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Effect of chronic Sildenafil treatment on the prostate of C57Bl/6 mice

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ABSTRACT

Sildenafil is a potent and selective inhibitor of phosphodiesterase-5 (PDE5) and is considered first-line therapy for erectile dysfunction. Nowadays, Sildenafil is used extensively throughout the world on patients with pulmonary hypertension. However, few studies have evaluated the possible side effects of chronic Sildenafil treatment on the male reproductive system, specifically in the prostate. In the present study, it was demonstrated *via* morphological and ultrastructural analysis that chronic treatment with Sildenafil induced an enhancement of the glandular activity of the prostate. In addition, mice treated with Sildenafil showed a significant increase in testosterone serum levels. However, no statistically significant differences were observed in nitric oxide serum levels, or in sGC, eNOS, PSA and TGF-β prostatic expression. In conclusion, the present study suggests that chronic use of Sildenafil does not cause evident prostatic damage, and therefore, can be used pharmacologically to treat a variety of disorders.

1. Introduction

Inhibitor PDE-5, such as Sildenafil (Viagra), Vardenafil (Levitra), and Tadalafil (Cialis), has been used as a pharmacological vasodilator tool for several non-urological (pulmonary hypertension, systemic hypertension, diabetes, cardioprotection and endothelial function) and urological (erectile dysfunction, lower urinary tract symptoms, benign prostatic hyperplasia, priapism, premature ejaculation, and Peyronie's disease) disorders (Bella et al., 2007).

Phosphodiesterases (PDEs) are enzymes that are widely distributed in the body, hydrolyzing cyclic nucleotides, cAMP and cGMP to their inactive 5′-monofosfatos forms. Experimental studies using immunohistochemical methods have detected PDE isoenzymes 4, 5 and 11 in the fibromuscular prostatic stroma, as well as in the glandular structures of the transition zone of the prostate, suggesting that PDE enzymes play an important role in dynamic activity, secretory function and prostatic tissue proliferation (Ückert et al., 2006a,b).

The PDE-5 enzyme is responsible for the hydrolysis of the GMPc, which restores GMP levels. Endothelial-derived nitric oxide (NO) activates soluble guanylyl cyclase (sGC) in vascular smooth muscle, leading to an increase in intracellular cGMP, which activates cytosolic cGMP-dependent protein kinase (PKG). The ability of Sildenafil to compete kinetically with PDE5 by the cGMP catalytic site makes it a select inhibitor of PDE5 (Francis and Corbin, 1999). The accumulation of cGMP induced by Sildenafil promotes relaxation of the smooth muscle cells, not only in the corpus cavernosum, but also in the bladder neck, urethra and prostate, by decreasing intracellular calcium concentration (Wang, 2010; Liu et al., 2007).

McVary et al. (2007) reported satisfactory results in a randomized, double blind, placebo-controlled study of 12-week, once-daily dosing of 50 and 100 mg of Sildenafil in 369 men with ED and LUTS. Other authors also demonstrated a positive effect on urinary obstruction and irritation symptoms (Ying et al., 2004; Mulhall et al., 2006; Roehrborn et al., 2008; Stief et al., 2008).

Studies support the idea that PDE-5 expression increases in several types of human carcinoma, such as colon adenocarcinoma, bladder squamous carcinoma and lung cancers, suggesting the

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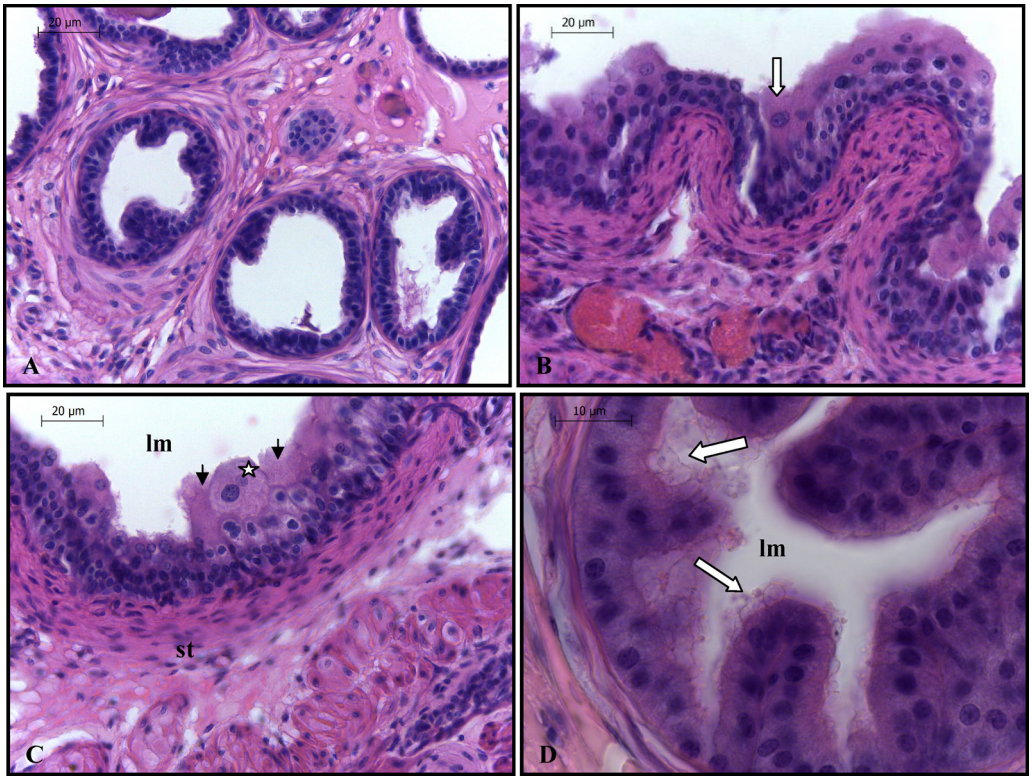

Fig. 1. Histological analysis of mice prostates following Sildenafil treatment. Stained with hematoxylin-eosin. (A) Prostate of the control group showed well-preserved acini and ducts composed of a single layer of secretory epithelial cells. (B) Group treated with 25 mg/kg of Sildenafil showed evident proliferation (arrows). (C) Preserved stromal region and round hypertrophied cells (star). The absence of nuclei in some cells (arrowheads) can be observed. (D) Secretion vesicles above the epithelial glandular cells (arrows). (ep, epithelium; lm, lumen; st, stroma. $n = 20$ mice from each group.

involvement of these enzymes in the control of cell proliferation and apoptotic mechanisms (Piazza et al., 2001; Moon et al., 2002; Whitehead et al., 2003; Sarfati et al., 2003). Additionally, in human prostate cancer cell lines, the increase of intracellular second messengers (cAMP and cGMP) initiates morphologic differentiation, inhibiting the growth and the invasive potential of these cells (Bang et al., 1994; Goto et al., 1999).

