

Mechanism of endothelial cyto-protective and thrombo-resistance effects of sildenafil, vardenafil and tadalafil in male rabbit

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Abstract

Introduction: PDE5 inhibitors (PDE5_{inhs}) have proven to be of great impact in the treatment of numerous human extra-sexual diseases and their chronic use may induce endothelial rehabilitation. This study aimed to assess the effects of PDE5_{inhs} at chronic administration to explore the possible endothelial cyto-protective and thrombo-resistance effects.

Material and methods: One hundred New Zealand white male rabbits were divided into four groups. The first group (control, C) received 1 ml saline/kg, the second group (S) received 10 mg/kg sildenafil, the third group (V) received 2 mg/kg vardenafil, and the fourth group (T) received 2 mg/kg tadalafil in saline I.P. three times weekly for 4 weeks. Blood samples were collected and plasma was isolated for determination of 2,3-dinor-6-keto-prostaglandin F-1 α (PGF_{1 α}), 11-dehydro-TXB₂ (TXB₂), fibrinogen, calcium levels, prothrombin (PT), and thrombin times (TT).

Results: PDE5_{inhs} significantly increase PGF_{1 α} , calcium levels, PT and TT ($p < 0.001$) when compared with baseline data or with the saline group at the end of treatment. In contrast, PDE5_{inhs} significantly decrease TXB₂ and fibrinogen levels ($p < 0.001$) when compared either with their baseline data or with the saline group at the end of treatment. The tadalafil group showed a lower increase in PGF_{1 α} ($p < 0.001$), lower decrease in TXB₂ ($p < 0.001$), and higher increase in calcium levels ($p < 0.01$, $p < 0.05$), lower increase in PT and TT levels ($p < 0.001$) when compared with sildenafil or vardenafil.

Conclusions: The prolonged use of PDE5_{inhs} has time-dependent mild to moderate endothelial cyto-protective, thrombo-resistance anti-inflammatory and anti-nociception effects via activation of endothelial NOS (eNOS), increase of PGI₂ synthesis and decrease of fibrinogen with significant increase in PT and TT.

Key words: phosphodiesterase-5 inhibitors, cyto-protective, thrombo-resistance, prostacyclin, thromboxane A₂.

Introduction

Erectile dysfunction (ED) could be a manifestation of increased risk for cardiovascular diseases due to loss of endothelial cell (EC) integrity resulting in endothelial cell dysfunction (EDys) affecting vessel walls and circulation [1]. Intact ECs inactivate plasminogen activator inhibitor-1 (PAI-1) through binding thrombin resulting in protein-C activation; therefore ECs work as an anticoagulant membrane [2].

Prostacyclin (PGI₂), tissue plasminogen and nitric oxide (NO) are synthesized and released from intact ECs, which enables ECs to have a cru-

cial role in thrombosis control [3]. Nitric oxide is synthesized from the amino acid L-arginine by nitric oxide synthase (NOS) [4].

Endothelial cell dysfunction induce platelet activation releasing thromboxane A₂ (TXA₂) to promote platelet aggregation and coagulation triggering vasoconstriction which is counteracted by PGI₂, and this explains the altered TXA₂/PGI₂ ratio and thrombosis formation in cardiovascular and cerebrovascular diseases [3, 5–7].

At least 11 phosphodiesterase-5 enzyme (PDE5) families have been identified and characterized for their tissue, substrates and drug specificity. Phosphodiesterase-5 enzyme inhibitors (PDE5_{inhs}) such as sildenafil, vardenafil and tadalafil are widely used in male ED treatment. They increase the intracellular cGMP level by inhibition of PDE5 and enhancement of nitric oxide (NO), leading to corpus cavernosum smooth muscle relaxation and improved penile erection [8].

PDE5_{inhs} have proven effective in treatment of several human extra-sexual diseases and their chronic use might induce endothelial rehabilitation and amelioration of endothelial dysfunction, hemodynamics and exercise tolerance improvement. They have been investigated for the treatment of several extra-sexual diseases including pulmonary hypertension and asthma in lungs, ischemic acute stroke and systemic inflammation, and their clinical effects were approved for treating several cardiovascular and chronic obstructive pulmonary diseases [9–11]. Regardless of the ED degree, chronic therapy for four weeks with tadalafil in ED patients with cardiovascular risk improved their endothelial function and after therapy discontinuation this clinical effect lasted for at least 2 weeks [12]. PDE5_{inhs} are dose dependent with variable half-maximal inhibition (IC₅₀); sildenafil is mostly selective for PDE5 (at 3.5 nmol/l IC₅₀ concentration), retinal PDE6 (at 34 to 38 nmol/l IC₅₀ concentration), PDE1 (at 280 nmol/l IC₅₀ concentration) and PDE2,4,7-11 (at > 2600 nmol/l IC₅₀ concentration) [13].

