Journal of Advances in Medicine and Medical Research

26(12): 1-9, 2018; Article no.JAMMR.41829
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

Role of Paricalcitrol as an Active Vitamin D Analogue on Erectile Function and Penile NOS Expression in Diabetic Rats

Amany Shalaby[1*], Gehan A. Shaker[2] and Eman H. El-Adawy[3]

[1]Department of Clinical Pharmacology, Faculty of Medicine, Mansoura University, Egypt.
[2]Department of Medical Physiology, Faculty of Medicine, Mansoura University, Egypt.
[3]Department of Internal Medicine, Faculty of Medicine, Mansoura University, Egypt.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMMR/2018/41829
Editor(s):
(1) Muhammad Torequl Islam, Nuclear of Pharmaceutical Technology (NTF), Federal University of Piaui, Brazil.
Reviewers:
(1) Geraldo Munguba Macedo, Santa Casa Teaching Hospital, Brazil.
(2) Sumanta Kumar Goswami, Drexel University School of Medicine, USA.
Complete Peer review History: http://www.sciencedomain.org/review-history/25304

Original Research Article

Received 23rd March 2018
Accepted 5th June 2018
Published 28th June 2018

ABSTRACT

Objectives: To investigate the effect of paricalcitrol as a vitamin D analogue on erectile dysfunction and on the penile expression of superoxide anion generationand nitric oxide synthase (NOS) isoforms instreptozotocin-induced diabetic rats models.
Methods: Male Sprague-Dawley rats were used in this study. Diabetes was induced by a one-time intraperitoneal injection of streptozotocin (60 mg/kg). The rats with blood glucose levels above 300 mg/dL were selected for the study. The erectile function of the rats was assessed by recording frequency of erection after subcutaneous injection of apomorphine (80 ug/kg). Those were divided into 2 groups (N = 10 per group): i) EDDM (erectile dysfunction diabetes mellitus) group fed with saline and ii) EDDM + paricalcitrol treated group receiving paricalcitrol (0.4 µg/k/day) by intraperitoneal injection. 10 animals were used as a control group and received no streptozotocin.
4 weeks later, the erectile function of the rats was assessed by recording frequency of erection after subcutaneous injection of apomorphine (80 ug/kg). Superoxide anion generation and mRNA expression of of nNOS and eNOS were evaluated in corpora cavernosum tissue.
Results: Penile erection, the expression of both nNOS, eNOS generation was significantly elevated however the superoxide generation was significantly decreased in paricalcitrol treated group compared to diabetic group.

**Corresponding author: E-mail: aashalaby1967@hotmail.com;*

Conclusion: Treatment with paricalcitrol for 4 weeks improves erectile dysfunction in diabetic rats probably by improving expression of nitric oxide synthase expression and decreasing superoxide production.

Keywords: STZ rats; erectile dysfunction; nitric oxide synthase and paricalcitrol.

1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemic condition resulting from damages in insulin secretion and/or insulin resistance, and disturbance of carbohydrate metabolism [1]. The World Health Organization estimates DM affects 220 million people worldwide and that number is projected to double by 2030 [2]. Erectile dysfunction (ED) is one of the most common complications in male patients with diabetes mellitus. The prevalence of ED is approximately 35–75% in diabetic patients, 3 times more than that in non diabetics [3]. The Pathophysiological mechanism underlying diabetes-associated ED is mainly due to endothelial dysfunction, which could lead to the inability of the endothelium to produce vasorelaxing messengers and to maintain vasodilation and vascular homeostasis [4]. Nitric oxide (NO) is one of the most important vasorelaxing messengers in the process of erection. NO generated by neuronal NOS (nNOS) is considered the main factor responsible for the immediate relaxation of corpus cavernosum, while NO from endothelial NOS (eNOS) is essential for maintaining relaxation [5]. Conditions that are associated with reduction of nNOS and eNOS levels can cause circulatory and structural changes in penile tissues, resulting in erectile dysfunction.