Studies have also demonstrated that Sildenafil attenuated pulmonary hypertension by increasing the supply of blood to the lungs reducing the right ventricular systolic pressure, right ventricular hypertrophy, the pulmonary artery muscularization, suggesting that the NO-cGMP pathway contributed to the drug response (Zhao et al., 2001, 2003).

Based on these evidences, in 2005, Sildenafil (Revatio, Pfizer) was approved for the chronic treatment of pulmonary hypertension. Recently, the Food and Drug Administration (FDA) and European Medical Agency (EMA) approved the daily use of the PDE5 inhibitor as a new opportunity for men with BPH/LUTS with coexisting ED.

Therefore, Sildenafil have a potential therapeutic indication for several chronic diseases; however the safety, efficacy and cost-effectiveness need to be ascertained. There is a paucity of data on the long-term effects of chronic PDE-5 inhibitor use on the prostatic function. Since Sildenafil has a vasodilatation action as a result of its effect on NO/sGC/GMPc/PKG pathways, the aim of the present study was to investigate the effect of chronic Sildenafil treatment on prostate model mice. The following end points were achieved: (1) prostate histopathology (histology and ultrastructure), (2) detection of sGC, PSA and TGF-β (immunohistochemical), (3) nitric oxide (NO) synthesis (nitrite concentration), (4) expression of sGC, eNOS and TGF-β (western blot), and (5) hormonal assays (testosterone dosage).

2. Materials and methods

2.1. Animals

Forty pubertal male C57BL/6 mice (obtained from the Centro de Pesquisas Aggeu Magalhães/FIOCRUZ, Recife, Brazil) aged 25 days and weighing 15–20 g were used in all experiments. Mice were examined for health status and acclimated to the laboratory environment, which had a temperature of 23 °C and a 12 h light:12 h dark photoperiod. The animals were housed in metal cages and fed a standard diet and water *ad libitum*. The experimental group was composed of 11 animals, which received a dose of 25 mg/kg body weight of Sildenafil (Pfizer Inc., New York, NY, USA) for 4 weeks, administered through drinking water (Zhao et al., 2003). Body weight was recorded every day and the drug concentration in the water was adjusted to maintain the dose. The control group was also composed of 20 animals, which received only pure water, using the same procedure as described above. All experiments were performed according to ethical guidelines (L-0035/08 – CEUA/FIOCRUZ). After treatment with Sildenafil, the experimental and control animals were anaesthetized with ketamine (115 mg/kg, i.m.) and xylazine (10 mg/kg, i.m.) (Sespo Comércio e Indústria Ltda., Sao Paulo, Brazil), before blood collection by cardiac puncture without anticoagulant. The serum was separated and stored at −70 °C for testosterone hormone radioimmunoassay. The prostates were quickly dissected and fixed for morphological analysis.

2.2. Light microscopy

The prostates were fixed in Bouin's solution for 8 h. Next, they were dehydrated in an ethanol series and embedded in paraffin

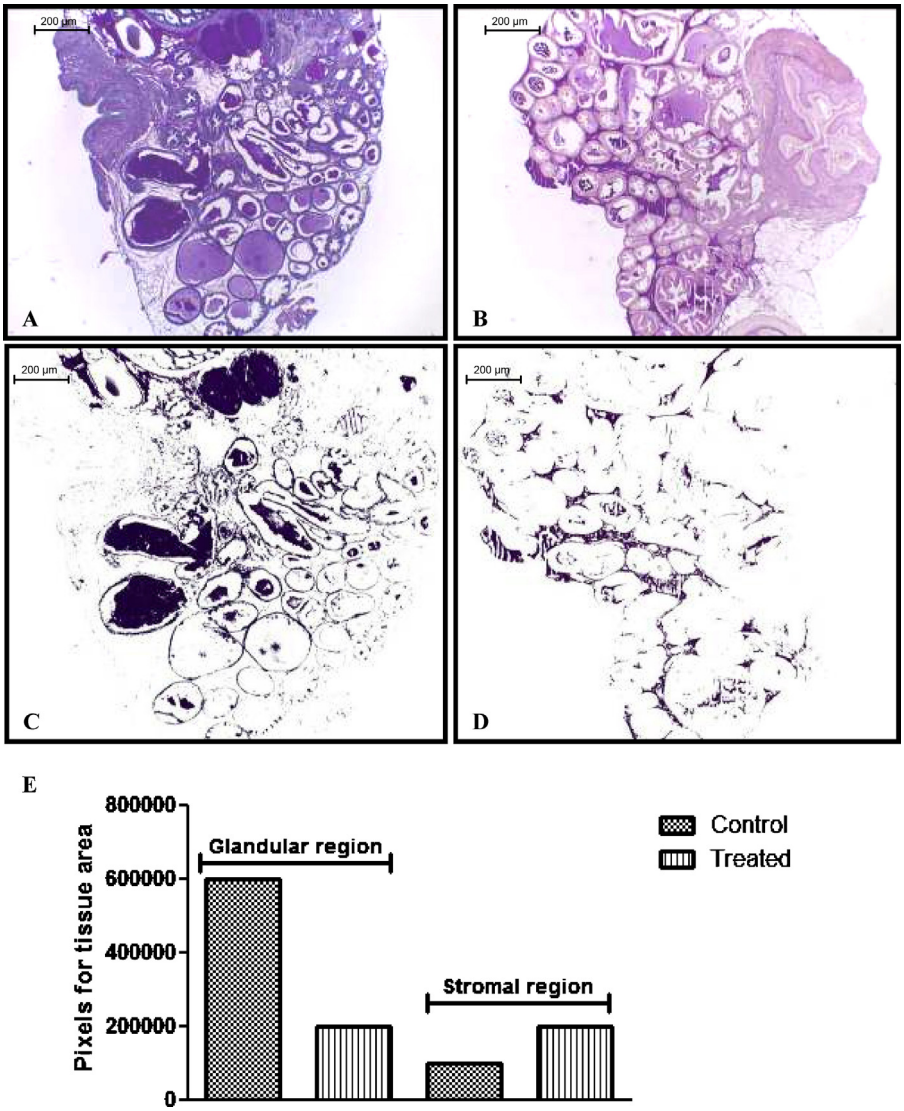

Fig. 2. Effect of Sildenafil treatment on prostatic carbohydrate content – PAS (Periodic acid-Schiff). (A, C) Control group; (B, D) Sildenafil group. $n = 20$ mice from each group; (E) quantification of tissue area in pixels.

