Investigating the effect of paricalcitol on serum FGF23 in vitamin D deficient rats

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Research article

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Abstract

Background: We conducted this study to discover changes of serum FGF23 in non-uremic rat model of vitamin D deficiency without secondary hyperparathyroidism using paricalcitol.

Methods: 30 adult male rats weighting 300±20 grams were enrolled. They were divided into three groups of 10 rats including Control, Vitamin D deficient(VDD), and Vitamin D deficient diet treated with paricalcitol(VDD+P). Serum biochemical were checked twice, at baseline and after the 22nd day of study.

Results: There was no significant difference in baseline laboratory data between groups. At the end of the study, 1,25(OH)D3 was reduced in VDD ($P = 0.019$) and VDD+P ($P < 0.001$) with a more significant decline in VDD+P group. Serum level of FGF23 was reduced in VDD+P group compared to the control group ($P = 0.011$) and VDD group ($P = 0.021$). serum PTH in VDD group was higher than the control and VDD+P group ($P = 0.036$ and $P = 0.038$, respectively).

Conclusion: The present study showed that paricalcitol could reduce FGF23 in vitamin D deficient rats without any changes in serum calcium, phosphorous and fractional excretion of phosphorous, which might be due to low PTH and 1,25(OH)2 D3.

Background

Fibroblast growth factor (FGF23) is a bone-derived hormone, which plays an important role in mineral metabolism (1). The main function of serum FGF23 is to suppress phosphate reabsorption and vitamin D activation in kidneys (2). However, several new klotho-dependent and klotho-independent roles including blood pressure regulation, bone mineralization and affecting innate immune system have been uncovered for FGF23 (3). Although recent studies have revealed major new functions of FGF23, it rises many new questions regarding its details (2). One of these debates has considered the effect of paricalcitol on serum FGF23 (4).

Paricalcitol is a selective vitamin D receptor (VDR) activator, which has been approved since 1998 for the treatment of secondary hyperparathyroidism (SHPT) in patients with chronic kidney disease (CKD) (5). Paricalcitol was introduced due to the need for a treatment that could inhibit high serum PTH in patients with SHPT, with a minimal effect on calcium-phosphorous product (Ca × P) in CKD patients, without renal toxicity (6). Nowadays, it is being considered as an anti-parathyroid agent rather than vitamin D analogue (7). Previous studies evaluated the effect of paricalcitol on serum FGF23 in SHPT patients with CKD (4,8). Some revealed that paricalcitol could increase serum FGF23 in hemodialysis patients through an increase in serum phosphate and calcium (4,8); however, others revealed that in non-uremic vitamin D deficient rats, paricalcitol could induce vitamin D deficiency state with unchanged PTH, Ca, and phosphate level (9). In addition, it is well known that serum FGF23 rises progressively as kidney function declines due to some known and unknown mechanisms (10,11), which consequently influences the conclusion regarding the exact effect of paricalcitol on serum FGF23 in non-uremic patients.

Hence, we conducted this study to discover changes of serum FGF23 in non-uremic rat model of vitamin D deficiency without secondary hyperparathyroidism using paricalcitol. Furthermore, we compared the serum minerals and hormones between vitamin D deficient rats (vitamin D deficiency + SHPT) and vitamin D deficient rats using paricalcitol (vitamin D deficiency without SHPT).

Methods

A total of 30 adult male spargue-dawley rats (10 weeks old), weighting 300±20 grams were purchased from the animal laboratory center of Shiraz University of Medical Sciences. Subjects underwent one-week acclimatization to the animal laboratory facilities before the study. They were housed in standard cages, five per cage, with 12:12 hours light-dark cycles at temperature of 23±2°c. All rats were randomly divided into three groups of 10 rats as below:

A: Control group(C): received normal standard rodent chow diet and had free access to tap water

B: Vitamin D deficient group (VDD group): received standard vitamin D deficient diet (TD.87095 Brown C. C. vitamin D deficient diet, containing 20% lactose, 2% calcium and 1.25% phosphate)

C: Vitamin D deficient diet + paricalcitol (VDD+P group): received the above mentioned standard vitamin D deficient diet plus intraperitoneal injections of 32 ng of 19-nor–1,25-dihydroxy vitamin D_2 (paricalcitol; Zemplar) on days 1,3,5,8,10 and 12 of the study, according to stavenuiter protocol (F). Rats' weight was checked on days 1,10,15 and 22 of the study.