In this study we aim to assess the effects of commonly and widely used PDE5_{inhs} (at chronic administration) on PGI₂, TXA₂, fibrinogen, calcium and coagulation parameters in order to explore the possible extra-sexual therapeutic effects – endothelial cytoprotection, thrombo-resistance, anti-inflammatory and anti-nociception – in male rabbits.

Material and methods

Chemicals, reagents, and equipment

All drugs were purchased from Sigma-Aldrich (St Louis, USA) unless otherwise indicated. Sildenafil (Viagra 100 mg; Pfizer), tadalafil (Cialis 20 mg; Eli Lilly, Indianapolis, Indiana), and vardenafil (Levitra 20 mg; Schering-Plough, Kenilworth, New Jersey)

solutions were prepared according to Behn and Potter [14]. Tablets were ground into a fine powder and mixed with saline then filtered twice through 40 µm filters and the filtered solution was chilled at 4°C. Working solutions used to prepare doses were brought to room temperature 2 h before injections.

Kits for measuring TXA₂ stable metabolite [11-dehydro thromboxane B₂ (Cayman, Cat. No. = 519510)] and PGI₂ stable metabolite [2,3-dinor-6-keto prostaglandin F_{1α} (Cayman, Cat. No. = 515121)] were used. Ultra Pure water free of organic contaminant traces and deionized was used to prepare all ELISA reagents and buffers (Ultra Pure) (Cayman Item No. = 400000). Calcium was measured by colorimetric kit (QCA company ref. 99-59-36).

Plate ELISA reader (Humareader Human Compay 2106/1682) capable of measuring absorbance at 405-420 nm, and adjustable pipettes. Centrifuge 6000 rpm (Hitachi, Germany), syringes, Vacutainer tubes, tourniquet, Eppendorf tubes, micropipettes, plastic tubes, and cylinders. Coagulometer (Biomatic Biosarstedt, Freiburg, Germany) and UV/visible spectrophotometer (Shimadzu).

Animals

To define the minimum total sample size and the average number for each group the *a priori* test with the following parameters was conducted: anticipated effect size (Cohen's *d*): 0.7; desired statistical power level: 0.8; probability level: 0.05. The *a priori* test calculation returned the following values: minimum total sample size (one-tailed hypothesis): 52; minimum sample size per group (one-tailed hypothesis): 26.

According to *a priori* test results, experiments were conducted on 100 male New Zealand white rabbits with body weight range 1.8-2.1 kg and at 6 or 7 months of age. The animals were maintained in aluminium cages in an animal room under controlled conditions of temperature, relative humidity, and ventilation. Animals were fed ad libitum with standard laboratory chow and allowed free access to water. This investigation conforms to the ethical Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised in 1996).

Experimental protocols

After a 1 week acclimation period, the animals were fasted for 24 h before starting the experiments. Animals were assigned to four groups, 25 animals in each group. The first group (Control, C) received 1 ml/kg saline (I.P.), the second group (S) received 10 mg/kg sildenafil in saline (I.P.), the third group (V) received 2 mg/kg vardenafil in saline (I.P.), and the fourth group (T) received 2 mg/kg tadalafil in saline (I.P.) three times weekly for 4 weeks [15, 16].

The animal protocol is in accordance with the Animal Ethical Care regulations in the Faculty of Pharmacy, Helwan University.

Biochemical assay

The blood samples were obtained from the auricular arteries while the animals were in a fasting state before experimentation (day 0) and at 2 and 4 weeks after PDE5_{inhs} injection. The blood samples from each animal were collected and the first 3-ml aliquot of blood was taken on sodium heparinised sampling vials for plasma separation to be refrigerated at -20°C until used for measuring of PGI₂ stable metabolite, 2,3-dinor-6-keto-prostaglandin F-1α (2,3-dinor-6-keto-PGF-1α) [17], 11-dehydrothromboxane B₂ (11-dehydro-TXB₂, a stable metabolite of TXA₂) [18] by enzyme immunoassay, and calcium was estimated by the O-cresolphthalein direct method using a colorimetric kit (QCA company ref. 99-59-36) [19]. The second 3-ml aliquots of blood samples were taken into citrated blood sampling vials and immediately sent to the lab to be assayed immediately after collection, for measuring fibrinogen levels (mg/dl) using modified Clauss assay [20], prothrombin time (PT), and thrombin time (TT) as described by Dacie and Lewis [21] using a coagulometer (an automated system in which the formation of the clot is detected electronically) (Biomatic Biosarstedt, Freiburg, Germany).