wax. Serial sections of 5 µm were cut using a microtome (Leica RM 2125RT), stained with hematoxylin–eosin and PAS (periodic acid-Schiff), and evaluated with an inverted microscopy (Observer Z1, Zeiss Micro Imaging GmbH) equipped with a camera and 4.7.4 image analysis program (AxionCam MRm Zeiss) at a magnification of 400×.

2.3. Electron transmission microscopy

The fragments of prostate were fixed overnight in a solution containing 2.5% glutaraldehyde and 4% paraformaldehyde in 0.1 M cacodylate buffer. After fixation, the samples were washed twice in the same buffer and were then post-fixed in a solution containing 1% osmium tetroxide, 2 mM calcium chloride and 0.8% potassium ferricyanide in 0.1 M cacodylate buffer, pH 7.2, dehydrated in acetone, and embedded in Embed 812. Polymerization was performed at 60 °C for 3 days. Ultrathin sections were collected on 300-mesh nickel grids, counterstained with 5% uranyl acetate and lead citrate, and examined using a FEI Morgani 268D transmission electron microscope.

2.4. Immunohistochemical assays for sGC, PSA and TGF-β

Ultrathin sections (5 µm in thickness) of each group were cut and adhered to slides treated with 3-amino-propyl-trietoxi-silane (APES [Sigma, USA]). Briefly, sections were deparaffinized with xylene and rehydrated in graded ethanol (100–70%). The sections were heated for 30 min in a sodium citrate buffer (0.01 M, pH 6.0) to increase epitope exposure. To minimize endogenous peroxidase activity, the slides were treated with 0.3% (v/v) H_2O_2 in water for 5 min. The sections were washed with 0.01 M PBS (pH 7.2) and then blocked with 1% BSA, 0.2% Tween 20 in PBS for 1 h, at room temperature. The sections were then incubated for 12 h at 4 °C with rabbit polyclonal antibody against anti-guanylyl cyclase β1 soluble (sGC) (Sigma, USA), polyclonal antibody prostate-specific antigen (PSA) (ABCAM, CA, USA), and rabbit polyclonal transforming growth factor βs (TGF-β) (Santa Cruz Biotechnology, Santa Cruz, CA). The optimal concentration used for these antibodies was 1:100. The antigen–antibody reaction was visualized with avidin-biotin peroxidase (Dako Universal LSAB® + Kit, Peroxidase) using 3.3-diaminobenzidine as the chromogen. The slides were counterstained in hematoxylin. Positive staining resulted in a brown