Paricalcitrol (19-nor-1,25-dihydroxyvitamin D2) is an active, non-hypercalcemic vitamin D analogue that shows biological activity similar to vitamin D, but has less adverse effects [6]. In addition to its primary role in calcium metabolism and bone mineralisation, vitamin D and its non-hypercalcemic analogue paricalcitrol have pleiotropic and antioxidant effects on cellular homeostasis [7].

Recently it has been shown that paricalcitrol has antioxidant effects on the myocardium [8]. Vitamin D therapy also ameliorates oxidative stress injury in some experimental models. *In vitro*, vitamin D reduces interleukin (IL)-6 synthesis and nuclear factor-κB activity, and prevents advanced glycation end-product-induced inhibition of endothelial nitric oxide-synthase production [9]. However; the effect of paricalcitrol on penile NOS isoform expression and/or erectile function in diabetics has not been evaluated to date. So the present study was done to evaluate the effect of paricalcitrol on erectile function as well as the expression of NOS isoforms and superoxide production in penile tissue of rat model of STZ- diabetic erectile dysfunction.

2. ANIMALS AND TREATMENTS

12 weeks old Male Sprague-Dawley rats weighing 300-350 g were included in this study. The study protocol was approved by the local ethics committee for animal experiments, Mansoura faculty of medicine.

Overnight fasted rats were injected intraperitoneally with a freshly prepared streptozocin (STZ, Sigma Chemical Co, St Louis, MO, USA) (60 mg/kg) or vehicle (0.130 mol/L citrate-phosphate buffer solution, pH 4.5) [10,11]. Blood glucose levels were monitored 72 h after STZ or vehicle injection, weekly during the whole experimental period, and immediately prior to euthanasia. Blood samples were obtained by tail prick, and blood glucose concentration was measured using a blood glucose meter (B. Braun, Germany). 72 h later, only rats with fasting glucose concentrations (≥ 300 mg/dL) were considered in the diabetic group. 4 weeks later with free diet, the diabetic rats were evaluated for erectile function and those whose erectile function decreased significantly were considered to be diabetic rats with ED. Those rats were randomly divided into 2 groups (N = 10 per group), i) EDDM group (erectile dysfunction diabetes mellitus group) fed with saline and ii) EDDM + Paricalcitrol treated group receiving paricalcitrol (ZemplarH; Abbott Laboratories, Abbott Park, IL) ip at a dose of 0.4 µg /kg per day once daily [12]. 10 animals used as a control group and received only standard husbandry care, 4 weeks later, erectile function was evaluated by apomorphine injection.

2.1 Erectile Function Evaluation

Subcutaneous injection of apomorphine was used to evaluate erectile function in an animal experiment [13]. Briefly the room light was

dimmed apart from some indirect light sufficient for observation. After a 10 min habituation period, rat was injected with apomorphine (80 ug/kg) subcutaneously in the loose skin area at the back of the neck. It was observed for 30 min to record frequency of erection. Emergence of an engorged glans penis and distal shaft was only counted as an erection.

2.2 Tissue Preparation

Rats were anesthetized by intraperitoneal injection of 25 mg/kg of pentobarbital, the rat penis was amputated. Penis' skin, subcutaneous tissue, and penile bone were carefully removed. The corpora cavernosum (CC) was divided into 2 samples (50 mg for each), which were immediately frozen in liquid nitrogen [14].

2.3 Estimation of Superoxide Anion Generation

Superoxide anion generation of the corpora cavernosum (CC) was estimated in terms of reduced nitroblue tetrazolium (NBT) as described in the methodology of Wang et al. [14] Briefly y, CC homogenate react with NBT under certain chemical condition to form formazan as an index of superoxide anion generation. Formazan absorbance was determined spectrophotometrically at 540 nm.

The quantity of NBT reduction = $A \times V/(T \times Wt \times \varepsilon \times l)$,

Where A is the absorbance of blue formazan at 540 nm, V is the volume of the solution, T is the time period (90 min) during which rings were incubated with NBT, Wt is the blotted wet weight of the CC, ε is the extinction coefficient of blue formazan (i. e., 0.72 l/mmol/mm), and l is the length of the light path. The results were reported as picomoles / minute /milligram wet weight of CC.