Statistical analysis

Data for the parameters are presented as the means ± S.E.M. of the values recorded in each group. The above parameters were analyzed using a one-way analysis of variance (ANOVA), with Tukey post hoc correction for multiple comparisons being performed; a probability level of *p* < 0.05 was regarded as significant (using InStat3 version software).

Results

PDE5_{inhs} (sildenafil, tadalafil, and vardenafil) were injected intra-peritoneally into rabbits to be compared with saline for their effects on PGI₂, TXA₂, fibrinogen, calcium levels, PT and TT to assess additional extra-sexual effects.

Data of the current study showed that PDE5_{inhs} significantly increase 2,3-dinor-6-keto prostaglandin F_{1α} (as PGI₂ stable metabolite) levels when compared either with their baseline data or with the saline group at the end of treatment. The tadalafil group showed a lower increase in 2,3-dinor-6-keto prostaglandin F_{1α} when compared either with sildenafil or vardenafil. In contrast, PDE5_{inhs} significantly decrease 11-dehydro-TXB₂ (as TXA₂ stable metabolite) levels when compared either with their baseline data or with the saline group at the end of treatment. The tadalafil group showed a lower decrease in 11-dehydro-TXB₂ when compared with either sildenafil or vardenafil (data shown in Table I, Figure 1).

Data of the present study showed that PDE5_{inhs} significantly increase plasma calcium levels, PT and TT when compared either with their baseline data or with the saline group at the end of treatment. The tadalafil group showed a slightly higher increase in calcium levels and lower increase in PT or TT when compared with either sildenafil or vardenafil. In contrast, PDE5_{inhs} significantly decrease fibrinogen levels when compared either with their baseline data or with the saline group at the end of treatment (data shown in Table II, Figure 2). The postulated mechanisms of PDE5_{inhs} are presented diagrammatically in Figure 3.

Discussion

PDE5_{inhs} have proven effective in treatment of several human extra-sexual diseases and endothe-

Table I. Effects of PDE5_{inhs} on 2,3-dinor-6-keto prostaglandin F_{1α} and 11-dehydro-TXB₂ plasma levels in rabbit

Variables	Time/ treatment	Saline (1 ml/kg I.P./triweekly/ 4 weeks) (n = 25)	Sildenafil (10 mg in 1 ml saline/kg I.P. triweekly for 4 weeks) (n = 25)	Vardenafil (2 mg in 1 ml saline/kg I.P. triweekly for 4 weeks) (n = 25)	Tadalafil (2 mg in 1 ml saline/kg I.P. triweekly for 4 weeks) (n = 25)
Plasma 2,3-dinor-6-keto prostaglandin F _{1α} [pg/ml]	0-W	3.98 ±0.12	4.22 ±0.14	4.35 ±0.11	4.36 ±0.12
	2-W	4.13 ±0.10	8.87 ±0.31	9.36 ±0.25	6.28 ±0.27
	4-W	4.12 ±0.14	11.85 ±0.30***,∇∇∇	12.80 ±0.31***,∇∇∇	6.53 ±0.28***,∇∇∇,◆◆◆,ΦΦΦ
Plasma 11-dehydro-TXB ₂ [pg/ml]	0-W	1.75 ±0.056	1.66 ±0.064	1.58 ±0.11	1.70 ±0.061
	2-W	1.73 ±0.055	1.27 ±0.045	1.20 ±0.07	1.47 ±0.067
	4-W	1.74 ±0.058	0.94 ±0.060***,∇∇∇	0.89 ±0.06***,∇∇∇	1.38 ±0.058*,∇∇,◆◆◆,ΦΦΦ

Each value represents mean ± S.E.M. * Comparing each group at 4W vs. baseline (0 day), ∇ Comparing all tested groups at 4W vs. control (saline) at 4W, ◆ Comparing vardenafil or tadalafil group at 4W vs. sildenafil group at 4W, Φ Comparing tadalafil group at 4W vs. vardenafil group at 4W, **p* < 0.05, ∇*p* < 0.01, ***∇∇∇,◆◆◆,ΦΦΦ *p* < 0.001. PDE5_{inhs} – phosphodiesterase-5-inhibitors, W – weeks, I.P. – intra-peritoneally.