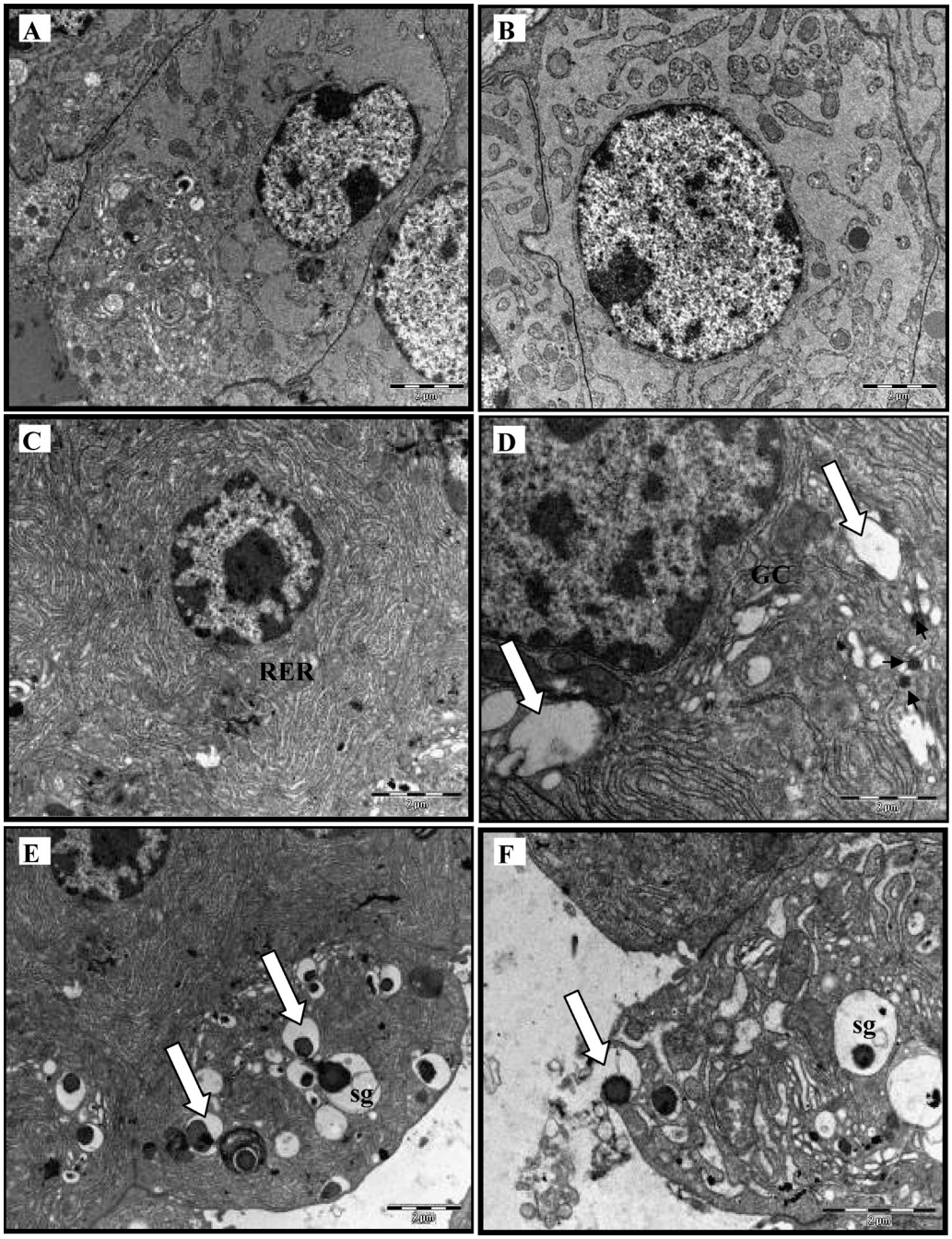

Fig. 3. Transmission electron microscopy. (A, B) Control group showed an epithelial cell morphology pattern with rough endoplasmic reticulum, apical Golgi complex and secretory vesicles. (C, D, E and F) Epithelial prostatic cells from the Sildenafil-treated group (25 mg/kg) had the following characteristics: (C) prominent rough endoplasmic reticulum (RER), (D) hypertrophied Golgi complex (GC) showing large lacunas (arrows), some of which contained electrodense material (arrowheads), (E) numerous secretory granules were observed in the apical region (sg) with electrodense material (arrows), and (F) exocitosis (arrow).

reaction product. Negative controls were treated as above, but with the omission of the first antibody. Five pictures taken at the same magnification were quantitatively analyzed using Gimp 2.6 software (GNU Image Manipulation Program, UNIX platforms).

2.5. Hormone assays

Serum testosterone was assayed using a solid-phase radioimmunoassay kit in accordance with the manufacturer's instructions (Coat-A-Count Total Testosterone; Diagnostic Products Corporation, Los Angeles, CA, USA). The sensitivity of the testosterone assay was 4 ng/dl and the intra- and inter-assay variation coefficients were 4–18% and 5.9–12%, respectively. The values were expressed in ng/ml. Data were analyzed using the Mann–Whitney test to compare testosterone levels of the controls and the organisms that underwent Sildenafil treatment (Zar, 1996).

2.6. Measurement of NO

Greiss colorimetric reaction, which detects nitrite (NO_2^-) and oxidation of NO in serum, was used to measure nitric oxide. Blood was obtained by cardiac puncture and centrifuged at 1000 × *g* for 10 min. Subsequently serum samples were diluted fourfold with distilled water, and deproteinized by adding 1/20th volume of

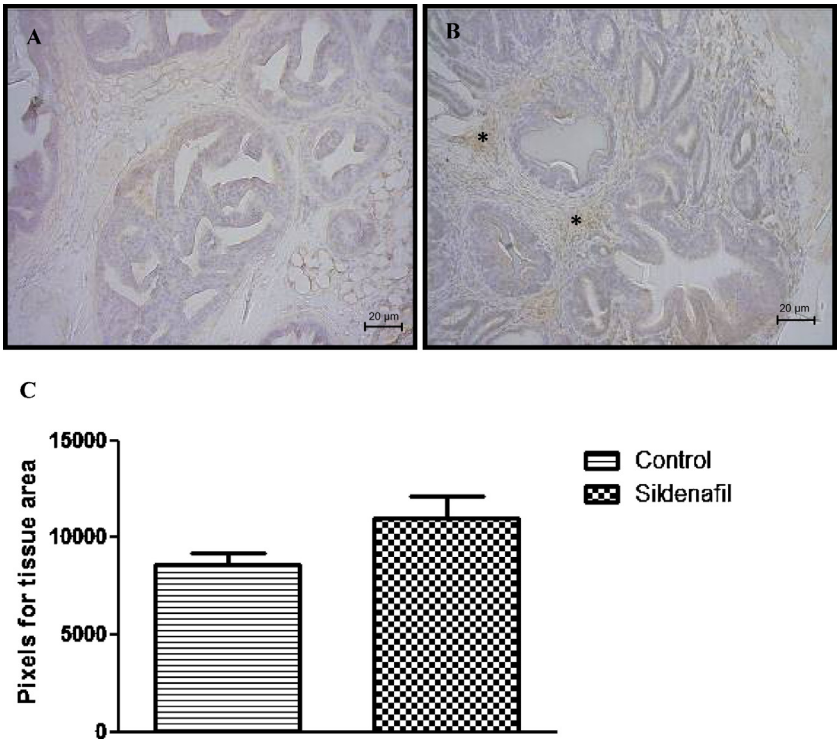

Fig. 4. Effects of Sildenafil on immunohistochemical localization of sGC: (A) control group with no positive staining for sGC, (B) treated group with positive staining for sGC when Sildenafil was administered for 30 days, and (C) pixel quantification of tissue area. $n = 10$ mice from each group.

a zinc sulfate solution (300 g/L), to give a final concentration of 15 g/L. After 3500 × *g* centrifugation for 10 min, 100 μL of samples were added to an ELISA plate (96 wells) in duplicate, followed by the same volume of Griess reagent. Griess reagent is composed of 1% sulfanilamide diluted in 2.5% H_3PO_4 (solution A) and N-1-naphtyl-ethtylenodiamina, also diluted in 2.5% H_3PO_4 (solution B). To prepare a standard curve, a solution of sodium nitrite in an initial concentration of 100 μM was serially diluted in PBS. After incubation for 10 min in the dark, a spectrophotometer reading was taken at 490 nm. The absorbance of different samples was compared with the standard curve, and the results expressed as mean ± standard error of the duplicate, using GraphPad Prism software (v. 5.0).