2.4 Reverse Transcriptase Polymerase Chain Reaction

Total RNA was extracted from the penile tissue using a modificationof the method of Chomczynski and Sacchi [15]. The RNA concentration was determined using spectrophotometry (OD 260).

The reverse transcriptase (RT) reaction was performed using aQIAGEN one-step RT-polymerase chain reaction (PCR) kit (Hilden,Germany) as previously described [16]. One microgram of total RNA was reverse transcribed into cDNA using Omniscript RT, SensiscriptRT, and primers. The sense primer sequence for eNOS was5′-TGCACCCTTCCGGGGATTCT-3′ and the antisense was 5′-GGATCCCTGGAAAAGGCGGT-3′. The sense primer sequence for nNOS was 5′-GGCACTGGCATCGCACCCTT-3′ and the antisense was5′-CTTTGGCCTGTCCGGTTCCC-3′. Amplifi cation was initiated at 50°C for 30 min, followed by 30 cycles consisting of denaturation at 94°C for 1 min, annealing at the appropriate primer-pair annealing temperature for 1 min, and extension at 72°C for 1 min, and then a final extension step of 10 min at 72°C. ß-actin (sense: 5′-TCTACAAT GAGCTGCGTGTG-3′ and antisense: 5′-GGTCAGGATCTTCATGAGGT-3′) was used as an internal control and standard. The RT-PCR products were electrophoresed on a 1.5% agarose gel and visualized by staining with ethidium bromide.

2.5 Statistical Analysis

Data was expressed as mean value ± SD. Comparisons were carried out by analysis of variance followed by Tukey's test for the parametric data, blood glucose and body weight data were non- parametric and were analyzed using Mann-Whitney test, using SPSS for Windows (20.0 Version). The differences were considered statistically significant when $P < 0.05$.

3. RESULTS

Table 1 illustrated changes in body weight and blood glucose levels of the 3 groups. After induction of diabetes, serum blood glucose level was measured throughout the study. Both diabetic groups showed progressive loss of body weight and significant hyperglycemia compared to control group, with non statistical changes in blood glucose and body weight in paricalcitrol treated group.

3.1 Effect of Paricalcitrol on Penile Erection

Penile erection was significantly reduced in diabetic rats compared to control rats. Paricalcitrol treatment significantly increased penile erection in diabetic rats (Fig. 1).

3.2 Effect of Paricalcitrol on Penile Expression of NOS

Penile expression of both nNOS and eNOS were significantly suppressed in EDDM group

compared with control group. However, Paricalcitrol significantly increased both nNOS and eNOS expression (Fig. 2).

3.3 Effect of Paricalcitrol on Penile Superoxide Production

Penile superoxide production was markedly increased in diabetic ED group as compared to control group. Paricalcitrol significantly reduced the superoxide production (Fig. 3).

4. DISCUSSION

Normal penile erection is a hemodynamic event that is dependent on penile smooth muscle relaxation mediated by NO/cGMP signaling, parasympathetic neurotransmission and other regulatory factors [17]. DM may lead to ED by a number of pathophysiological events including neuropathy, endothelial dysfunction, decreased bioavailability of nitric oxide, increased oxidative stress, disturbances in intracellular signal transduction, and activation of advanced glycation end products (AGEs) [18].

The current study demonstrated that erectile function and penile expression of NOS mRNA are markedly suppressed in diabetic rats compared with control rats and that ED and expression is markedly elevated by paricalcitrol treatment. Also, Penile superoxide production was markedly increased in diabetic ED group as compared to control group, paricalcitrol significantly reduced That superoxide production.