At the end of the experiment, all rats were anesthetized with ketamine 10 % (100 mg/kg, Alfasan, Netherlands) and xylazin 2 % (10 mg/kg, Alfasan, Netherlands) solution intraperitoneally and sacrificed by using thiopental (100 mg/kg).

Biochemical studies:

Serum calcium (Ca), phosphorous (P), Alkaline phosphatase (Alp), parathormone (PTH), 25-hydroxy vitamin D_3 (25OH2D3), 1,25-dihydroxy vitamin D_3 ($1,25(OH)_2D_3$) and serum FGF_{23} were checked for all 30 rats on 1^{st} and 22^{nd} day of the study.

All blood samples were centrifuged at 3500 rpm for 12 min, the plasma was stored at–70°C till further analysis. Serum Ca (mg/dl), P(mg/dl), creatinine(mg/dl), and ALP (Iu/L), were measured by colorimetric assays with a Biosystem, SA auto-analyzer, Spain. Fractional excretion of phosphorus (FEP) was assessed using this formula: (urine P x serum creatinine) ×100/(serum P x urine creatinine). Serum $25OHD_3$ (ng/ml) were checked using Electrochemolumicence method, Germany, with 2 ng/ml sensitivity, 3.3% intra assay CV and 5.1% inter-assay CV. Serum $1,25(OH)_2D_3$ (pmol/ml) were measured with ELISA method produced by Bioassay Technology laboratory, China with intra- and inter-assay CVs sensitivity of less than 8% and 10%, respectively. Serum PTH (pg/ml) was measured with ELISA kits with sandwich technology, MyBioSource, USA with intra- and inter-assay of <6% and <7%, respectively. Serum

FGF_{23}(pg/ml) was measured by sandwich technology of ELIZA method. The kit was produced by Bioassay Technology laboratory in China with intra- and inter-assay CVs less than 8 and 10%, respectively.

Statistical analysis:

Data were analyzed using SPSS, version 21. Data were presented as mean ± SD. The paired-samples *t*-test was used to analyze values within the same group at baseline and after 22^{nd} day of the study. One-way ANOVA test with Tukey post-hoc test was used to compare biochemical data between the 3 studied groups. P value less than 0.05 was considered to be statistically significance.

Compliance with Ethical Standards:

All authors declare that they have no conflict of interest. This study was approved by the local Ethics Committee and vice-chancellor of research at Shiraz University of Medical Sciences.

The study was done in accordance with the ARRIVE (Animal Research: Reporting of *in vivo* Experiments) guide line (12) on the use and care of research animals and all the applicable institutional and national guidelines for care and use of animals.

Results

Biochemical parameters were measured in all 3 groups at beginning of the experiment, and there was no significant difference in serum $25(OH)D_3$, $1,25(OH)_2D_3$, Ca, P, Alp, and serum FGF_{23} in all the 3 groups, $P<0.05$. All the data are summarized in table 1.

Daily food intake was about 21 grams (median; Inter Quartiles 19.8–23.3) per day in all rats, without any significant difference in the 3 groups ($P = 0.394$) regarding energy intake. Also, no obvious change was observed in rats' behavior. Mean body weight was similar in all 3 groups on the first day, summarized in figure–1. However, after 22 days the body weight in VDD and VDD+P groups was less than the controls.