2.7. Western blot for eNOS, sGC and TGFβ

The prostates were quickly dissected and then homogenized in a Wheaton Overhead Stirrer (n° 903475) in an extraction cocktail (10 mM ethylenediamine tetraacetic acid (EDTA), 2 mM phenylmethylsulfonyl fluoride (PMSF), 100 mM sodium fluoride, 10 mM sodium pyrophosphate, 10 mM sodium orthovanadate ($NaVO_4$), 10 mg of aprotinin and 100 mM tris(hydroxymethyl)aminomethane, pH 7.4). Homogenates were centrifuged at 3000 × *g* for 10 min and the supernatant was collected and stored at −70° C until used for immunoblotting. Protein levels were determined using the Bradford method taking bovine serum albumin as standard (Bradford, 1970). The proteins (40 mg) were separated in 10% (sGC, eNOS and TGF-β) sodium dodecyl sulfate–polyacrylamide by gel electrophoresis under reduced conditions and were electrophoretically transferred onto nitrocellulose membrane (Bio Rad, CA, USA, Ref. 162-0115). After blocking overnight at 4 °C with 5% non-fat milk in TBS-T (Tris-buffered saline 0.1% plus 0.05% Tween 20, pH 7.4), the membranes were incubated at room temperature for 3 h, with rabbit polyclonal antibody against eNOS (1:1000 dilution; BD Transduction Laboratories, USA), sGC (1:200 dilution, Abcam, CA, USA) and TGF-β (1:1000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA), and diluted in buffer solution TBS-T containing 3% non-fat milk. After washing (six times, 10 min each) in TBS-T, the membranes were further reacted with horseradish peroxidase-conjugated anti-rabbit secondary antibody (1:80,000 (Ref. A6154), diluted in TBS-T with 1% nonfat milk for 1 h 30 min at room temperature. An enhanced chemiluminescence reagent (Super Signal, Pierce, Ref. 34080) was used to make the labeled protein bands visible and the blots were developed on X-ray film (Fuji Medical, Kodak, Ref. Z358487-50EA). For quantification, the density of pixels of each band was determined using the Image J 1.38 program (available at http://rsbweb.nih.gov/ij/download.html; developed by Wayne Rasband, NIH, Bethesda, MD). For each protein investigated, the results were confirmed using three sets of experiments. Immunoblot for β-actin was used as a control for the protein blots. After protein blot visualization with enhanced chemiluminescence, the protein antibodies were stripped from the membranes, which were reprobed with monoclonal anti-β-actin antibody (1:2000 dilution, Sigma, USA), and protein densitometry was performed.

2.8. Statistical analysis

GraphPad Prism software, version 5 was used for statistical analysis. Data were expressed as mean ± standard deviation. The differences between the control and treated groups were analyzed used Mann–Whitney or *T*-test. Probability values less than 0.05 were considered significant.

3. Results

3.1. Morphological analysis

Histological analysis of the prostate glands of animals in the control group showed well-preserved acini and ducts composed

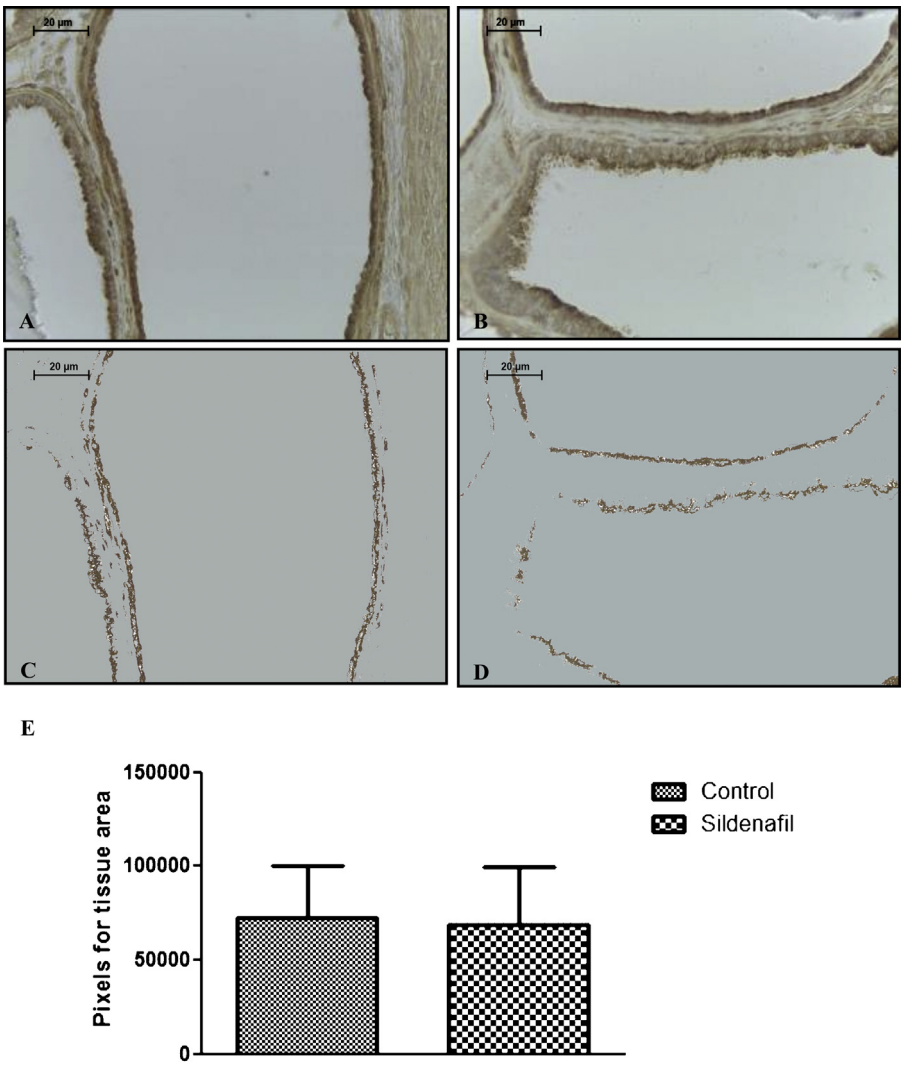

Fig. 5. Effects of Sildenafil on immunohistochemical localization of PSA in glandular region. Control group (A, C); Sildenafil-treated group for 30 days (B, D); and pixel quantification (E). $n = 10$ mice from each group.

of a single layer of secretory epithelial cells, characterized by columnar cells. Below the epithelium, there was a continuous layer of basal cells and a basal membrane. The stromal compartment, formed by a subepithelial region and a layer of smooth muscle cells surrounding the tubules, was also observed (Fig. 1A).

After 30 days of treatment with 25 mg/kg of Sildenafil, there was a clear difference between the Sildenafil treated and the control groups in the glandular and stromal regions. The secretory epithelium lining showed tall columnar cells in evident cellular proliferation (Fig. 1B), some of which were hypertrophied, with round profile and evident nuclei, while others had an absence of nuclei (Fig. 1C). Moreover, the glandular apical region showed evident secretion vesicles, indicating an increase in glandular activity (Fig. 1D).