Table 1. Changes of body weight and plasma glucose of the 3 groups

Group	Pre STZ injection	4 weeks	8 weeks
Body weight (gm)			
control	320±23	390±25*	430±33*
EDDM	310±21	250±20*	190±13*
Paricalcitrol treated	340±21	300±29*	310±30*
Blood glucose mg/dl			
control	103±8	108±5	112±5
EDDM	109±6	350+22 *	450±45*
Paricalcitrol treated	114±9	320±18*	390±51*

**significant statistical difference ($p < 0.05$) from the control*

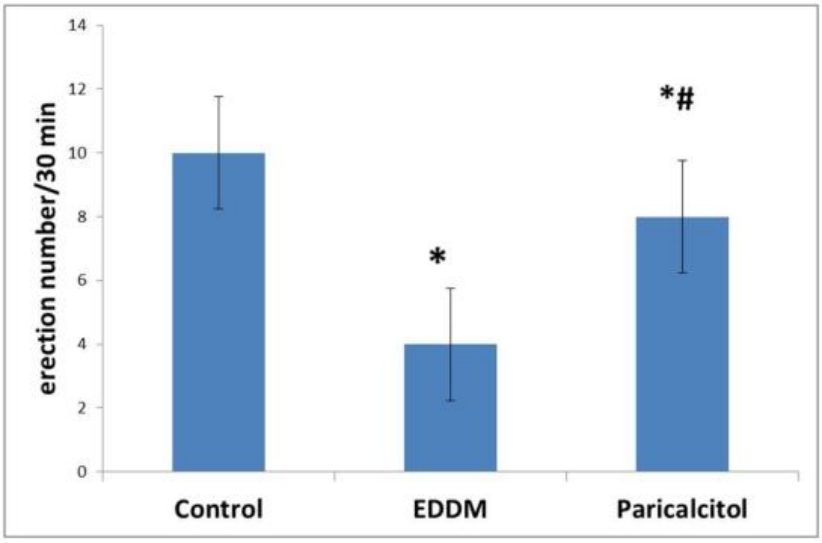

Fig. 1. Effect of paricalcitrol on erectile function in diabetic ED rats: Erectile function was evaluated in a penis erectile experiment with apomorphine

*Mean ± SD, *P < 0.05, vs. corresponding control rats; # P < 0.05, vs. corresponding EDDM group*

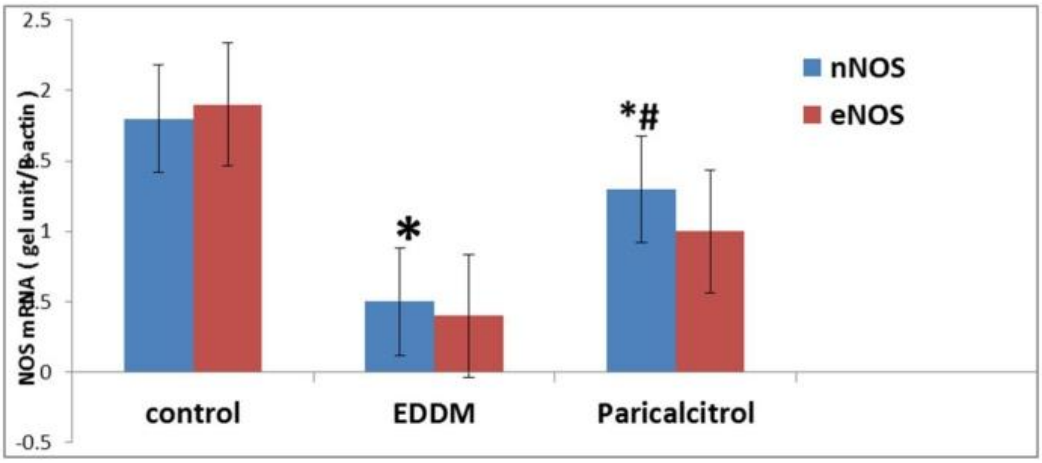

Fig. 2. Effect of paricalcitrol on NOS mRNA in diabetic ED rats: Densitometry and statistics of NOS mRNA (ratio to-B-actin) in the corpus cavernosum of the control group, EDDM group and EDDM group treated with paricalcitrol

*Mean ± SD, *P < 0.05, vs. Corresponding control rats; # P < 0.05, vs. corresponding EDDM group*