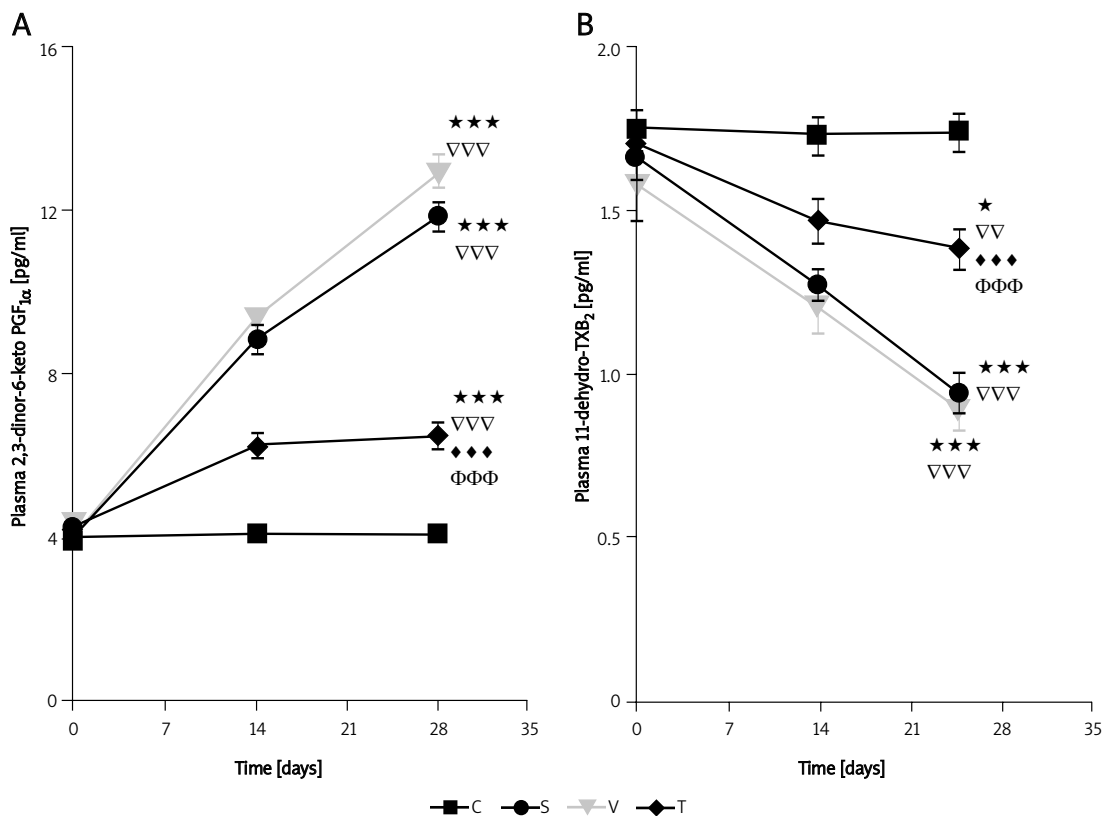


Figure 1. Effects of phosphodiesterase-5-inhibitors on 2,3-dinor-6-keto prostaglandin $F_{1\alpha}$ (A) and 11-dehydro-TXB₂ (B) plasma levels in rabbit
 Each value represents mean \pm S.E.M. * Comparing each group at 4W vs. baseline (0 day), ∇ Comparing all tested groups at 4W vs. control (saline) at 4W, \blacklozenge Comparing vardenafil or tadalafil group at 4W vs. sildenafil group at 4W, Φ Comparing tadalafil group at 4W vs. vardenafil group at 4W, * $p < 0.05$, $\nabla\nabla\nabla\nabla\nabla\nabla$ $p < 0.01$, $\blacklozenge\blacklozenge\blacklozenge\blacklozenge\blacklozenge\blacklozenge$ $p < 0.001$. PDE5_{inhs} – phosphodiesterase-5-inhibitors, W – weeks.