At the end of the study, all biochemical analysis was rechecked. Data are summarized in figure 2 and 3. There was significant vitamin D deficiency in VDD and VDD+P groups ($P < 0.001$). Also, serum $1,25(OH)D_3$ was reduced significantly in VDD group ($P = 0.019$) and VDD+P group ($P < 0.001$) with a more significant decline in VDD+P group. Serum level of FGF_{23} was reduced in VDD+P group compared to the control group ($P = 0.011$) and VDD group ($P = 0.021$), figure 2. Serum Ca, P, %FEP and ALP did not show significant difference between all groups ($P > 0.05$), figure 3. In addition, there were no significant difference in serum PTH between VDD+P and the control group; however, serum PTH in VDD group was higher than the control and VDD+P group ($P = 0.036$ and $P = 0.038$, respectively).

Discussion

The present study showed that paricalcitol could lower serum FGF_{23} and $1,25(OH)D_3$ in vitamin D deficient rats. Recent studies revealed the beneficial effects of paricalcitol in treating secondary hyperparathyroidism in chronic kidney disease, as it can lower serum PTH with a minimal effect on serum concentrations of Ca, P and calcium-phosphorous product (Ca × P), without damaging the kidneys (6, 13). However, its effect on FGF_{23} should be further investigated.

Donate-Correa et al. showed that in renal transplant recipients after 3-months of therapy with oral paricalcitol, serum KLOTHO concentration and KLOTHO mRNA level was increased. Also, they observed a significant reduction in serum PTH without any significant change in serum calcium and phosphorus (4). In addition, they showed a significant increase in the serum FGF_{23} after paricalcitol administration. They suggested that it might be due to the general effect of vitamin D analogs on FGF_{23} induction (4); however, they did not evaluate the serum 1,25(OH)D. Finch et al. investigated the effect of paricalcitol and cinacalcent on mineral metabolism in rats with chronic kidney disease (14). They showed that paricalcitol could reduce serum $1,25(OH)_2D_3$ and PTH; however, they showed that serum FGF_{23} was increased. They stated that this paradox might be due to vitamin D analogs role in activating FGF_{23} release (15). In contrast, Wetmore et al. found that paricalcitol and doxercociferol did not change serum FGF_{23} in end stage renal disease (16). These discrepancies might be due to differences in nature of vitamin D analogues, or might be due to some other unknown factors (8). Previous studies revealed that serum FGF_{23} increases as kidney function declines (10,17), but the reason for this increase is not fully understood (18). Hence, effect of paricalcitol on FGF_{23} in treating secondary hyperparathyroidism should be investigated in non-uremic states affected vitamin D deficiency. In the present study, we showed that using paricalcitol in vitamin D deficient states could reduce serum PTH and $1,25(OH)_2D_3$ compared to vitamin D deficient rats, without any significant changes in serum calcium, phosphorous and fractional excretion of phosphorous. In addition, we showed that serum FGF_{23} was reduced after using paricalcitol in vitamin D deficient rats. The present study suggests that reduced FGF_{23} in vitamin D deficient rats, using paricalcitol could be explained by two mechanisms. The first mechanism might be low PTH level (2), which decreases serum FGF23, since PTH stimulates FGF_{23} secretion in bone (19). The second mechanism of low FGF_{23} in vitamin D deficient states treated by paricalcitol might be due to decrease in $1,25(OH)_2D_3$. Previous studies showed that $1,25(OH)_2D_3$ stimulates the expression of FGF_{23} *in vitro* in bone-derived cell cultures (15,20,21). Hence, low $1,25(OH)_2D_3$ in paricalcitol treated patients can negatively affect FGF_{23} expression from bones (15). In spite of FGF_{23} reduction, we did not observe any significant change in fractional excretion of phosphorous. This might be due to low serum PTH level in this group. Clarke et al. showed that FGF_{23} regulation of phosphate homeostasis depends on PTH (22). Hence, using paricalcitol to reduce PTH might influence on the phosphaturic action of FGF_{23} in vitamin D deficient rats treated with paricalcitol. Therefore, the present study supports Clarke's claim.