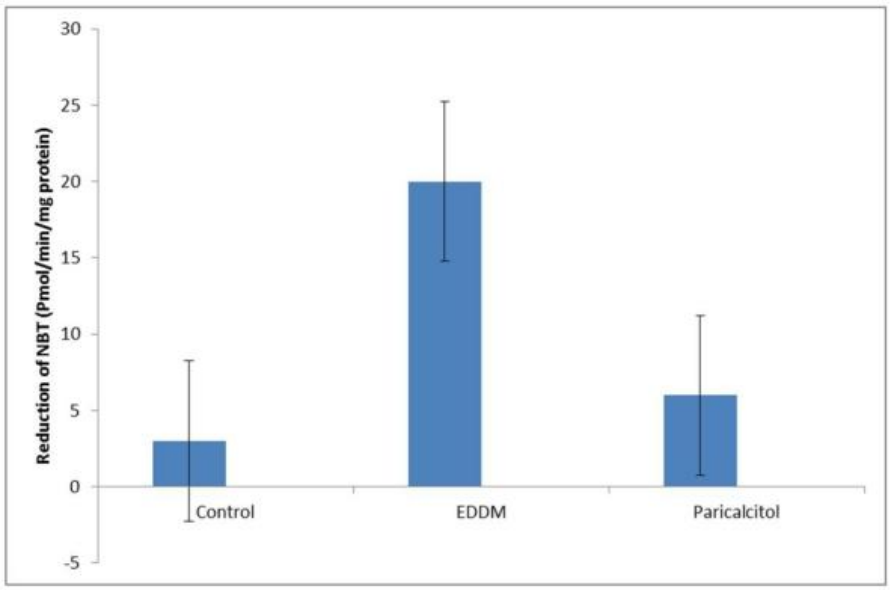

Fig. 3. Effect of paricalcitrol on superoxide production in diabetic ED rats: Superoxide production was evaluated in corpus cavernosum by measuring level of NBT (pmol/min/mg protein)

*Mean ± SD, *P < 0.05, vs. Corresponding control rats; # P < 0.05, vs. corresponding EDDM group*

The elevated penile expression of NOS mRNA with improved erectile function is in agreement to that reported in the penis of type 1 diabetic animals where a selective nitrergic degeneration has been demonstrated with loss of nNOS in the nerve fibers [19,20]. Also [21], reported that activated VD stimulates the production of NO in endothelial cells and NO synthases which catalyze the production of NO from L-arginine.

Earlier research in the USA showed that low serum VD levels were associated with higher prevalence of peripheral arterial disease [22]. Likewise, in a very recent cross-sectional analyses (3,390 men aged >20 years, free of ASCV disease) it has been also reported that vitamin D deficiency (VDD) was associated with an increased ED prevalence [23]. It was also reported that VD receptor mutant mice are

characterized by lower bioavailability of the vasodilator NO. It was demonstrated that VD is a direct transcriptional regulator of eNOS [24]. This may clarify why endothelium derived, and NO-evoked dilation is reduced nearly 50% in arteries from VD deficient male rats, that explains the high prevalence of ED among VD deficient patients [25].

The improved ED may be explained by the fact that, Vitamin D as a potent steroid hormone which is positively correlated with testosterone, exhibits a concordant seasonal fluctuation[38]3, and elevates when testosterone is supplemented in androgen deficient men [26]. Amazingly, the reverse situation is also true, suggesting that VD supplementation might increase testosterone levels [27]. Although such a possible association between serum VD and testosterone has been reported, conflicting findings still exist [28].

Vitamin D supplement may protect the cells through suppressing inflamatory factors and alleviating apoptosis, as well as upregulating the expression of genes related to reproduction and testosterone synthesis. Vitamin D also played a protective role against testicular damage induced by diabetes, and the possible mechanism might be effective through attenuation of inflammation and inactivating caspase cascade [29].

Andress [30] reported that, the selective vitamin D receptor activation agents including paricalcitrol are reported to have anti-inflammatory and antithrombotic effects, and could inhibit vascular smooth muscle cell proliferation, and vascular calcification and stiffening, and could regress left- ventricular hypertrophy. Which may explain improved ED after paricalcitrol treatment.