Table II. Effects of PDE5_{inhs} on coagulation parameters and calcium in rabbit

Variables	Time/treatment	Saline (1 ml/kg I.P./tri-weekly/ 4 weeks) (n = 25)	Sildenafil (10 mg in 1 ml saline/kg I.P. triweekly for 4 weeks) (n = 25)	Vardenafil (2 mg in 1 ml saline/kg I.P. triweekly for 4 weeks) (n = 25)	Tadalafil (2 mg in 1 ml saline/kg I.P. triweekly for 4 weeks) (n = 25)
Calcium [mg/dl]	0-W	14.13 \pm 0.23	14.20 \pm 0.29	14.05 \pm 0.22	14.20 \pm 0.28
	2-W	14.14 \pm 0.19	15.10 \pm 0.28	15.02 \pm 0.27	15.28 \pm 0.25
	4-W	14.23 \pm 0.16	16.02 \pm 0.32***, $\nabla\nabla\nabla$	16.17 \pm 0.25***, $\nabla\nabla\nabla$	17.42 \pm 0.21***, $\nabla\nabla\nabla$, \blacklozenge , Φ
Fibrinogen [mg/dl]	0-W	252.44 \pm 3.31	253.68 \pm 3.50	253.64 \pm 3.05	255.36 \pm 3.23
	2-W	253.80 \pm 3.20	189.80 \pm 4.83	194.12 \pm 2.80	217.56 \pm 4.07
	4-W	254.92 \pm 3.06	179.32 \pm 4.13***, $\nabla\nabla\nabla$	182.20 \pm 3.60***, $\nabla\nabla\nabla$	195.04 \pm 8.22***, $\nabla\nabla\nabla$
Prothrombin time (PT) [s]	0-W	9.32 \pm 0.25	9.28 \pm 0.18	9.40 \pm 0.29	9.48 \pm 0.36
	2-W	9.24 \pm 0.19	18.80 \pm 0.52	19.16 \pm 0.69	13.92 \pm 0.43
	4-W	9.48 \pm 0.19	29.52 \pm 0.80***, $\nabla\nabla\nabla$	26.32 \pm 1.03***, $\nabla\nabla\nabla$, \blacklozenge	17.40 \pm 0.64***, $\nabla\nabla\nabla$, \blacklozenge , Φ , Φ , Φ
Thrombin time (TT) [s]	0-W	18.20 \pm 0.33	18.32 \pm 0.35	18.60 \pm 0.53	18.52 \pm 0.54
	2-W	18.32 \pm 0.33	28.60 \pm 0.71	27.36 \pm 0.74	23.32 \pm 0.67
	4-W	18.36 \pm 0.35	36.12 \pm 0.78***, $\nabla\nabla\nabla$	34.20 \pm 0.87***, $\nabla\nabla\nabla$	27.28 \pm 0.72***, $\nabla\nabla\nabla$, \blacklozenge , \blacklozenge , Φ , Φ , Φ

Abbreviations – see Table I; $\Phi p < 0.05$, $\blacklozenge p < 0.01$.