In addition to strengths of this study, we had some limitations. We did not investigate FGF_{23} gene expression and KLOTHO pathway in vitamin D deficient rats treated with paricalcitol, which should be further evaluated to find out more details about FGF_{23} and paricalcitol.

Conclusion

The present study showed that paricalcitol could reduce PTH, 1,25$(OH)_2$ D3 and FGF_{23} in vitamin D deficient rats without any changes in serum calcium, phosphorous and fractional excretion of phosphorous. FGF23 reduction might be due to low PTH and 1,25(OH)2 D3. In addition, it supports the previous studies that claimed FGF_{23} regulation of phosphorous homeostasis depends on PTH. Further investigation evaluating the FGF_{23} gene expression and KLOTHO pathway should be considered in vitamin D deficient rats treated with paricalcitol, to find out more details about FGF_{23} and paricalcitol.

Abbreviation

Fibroblast growth factor–23 (FGF23)

25-hydroxy vitamin D3 (25OH2D3)

1,25-dihydroxy vitamin D3 (1,25(OH)2D3)

Vitamin D deficient diet (VDD)

Vitamin D deficient diet+ paricalcitol(VDD+P)

vitamin D receptor(VDR)

secondary hyperparathyroidism(SHPT)

chronic kidney disease (CKD)

parathormone (PTH)

Calcium(Ca)

phosphorous (P)

Alkaline phosphatase(ALP)

Statistical Package for Social Sciences(SPSS)

Standard deviation(SD)

one-way analysis of variance (One-way ANOVA)

Animal Research: Reporting of in vivo Experiments (ARRIVE)

Fractional excretion of phosphorus (FEP)

Declarations

Ethics approval and consent to participate: This study was approved by the local Ethics Committee of Shiraz University of Medical Sciences (SUMS). Vice chancellor of research at SUMS approved this study with ID: 97-01-33-17348. The present study is in line with the ARRIVE (Animal Research: Reporting of *in Vivo* Experiments) guidelines about using and coring of research animals.

Consent for publication: The present study is in line with the ARRIVE (Animal Research: Reporting of *in Vivo* Experiments) guidelines about using and coring of research animals.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request

Competing Interests: The authors, declare that they have no conflict of interest.

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Authors contribution:

1. **FS,** design, data gathering, preparing the manuscript
2. **GHRO**: design, data gathering, preparing the manuscript
3. **FK:** design, data gathering, preparing the manuscript and the correspondence. All authors have read and approved the manuscript

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Table

Table 1: It describes the serum minerals, vitamin D and FGF23 levels (Mean ± SD) in first day of study in all three groups

Groups	25(OH)D3 (nmol/ml)	1,25(OH)$_2$ D3 (nmol/ml)	Ca (mg/dl)	Ph (mg/dl)	ALP (IU/L)	FGF23 (pg/ml)
Control	89.25±3.95[a]	60.76±5.90[a]	9.76±0.13[a]	6.10±0.18[a]	482.80±31.37[a]	65.90±3.20[a]
VDD	79.7±4.25[a]	60.91±5.30[a]	9.26±0.38[a]	5.88±0.29[a]	388.80±24.68[a]	63.54±4.46[a]
VDD+P	80.37±3.95[a]	61.33±5.00[a]	9.01±0.56[a]	6.34±0.51[a]	410.5±40.66[a]	62.04±3.78[a]

[a] There was no significant difference between the experimental groups ($P<0.05$)
VDD: Vitamin D deficient group
VDD+P: Vitamin D deficient group treated with Paricalcitol