3.2. Carbohydrates

The distribution of carbohydrates was analyzed using the Periodic acid-Schiff technique. In the control group, the presence of carbohydrates was identified in the glandular region (Fig. 2A). Contrastingly, there was increased labeling in the stromal region in the group treated with Sildenafil (Fig. 2B).

3.3. Ultrastructural analysis

Ultrastructural analysis of the prostate gland showed a columnar epithelium morphological pattern with evident elliptical nuclei, with rough endoplasmic reticulum (RER), apical Golgi complex and secretory vesicles (Fig. 3A and B). There were a small number of secretion vesicles with electron-lucent content with spherical eletrodense condensations.

Contrastingly, ultrastructural analysis of prostatic cells from the group treated with Sildenafil showed several characteristics of cellular activation, such as hypertrophied rough endoplasmic reticulum (Fig. 3C), dilated cistern of Golgi complex occupying the apical cellular region (Fig. 3D), containing electrodense secretion and numerous secretory vesicles (Fig. 3E). Exocytocis of the granular content was also observed (Fig. 3F).

3.4. Immunohistochemical analysis for sGC, PAS and TGF-β

In the present study sGC expression in the prostate tissue was evaluated by immunohistochemical detection. Tissue sections obtained from mice from the control group demonstrated no positive staining for sGC in the stromal and glandular region (Fig. 4A). Slight staining for sGC was found in the stromal region

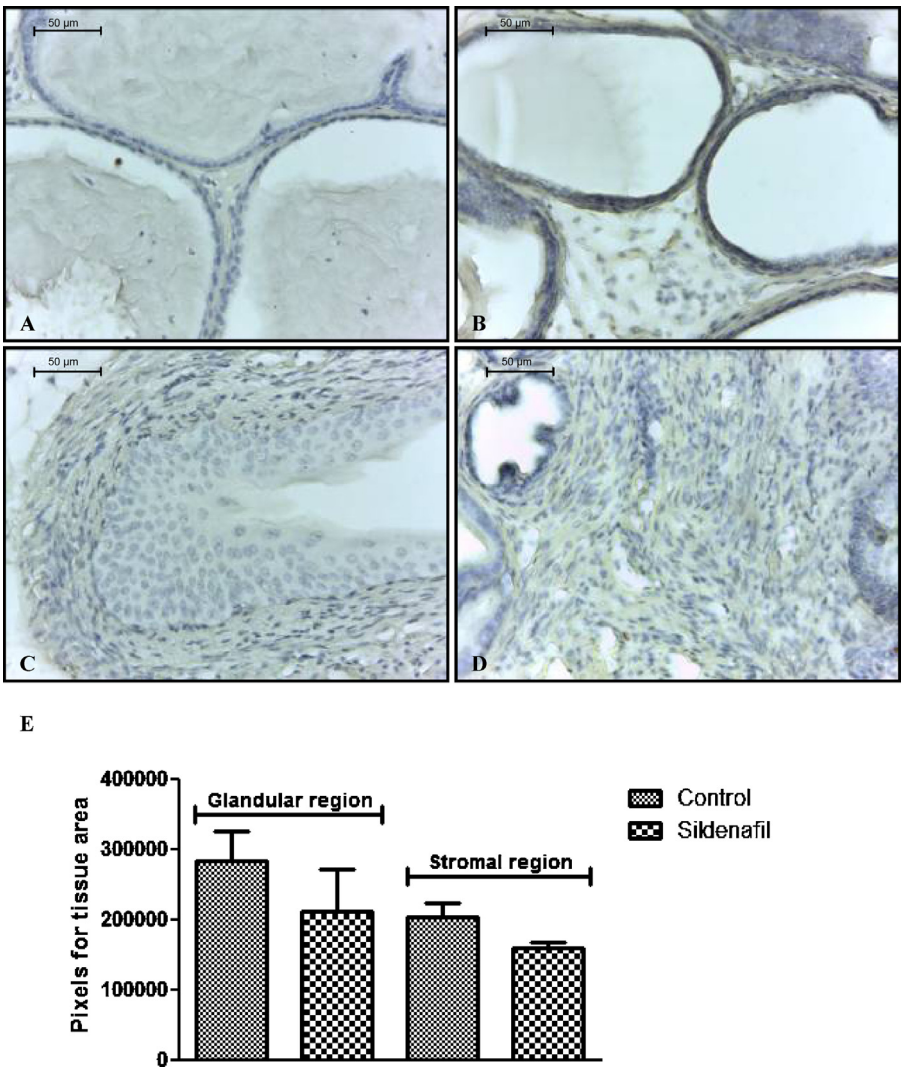

Fig. 6. Effects of Sildenafil on immunohistochemical localization of TGF-β. Control group, glandular (A) and stromal region (C). Sildenafil treated group, glandular (B) and stromal region (D). Pixel quantification (E). $n = 10$ mice from each group.

of the prostate of mice treated with Sildenafil 25 mg/kg. However, pixel quantification did not indicate statistically significant difference (Fig. 4B and C).

Similarly, prostate tissue sections from the control and Sildenafil treated groups showed no significant differences for PSA (Fig. 5A–E) and TGF-β (Fig. 6A–D) in the stromal and glandular regions.

3.5. Hormone assay

Serum testosterone levels were significantly higher in 25 mg/kg Sildenafil administered mice when compared with animals from the control group (Mann–Whitney, $P = 0.0057$). The parameters of the two groups are shown in Table 1.

3.6. Measurement of NO

NO levels in serum were analyzed using the Greiss reaction test. NO level was slightly higher in the Sildenafil 25 mg/kg group than in the control group, however the difference was not significant (Fig. 7).

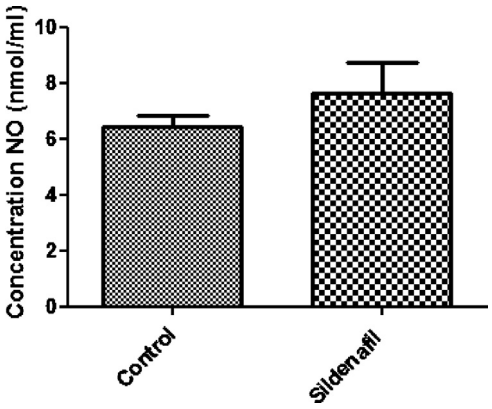

Fig. 7. Effect of Sildenafil on NO production in serum. Nitrite and nitrate levels and quantity of stable NO metabolites were higher in serum after treatment for 30 days, however the difference was not significant.