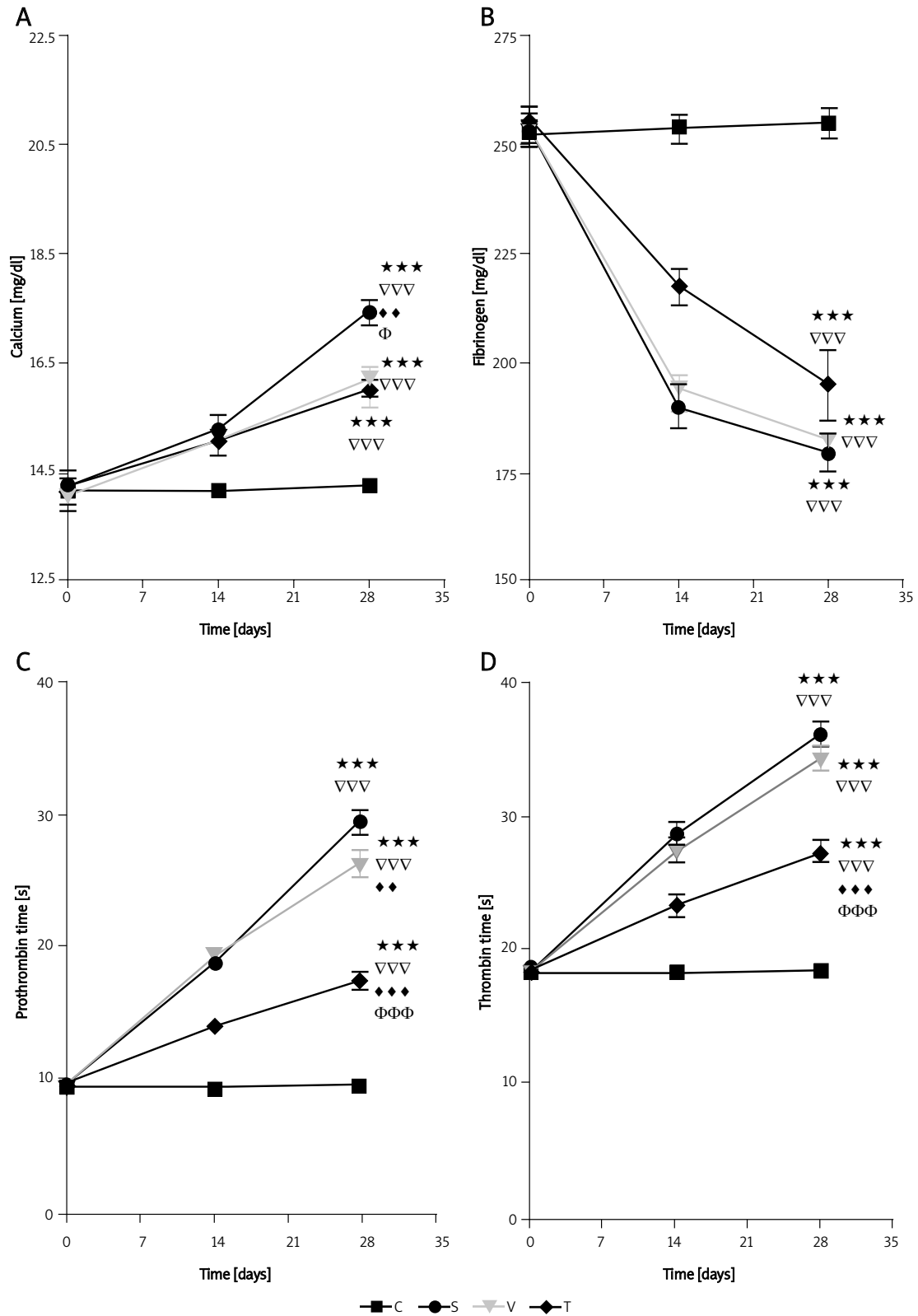


Figure 2. Effects of phosphodiesterase-5-inhibitors on calcium (A), fibrinogen (B), prothrombin (C) and thrombin (D) times in rabbit
 Abbreviations – see Table I; Φ $p < 0.05$, ♦ $p < 0.01$.