Figures

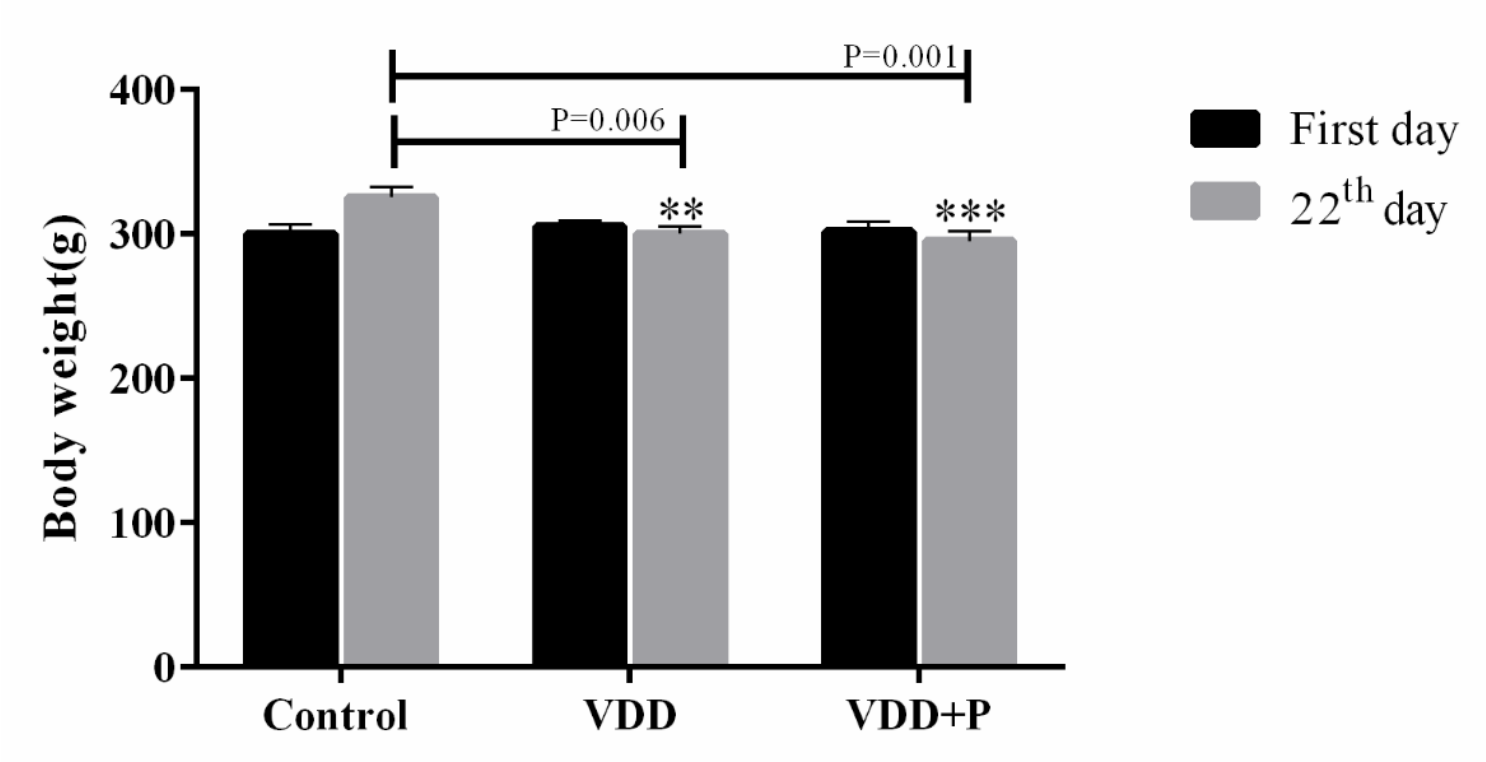

Figure 1

Body weight of studied rats in all three groups during the study