3.7. Expression of eNOS, sGC and TGFβ

The expression of eNOS, sGC and TGFβ in prostate was analyzed using the Western blot technique. Chronic Sildenafil treatment did

Table 1
Effect of Sildenafil treatment on mice serum testosterone levels (ng/ml).

	Serum testosterone levels (ng/ml)					
	N	Mean	Minimum	Maximum	SD	Mann–Whitney
Control	11	0.74	0.19	2.4	0.70	18
Sildenafil 25 mg/kg	11	5.74	0.41	11	4.71	

Significant difference between Sildenafil 25 mg/kg and control samples, $P = 0.0057$.

not result in a significant difference in sGC, eNOS and TGFβ expression (Fig. 8).

4. Discussion

Sildenafil has been used as a pharmacological strategy in the treatment of several urological and non-urogical disorders. However, there are few detailed studies of the possible effects of chronic treatment with Sildenafil on the male reproductive system.

Saraiva et al. (2009) undertook an *in vivo* investigation of the effects of chronic Sildenafil treatment (25 mg/kg) on male *Swiss Webster* mice. This study demonstrated that Leydig cells had alterations in the smooth endoplasmic reticulum, large vacuoles scattered through the cytoplasm, enlarged mitochondria and cells with intense secretory activity and hormonal production. Other

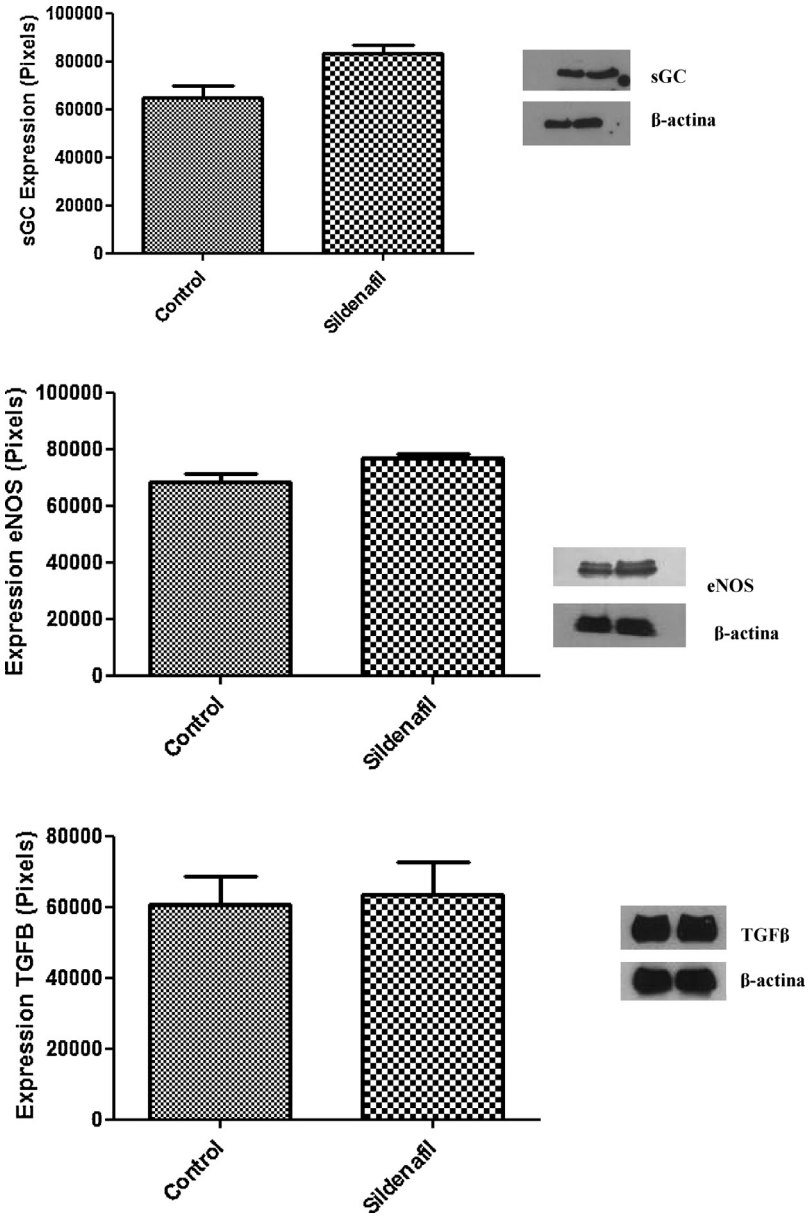

Fig. 8. Western blot analysis of sGC, eNOS and TGFβ expression. Content measured by pixel quantification of Western blot bands showed no significant difference between Sildenafil and control groups.

in vitro studies found evidence of the antiproliferative effect of PDE inhibitors in smooth muscle cells from human BPH tissue (Wong et al., 2009; Adolfsson et al., 2002; Cook and Haynes, 2004).

The prostatic gland is composed of epithelial and stromal cells. Interactions of these cells with androgens have a fundamental role in the growth, development and differentiation of the prostate (Chung and Davies, 1996; Thomson et al., 2002; Hayward et al., 1997; Cunha et al., 2004).

In the present study, the effects of chronic treatment with Sildenafil on the prostrate of C57Bl/6 mice were evaluated. Histological analysis showed no pathological alteration of the glandular and stromal region. However, epithelial cells showed some morphological characteristics of exacerbated activity. This hypothesis was confirmed by ultrastructural analysis as hypertrophied RER, prominent Golgi complex and secretory vesicle formation were identified. Histological glycogen staining using the periodic acid-Schiff (PAS) technique also confirmed that chronic treatment with Sildenafil induced prostatic secretion enhancement.

Differentiated prostatic epithelial cells can directly influence fibroblasts, vascular endothelial and inflammatory cells, to generate a microenvironment favorable to the onset of carcinogenesis (Cano et al., 2007).

