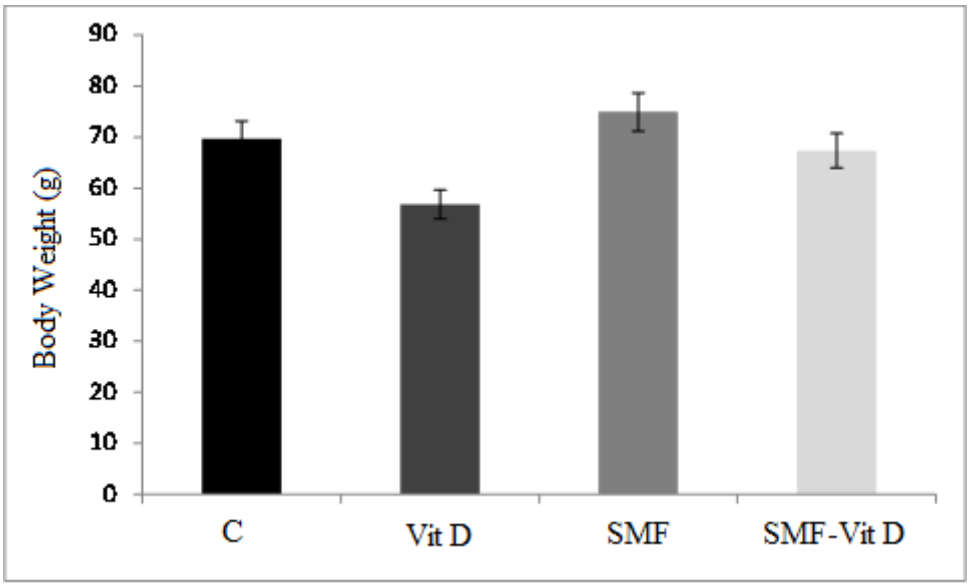

Fig 4: Data represent the means ± SEM of six animals per group. SMF: static magnetic field and vit D: vitamin D. p> 0.05, compared to control (C).

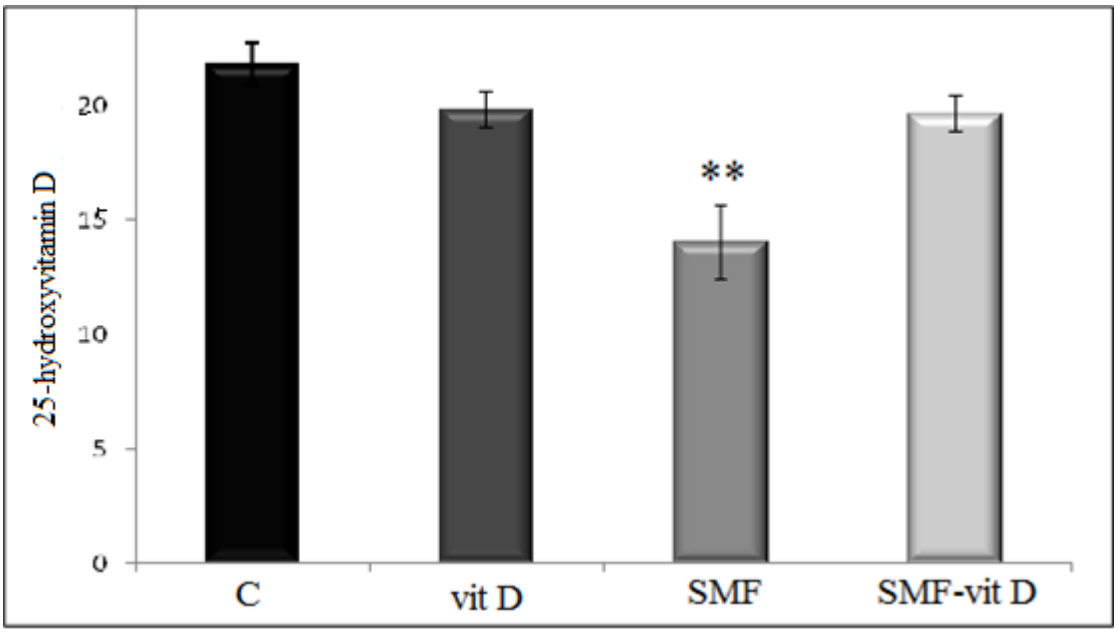

Fig 5: Data represent the means ± SEM of six animals per group. SMF: static magnetic field and vit D: vitamin D. p < 0.05, compared to control (C).

Table I: Effect of exposure to SMF on relative liver and kidney weights.

	C	***Vit D***	***SMF***	***SMF+ vit D***
Liver relative weight (g/100g)	4.57 ± 0.43	4.93 ± 0.27	4.10 ± 0.18	3.96 ± 0.04
Kidney relative weight (g/100g)	0.47 ± 0.02	0.57 ± 0.05	0.42 ± 0.03	0.40 ± 0.04

Data represents the means ± SEM of six animals per group. SMF: static magnetic field and vit D: vitamin D. $p > 0.05$, compared to control (C).