In the present study an increased superoxide concentration is in agreement with previous study [31] and the superoxide production by vascular tissues and its interaction with NO might generate the powerful oxidative and highly toxic peroxynitrite radical. Therefore; an increased superoxide introduces the concept of oxidative neurodegeneration in the pathophysiology of arteriogenic ED [32].

The reduced superoxide production in response to paricalcitrol could be explained The increased levels of superoxide dismutase (SOD) and reduced glutathione (GSH) following preclinical findings with this selective VDR agonist. Since both GSH and SOD are the main contributors to cellular redox homeostasis, it is rather relevant that paricalcitrol treatment seemed to increase the levels of these ROS scavengers, [33]. Paricalcitrol also has shown a protective effects against oxidative stress in the cardiac tissue of uremic rats, through inhibition of NADPH oxidase activity [8], An increasing evidence shows the prevalent role of vitamin D signaling pathways in redox homeostasis and cardiovascular disease prevention [34-37]. Moreover, a recent clinical report indicated that intravenous calcitriol, as a Vitamin D receptor agonist (VDRA), reduces oxidative stress in hemodialysis patients [35].

The antioxidant effect of paricalcitrol is further observed with the reduction of carbonyl groups and nitrites. The latter may be due to a previously described down regulation of eNOS and iNOS activity, which would respond both to an antioxidant and anti-inflammatory effect of VDR activation [38,39].

The induction pathway of many antioxidant molecules by paricalcitrol remains unknown, and further exploration of their signaling pathways could provide more information on the different paricalcitrol mechanisms of action. However, *in vitro* paricalcitrol treatment is consistent with the increase in other components of the antioxidant arm. The altered NOS function and NO bioavailability has been mostly attributed to vascular superoxide production [40].

Furthermore, hyperglycemia is recognized as a primary cause in the pathogenesis of the endothelial dysfunction of the vasculature in DM [41], and glycemic controle displayed a significant lower incidence of ED in diabetic patients [42], paricalcitrol could be beneficial as an adjuvant theraby with antidiabetic drugs to control hyperglycemia and prevent ED associated with DM.

5. CONCLUSION

Paricalcitrol may have a potency to improve the erectile function in streptozotocin-induced diabetic rat erectile dysfunction model. Paricalcitrol could produce notable clinical benefits for ED, especially of diabetic origin.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee.