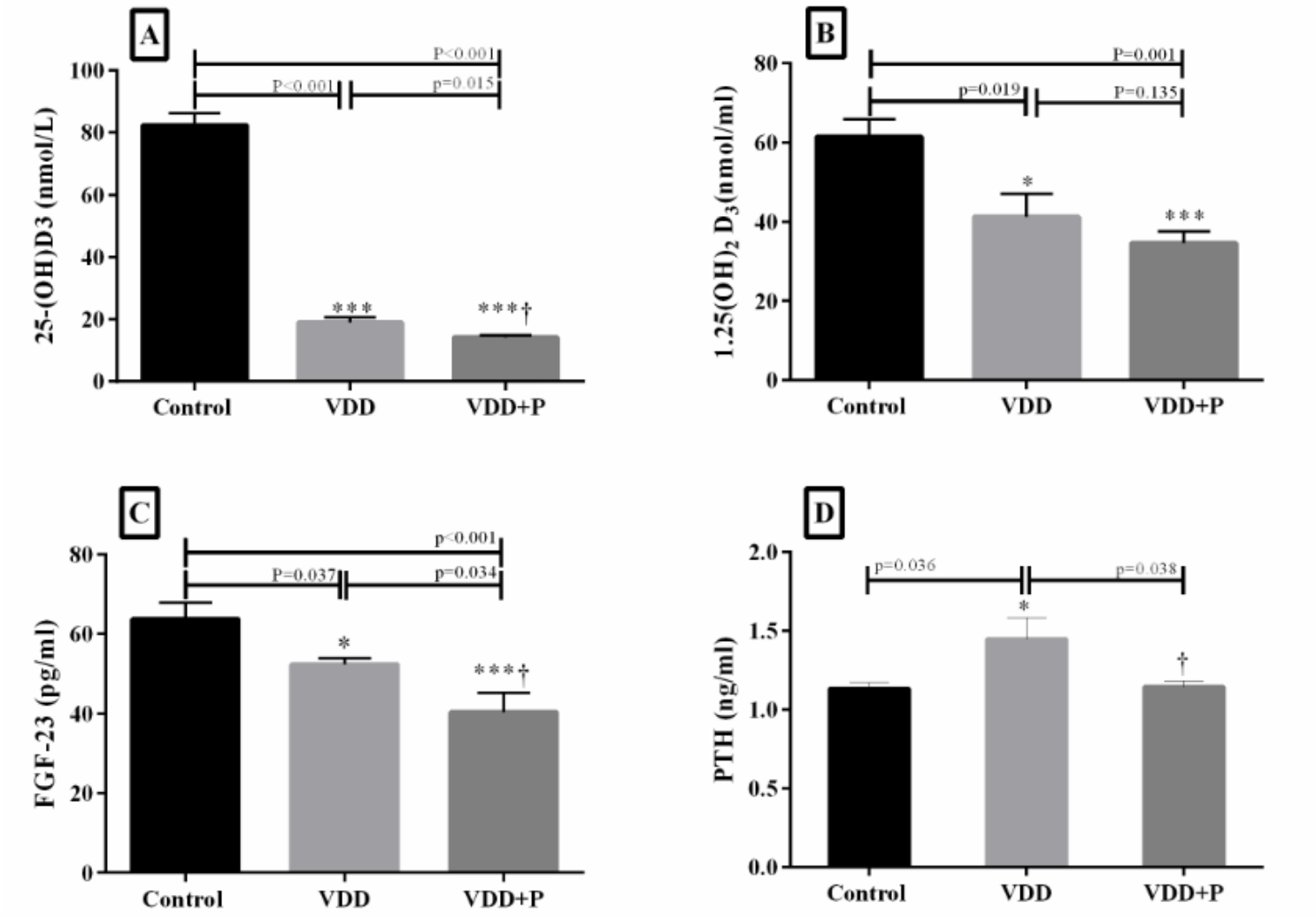

Figure 2

serum 25OHD3, 1, 25(OH)2D3, PTH and FGF23 serum level in all rats at the end of the study (22nd day)