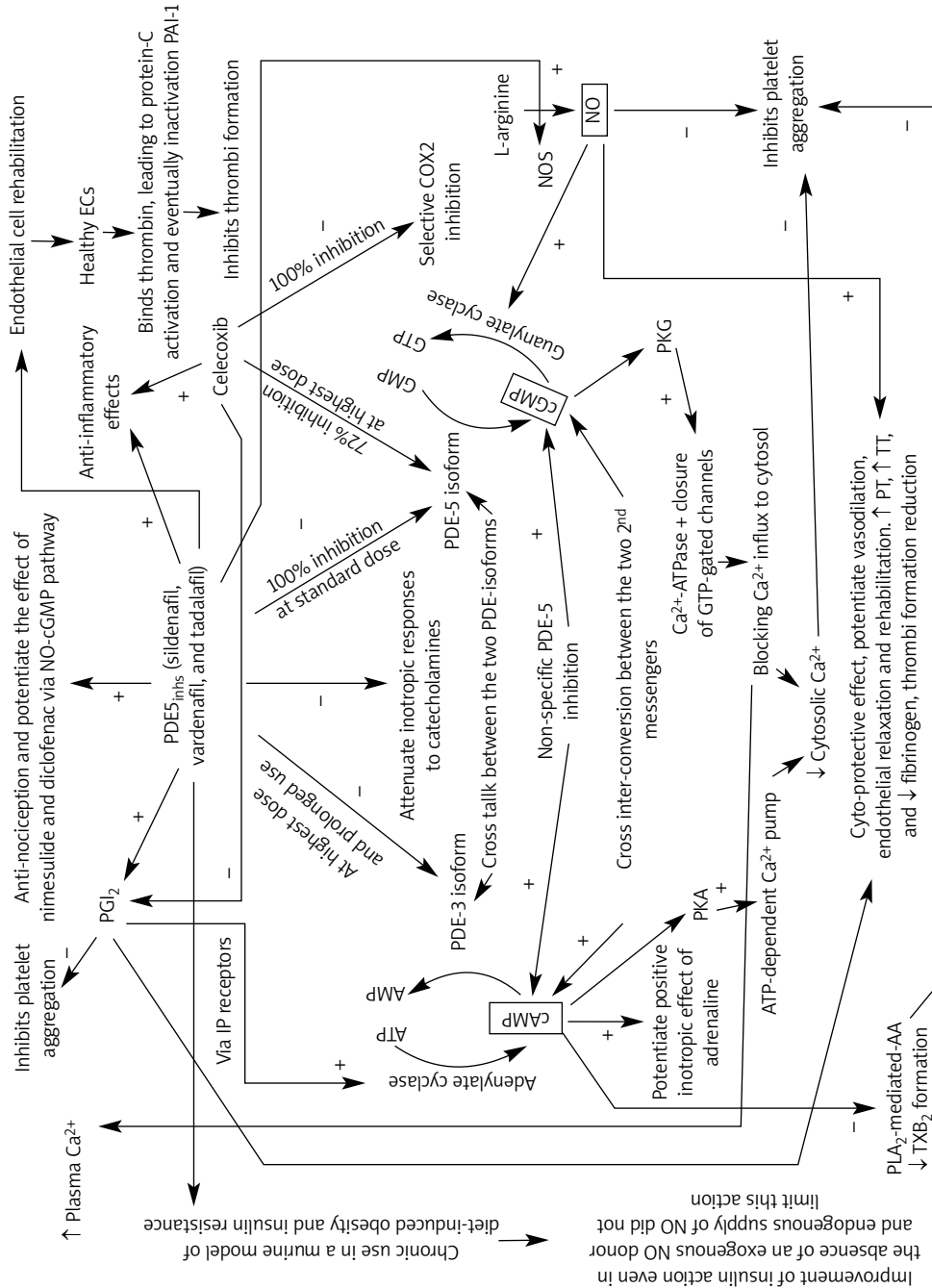


Figure 3. The description diagram of postulated cyto-protective, thrombo-resistance, anti-inflammatory, anti-nociception mechanisms of PDE5-inhibitors AA – arachidonic acid, ATPase – adenosine tri-phosphatase, Ca²⁺ – calcium, cAMP – cyclic adenosine mono-phosphate, COX-1 & -2 – cyclo-oxygenase-1 & -2, ECs – endothelial cells, GTP – guanosine tri-phosphate, NO – nitric oxide, NOS – nitric oxide synthase, PAI – plasminogen activator inhibitor-1, PGI₂ – prostaglandin I₂, PKA – protein kinase A, PKG – protein kinase G, PLA₂ – phospho-lipase-A₂, PT – pro-thrombin time, TT – thrombin time, TXA₂ – thromboxane A₂, + activation, - inhibition.

lial systemic effects [9, 13]. Imbalance of eicosanoids, namely TXA₂ and PGI₂ biosynthesis, leads to myocardial ischemia (CVD) due to disrupted homeostasis [22].

The studied PDE5_{inhs} significantly increased PGF_{1α} levels when compared either with their baseline data or with the saline group at the end of treatment. In contrast, PDE5_{inhs} significantly decreased TXB₂ levels when compared either with their baseline data or with the saline group at the end of treatment. The tadalafil group showed a lower increase in PGF_{1α} and lower decrease in TXB₂ when compared with either sildenafil or vardenafil.

Sildenafil induces an insignificant blood pressure decrease through vasodilatation [23] and cardioprotection when infused in rabbits with ischemic injury [24]. Sildenafil and tadalafil have therapeutic benefits in patients with pulmonary arterial hypertension (PAH) [11] and congestive heart failure [25].

High levels of circulating systemic inflammatory markers are indicative for ED and its severity associated with endothelium-dependent vasoreactivity [26]. Sildenafil induces anti-nociception, and mimics the effect of the cyclooxygenase inhibitors through NO-cGMP pathway activation and cyclic GMP degradation inhibition [27].

Celecoxib, a selective COX-2 inhibitor, can induce PDE5 blockade in rat aorta, affecting the NO/cGMP signaling pathway and in guinea-pig heart induces coronary vasodilatation and increases coronary flow (CF) up to 72% by the administered dose (200 nmol) in comparison with 100% CF increment by sildenafil [28]. COX-2 inhibitors bind with their polar sulfonamide side chain to a hydrophilic side COX-2 pocket and the effect of COX-2 inhibitors is time dependent [29]. Sildenafil and vardenafil have a sulfonyl side chain similar to that of celecoxib and that could suggest the anti-inflammatory effect of them via COX-2 inhibition through fitting the COX-2 pocket while tadalafil has a more bulky side chain without a sulfonyl group and this might explain the time-dependent lowering effect of tadalafil on TXB₂. TXA₂ and its stable metabolite TXB₂ are essential regulators to amplify platelet activation, secretion and aggregation [6].

The significant decrease of TXB₂ in this study could be explained as PDE5A inhibition augmenting cGMP leading to competitive inhibition of PDE3 elevating cAMP. Phospholipase-A-mediated arachidonic acid (AA) and TXB₂ formation will be inhibited by an elevation of cAMP. Consequently, cardioprotection of sildenafil is due to NOS enhancement, cGMP elevation and therefore cGMP-dependent protein kinase (PKG) activation through K⁺/ATP channel stimulation [30].