The secretory function of the prostate is dependent upon direct stimulation of the prostatic epithelial cells by androgens (Hayward and Cunha, 2000). The importance of the interaction between steroid hormones and prostatic function has been studied in several prostatic pathologies in recent years. Androgen deprivation leads to loss of secretory function and a reduction in glandular size. This regression is caused by widespread apoptosis in the prostate (Kerr et al., 1972). Oliver et al. (2010) showed that in lower concentrations of testosterone the cyclic adenosine monophosphate (cAMP) is more active in human cultured prostatic stromal cells (HCPSC). Many authors have showed the association between testosterone serum levels and the risk of prostate cancer, while others have demonstrated opposing results (Hoffman et al., 2000; Gill et al., 2010).

Prostate-specific antigen (PSA) is a glycoprotein produced by the prostatic epithelial cells that is considered the most useful marker of prostate cancer (Bok and Small, 2002; Vermassen et al., 2012). Its regulation has important clinical implications on cleavage semenogelins and fibronectin in coagulated semen, causing liquefaction, and aiding fertilization (Lilja et al., 1987). The present study showed that chronic treatment with Sildenafil can stimulate prostatic activity possible by elevating testosterone levels. According to data from literature, testosterone can directly influence PSA levels; however, the results of the present study showed that although high testosterone serum levels were detected after chronic Sildenafil treatment, no significant expression of PSA was observed.

Several studies indicate that PSA can accelerate carcinogenesis in the prostate. PSA can directly affect proteolysis components of the basement membrane, which can aide tumor-cell invasion and metastasis (Webber et al., 1995). In the case of advanced prostate cancer, a decrease in PSA level after systemic therapy has been shown to correlate with an improved outcome (Small et al., 2001).

PSA is thought to cleave insulin-like growth-factor-binding protein 3 (IGFBP3), thereby liberating insulin-like growth-factor 1 (IGF1), which is a mitogen to the prostatic stromal and epithelial (Cohen et al., 1992; Sutkowski et al., 1999; Djavan et al., 2001). PSA can also activate latent transforming growth factor (TGF)-β, which can stimulate cell detachment and facilitate the spread of tumor-cells (Killian et al., 1993). Based on these observations, the expression of TGF prostatic tissue was evaluated by western blot. No significant difference was detected after Sildenafil treatment, which is consistent with PSA results.

Metabolic syndrome (MetS) is a complex of clustering metabolic abnormalities and comprises a number of disorders such as insulin resistance, hypertension and obesity, which all act as risk factors for cardiovascular diseases. Recent studies have demonstrated that MetS, BPH/LUTS and prostatic cancer are often comorbid (Hammarsten and Peeker, 2011). Hyperinsulinemia, hyperglycemia and insulin-like growth factor-1 (IGF-1) contribute to the development and progression of BPH/LUTS. Hyperinsulinemia is also associated with increased sympathetic nervous system activity *via* enhanced glucose metabolism. This process promotes the increase in α-adrenergic receptors leading to increased smooth muscle tone of the male genitourinary tract (McVary, 2006; Ozden et al., 2007). The is a clear association between autonomic neural input and prostate growth rate (McVary et al., 1994). Besides, fasting plasma insulin, in particular, has been linked to BPH and lethal prostate cancer (Hammarsten and Peeker, 2011).

Another association between insulin resistance and BPH is related to IGF-1. Since these molecules have similar structure, insulin can bind to IGF-1 receptors and activate the signaling pathway for growth and proliferation of epithelial and stromal prostatic cells (Nunzio et al., 2012).

Chronic inflammation is one of the putative links between MetS and BPH/LUTS. Recently, Vignozzi et al. (2013) demonstrated that PDE5 blockade exerts anti-inflammatory effects on myofibroblast prostatic cells, blunting inflammatory and metabolic insults. These authors showed that treatment with tadalafil or vardenafil suppressed IL-8 and IP-10 secretion induced by inflammatory (TNF-α) and metabolic (oxLDL, AGE and IGF-1) stimuli, also suppressing TNF-α genes related to inflammation or tissue remodeling.

Other studies suggest that PDE5i could be a pharmacological strategy for the treatment of ED and LUTS/BPH by modifying NO/cGMP signaling pathway and improving the RhoA/Rho-kinase (ROCK), besides reducing the hyperactivity of the autonomic nervous system and chronic pelvic ischemia (Gacci et al., 2013).

Nitric oxide is a gas that is synthesized intracellularly by three NOS isoforms: neuronal (nNOS), inducible (iNOS), and endothelial (eNOS) (Andersson, 2007; Aaltomaa et al., 2000; Uotila et al., 2001; Cronauer et al., 2007; Nanni et al., 2009; Sanli et al., 2011; Yu et al., 2013). It has been demonstrated that the isoform eNOS plays a predominant role in tumor growth, metastasis and angiogenesis in human prostate cancer (PC), as well as in maintenance of the vascular tone and mediating vascular endothelial growth factor (VEGF)-induced endothelial cell activation (Ying and Hofseth, 2007; Polytarchou et al., 2009; Ziaei et al., 2013).

The fibromuscular stroma is densely supplied by NO synthase-containing nerve terminals (Burnett et al., 1995). According to the data from literature, the NO plays an important role in the control of prostate function in mammals and humans, including the regulation of prostate smooth muscle tone, glandular secretory function and local blood flow (Hedlund, 2005; Andersson, 2007; Kedia et al., 2008).

Activators of the NO/GMPc signaling cascade may interfere with regulation of smooth stromal muscle tone (Waldkirch et al., 2007). Secondary messengers, cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) are synthesized by activation of adenylyl- and guanylyl-cyclases, respectively, and degraded by cyclic nucleotide phosphodiesterases (PDE) (Hall, 1993). Nitric oxide (NO) activates soluble guanylyl cyclase (sGC), leading to an increase in intracellular cGMP, which activates cytosolic cGMP-dependent protein kinase (PKG). Soluble guanylyl cyclase (sGC) is considered the most important receptor for the signaling molecule NO (Carvajal et al., 2000; Ückert et al., 2006a,b).

To evaluate if chronic Sildenafil treatment could influence the NO/cGMP cascade in prostate, levels of serum NO, immunohistochemistry for sGC and western blot for sGC and eNOS were

evaluated. No statistical significant differences were observed in nitric oxide serum level or in sGC and eNOS prostatic expression.

5. Conclusion

In summary, chronic treatment with Sildenafil (25 mg/kg) induced an enhancement of prostatic glandular activity, possibly due to increased testosterone production. However, there was no increase in the expression of PSA and TGF-β. This data suggest that extensive use of Sildenafil in non-urological disorders such as pulmonary hypertension may not damage the prostate.

Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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