ACKNOWLEDGEMENTS

The valuable suggestions of Dr. Amal Seleem, Prof. of Medical Biochemistry, Faculty of Medicine, Mansoura University, Egypt.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bacon CG, Hu FB, Giovannucci E, Glasser DB, Mittleman MA, Rimm EB. Association of type and duration of diabetes with erectile dysfunction in a large cohort of men. Diabetes Care. 2002;25:1458-63.
2. Blanco R, Saenz de Tejada I, Goldstein I, et al. Dysfunctional penile cholinergic nerves in diabetic impotent men. Journal Urology. 1990;144:278–280.
3. Richardson D, Vinik A. Etiology and treatment of erectile failure in diabetes mellitus. Current Diabetes Reports. 2002;2:501–509.
4. Akingba AG, Burnett AL. Endothelial nitric oxide synthase protein expression, localization, and activity in the penis of the alloxan-induced diabetic rat. Mol Urol. 2001;5:189-97.
5. Cartledge JJ, Eardley I, Morrison JF. Advanced Glycation end-products are responsible for the impairment of corpus cavernosal smooth muscle relaxation seen in diabetes. BJU Int. 2001;87:402-7.
6. Dru¨ eke TB. Which vitamin D derivative to prescribe for renal patients. Curr Opin Nephrol Hypertens. 2005;14:343–9.
7. Park JW, Bae EH, Kim IJ, Ma SK, Choi C, Lee J, et al. Paricalcitrol attenuates cyclosporine-induced kidney injury in rats. Kidney Int. 2010;77:1076–85.
8. Husain K, Ferder L, Mizobuchi M, Finch J, Slatopolsky E. Combination therapy with paricalcitrol and enalapril ameliorates cardiac oxidative injury in uremic rats. Am J Nephrol. 2009;29:465–72.
9. Talmor Y, Golan E, Benchetrit S, et al. Calcitriol blunts the deleterious impact of advanced glycation end products on endothelial cells. Am J Physiol Renal Physiol. 2008;294:1059–1064.
10. Chen Y, Yang R, Yao L, et al. Differential expression of neurotrophins in penises of streptozotocin-induced diabetic rats. Journal of Andrology. 2007;28:306–312.
11. Kang Sup Kim, Woong Jin Bae, Su Jin Kim, Kyong-Hwa Kang, Se-Kwon Kim, Hyuk Jin Cho, Sung-Hoo Hong, Ji Youl Lee, Sae Woong Kim. Improvement of erectile dysfunction by the active pepide from *Urechis unicinctus* by high temperature/pressure and ultra - wave assisted lysis in streptozotocin induced diabetic rats. Int Braz J Urol. 2016;42:825-37.
12. Ari E, Kedrah AE, Alahdab Y, Bulut G, Eren Z, Baytekin O, Odabasi D. Antioxidant and renoprotective effects of paricalcitrol on experimental contrast-induced nephropathy model. The British Journal of Radiology. 2012;85:1038–1043.
13. Heaton JP, Varrin S, Morales A. The characterization of a bioassay of erectile function in a rat model. Journal Urology. 1991;145:1099–1102.
14. Wang HD, Pagano PJ, Du Y, et al. Superoxide anion from the adventitia of the rat thoracic aorta inactivates nitric oxide. Circulation Research. 1998;82:810–818.
15. Chromczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium-thiocyanate-phenolchloroform extraction. Analytical Biochemistry. 1987;162:156–159.
16. Kim YW, Choi DW, Park YH, et al. Leptin-like effects of MTII are augmented in MSG-obese rats. Regulatory Peptides. 2005;127:63–70.
17. Carson CC, Lue TF. Phosphodiesterase type 5 inhibitors for erectile dysfunction. BJU Int. 2005;96:257-80.
18. Creager MA, Lüscher TF, Cosentino F, Beckman JA. Diabetes and vascular disease: Pathophysiology, clinical consequences and medical therapy: Party I. Circulation. 2003;108(12):1527-32.
19. Vernet D, Cai L, Garban H, et al. Reduction of penile nitric oxide synthase in diabetic BB/WORdp (type I) and BZ/WORdp (type II) rats with erectile dysfunction. Endocrinology. 1995;136:5709–5717.
20. Cellek S, Foxwell NA, Moncada S. Two phases of nitrergic neuropathy in streptozotocin-induced diabetic rats. Diabetes. 2003;52:2353–2362.