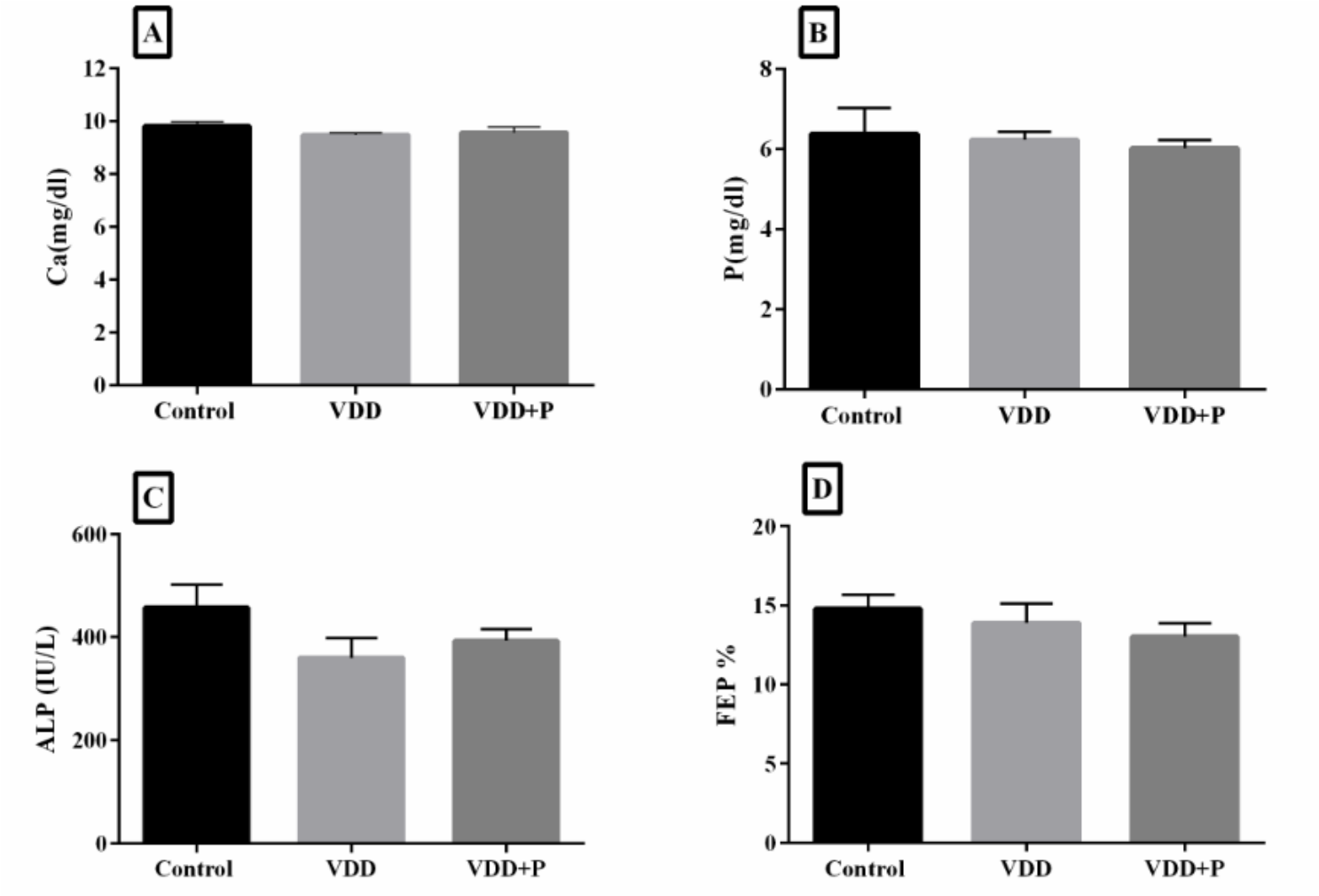

Figure 3

serum calcium, phosphorous, alkaline phosphatase and Fractional excretion of phosphorous in all rats at the end of the study (22nd day)