21. Molinari C, Uberti F, Grossini E, Vacca G, Carda S, Invernizzi M, et al. 1alpha,25-dihydroxycholecalciferol induces nitric oxide production in cultured endothelial cells. Cell Physiol Biochem. 2011;27:661-8. [CrossRef]
22. Melamed ML, Muntner P, Michos ED, Uribarri J, Weber C, Sharma J, et al. Serum 25-hydroxyvitamin D levels and the prevalence of peripheral arterial disease: Results from NHANES 2001 to 2004. Arterioscler Thromb Vasc Biol. 2008;28:1179-85. [CrossRef]
23. Farag YM, Guallar E, Zhao D, Kalyani RR, Blaha MJ, Feldman DI, et al. Vitamin D deficiency is independently associated with greater prevalence of erectile dysfunction: The National Health and Nutrition Examination Survey (NHANES) 2001-2004. Atherosclerosis. 2016;252:61-7. [CrossRef]
24. Andrukhova O, Slavic S, Zeitz U, Riesen SC, Heppelmann MS, Ambrisko TD, et al. Vitamin D is a regulator of endothelial nitric oxide synthase and arterial stiffness in mice. Mol Endocrinol. 2014;28:53-64. [CrossRef]
25. Tare M, Emmett SJ, Coleman HA, Skordilis C, Eyles DW, Morley R, et al. Vitamin D insufficiency is associated with impaired vascular endothelial and smooth muscle function and hypertension in young rats. J Physiol. 2011;589:4777-86. [CrossRef]
26. Francis RM, Peacock M, Aaron JE, Selby PL, Taylor GA, Thompson J, et al. Osteoporosis in hypogonadal men: Role of decreased plasma 1,25-dihydroxyvitamin D, calcium malabsorption, and low bone formation. Bone. 1986;7:261-8. [CrossRef]
27. Pilz S, Frisch S, Koertke H, Kuhn J, Dreier J, Obermayer-Pietsch B, et al. Effect of vitamin D supplementation on testosterone levels in men. Horm Metab Res. 2011;43:223-5. [CrossRef]
28. Heijboer AC, Oosterwerff M, Schroten NF, Eekhoff EM, Chel VG, de Boer RA, et al. Vitamin D supplementation and testosterone concentrations in male human subjects. Clin Endocrinol (Oxf). 2015;83:105-10. [CrossRef]
29. Ding C, Wang Q, Hao Y, Ma X, Wu L, du M, et al. Vitamin D supplement improved testicular function in diabetic rats. Biochem Biophys Res Commun. 2016;473:161-7. [CrossRef]
30. Andress D. Nonclassical aspects of differential vitamin D receptor activation: Implications for survival in patients with chronic kidney disease. Drugs. 2007;67:1999–2012.
31. Sánchez A, Contreras C, Martínez MP, et al. Role of Neural NO Synthase (nNOS) uncoupling in the dysfunctional nitrergic vasorelaxation of penile arteries from insulin-resistant obese zucker rats. PLoS One. 2012;7.
32. Azadzoi KM, Golabek T, Radisavljevic ZM, et al. Oxidative stress and neuro degeneration in penile ischaemia. Britich Journal of Urology International. 2010;105:404–410.
33. Zitman-Gal T, Golan E, Green J, Bernheim J, Benchetrit S. Vitamin D receptor activation in a diabetic-like environment: Potential role in the activity of the endothelial pro-inflammatory and thioredoxin pathways. J Steroid Biochem Mol Biol. 2012;132(1–2):1–7.
34. Baeke F, Takiishi T, Korf H, Gysemans C, Mathieu C. Vitamin D: Modulator of the immune system. Curr Opin Pharmacol. 2010;10(4):482–496.
35. Tanaka M, Tokunaga K, Komaba H, Itoh K, Matsushita K, Watanabe H, Kadowaki D, Maruyama T, Otagiri M, Fukagawa M. Vitamin D receptor activator reduces oxidative stress in hemodialysis patients with secondary hyperparathyroidism. Ther Apher Dial. 2011;15(2):161–168.
36. Judd SE, Tangpricha V. Vitamin D deficiency and risk for cardiovascular disease. Am J Med Sci. 2009;338(1):40–44.
37. Mathew S, Lund RJ, Chaudhary LR, Geurs T, Hruska KA. Vitamin D receptor activators can protect against vascular calcification. J Am Soc Nephrol. 2008;19(8):1509–1519.
38. Husain K, Suarez E, Isidro A, Ferder L. Effects of paricalcitrol and enalapril on atherosclerotic injury in mouse aortas. Am J Nephrol. 2010;32(4):296–304.
39. Zitman-Gal T, Golan E, Green J, Bernheim J, Benchetrit S. Vitamin D receptor activation in a diabetic-like environment: Potential role in the activity of the endothelial pro-inflammatory and thioredoxin pathways. J Steroid Biochem Mol Biol. 2012;132(1–2):1–7.

40. Touyz RM, Briones AM. Reactive oxygen species and vascular biology: Implications in human hypertension. Hypertension Research. 2011;34:5–1.
41. Hsueh WA, Anderson PW. Hypertension the endothelial cell, and the vascular complications of diabetes mellitus. Hypertension. 1992;20:253-63.
42. Romeo JH, Seftel AD, Madhun ZT, Aron DC. Sexual function in men with diabetes type 2: Association with glycemic control. J Urol. 2000;163:788-91.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/25304