The present study showed that PDE5_{inhs} significantly increase plasma calcium levels when com-

pared with either their baseline data or the saline group at the end of treatment. The tadalafil group showed a slightly higher increase in calcium levels when compared with either sildenafil or vardenafil groups.

These results reflect a decrease in cytosolic calcium concentrations through closure of cGMP-gated channels and blocking of Ca²⁺ influx. The selective inhibition of cGMP-specific PDE5 by studied PDE5_{inhs} results in elevation of cGMP concentrations activating PKG, which interacts with several proteins, including inositol triphosphate receptor, Ca²⁺-ATPase, closure of cGMP-gated channels and blocking of Ca²⁺ influx, decreasing cytosolic Ca²⁺ concentrations and leading to penile erection and inhibition of platelet aggregation [31, 32]. The PGI₂ decreases cytosolic levels of calcium due to adenylyl cyclase activation and synthesis of cAMP that lower levels of cytosolic calcium to inhibit platelet activation [33].

Healthy intact ECs works as an anticoagulant membrane with fibrinolytic, anti-aggregative and anticoagulant effects through inhibition of fibrin formation by heparin-like molecules enhancing anti-thrombin III expression, tissue-type plasminogen activator (tPA) and inactivation of the extrinsic pathway by tissue factor pathway inhibitor [2]. The cAMP is a major mediator in controlling platelet activation; elevated cAMP leads to inhibition of platelet activation and tissue adhesion [34]. The PGI₂ together with endothelium-derived relaxing factor (EDRF) shows synergistic action in inducing thrombo-resistance [35].

The current study showed that PDE5_{inhs} significantly increase PT and TT when compared either with their baseline data or with the saline group at the end of treatment. The tadalafil group showed a lower increase in PT or TT when compared with either sildenafil or vardenafil. In contrast, PDE5_{inhs} significantly decrease fibrinogen levels when compared either with their baseline data or with the saline group at the end of treatment.

These results are in disagreement with Michelakis *et al.* [36] and Robinson *et al.* [37], who found that sildenafil did not improve the fibrinolytic function and PT in coronary heart disease patients, even in patients using aspirin or warfarin. In addition, tadalafil had no significant hemodynamic effects on aspirin (bleeding time) and warfarin pharmacodynamics (prothrombin time) were not affected by vardenafil [38]. But the results of the current study are in line with Sheikh *et al.* [39], who reported a case of hemorrhoidal bleeding associated with sildenafil, Jackson [40], who showed that tadalafil has modest nitrate-like hemodynamic effects, and Berkels *et al.* [41], who found that 1 h after 100 mg sildenafil administration, bleeding time was significantly prolonged and after 4 h was resolved toward control values.

These paradoxical findings could be explained as being due to patients with chronic cardiovascular disease having damaged or partial intact endothelial cells with low capacity for expression of anti-thrombin III, heparin-like molecules, tissue factor pathway inhibitor, tissue-type plasminogen activator (tPA) and EDRF, and therefore the time-dependent mild to moderate cyto-protective effect of PDE5_{inhs} cannot affect hemodynamics or bleeding time in chronic CVD patients.

PDE5_{inhs} might have a certain degree of cross-talk between PDE5 and PDE3 isoforms through the inhibitory effect of cGMP on PDE3 that elevates the cAMP/PKA pathway [42], and this explains why the prolonged use of PDE5_{inhs} reduces thrombi formation as reported by Bischoff [8].

Chronic administration of sildenafil has no tachyphylaxis because it has no effect on NO bioavailability but it might activate the endothelial NOS by regulating the transduction pathway [43]. In CVD states, NO is also known to inhibit platelet activation/aggregation, vascular smooth muscle proliferation, leukocyte adherence, and low density lipoprotein oxidation [2]. Chronic use of PDE5_{inhs} may induce endothelial rehabilitation even in the absence of an exogenous NO donor [9]. Sildenafil induces a relaxation effect on carbachol- pre-contracted human bladder dome smooth muscle through K⁺/Ca²⁺ and K⁺/ATP channel activation and cGMP/cAMP signaling pathways [44].

Men with erectile dysfunction who did not smoke had higher levels of plasma fibrinogen compared to both smokers and non-smokers without erectile dysfunction [45]. The mild decrease of fibrinogen in this study could be explained by the anti-inflammatory effect of PDE5_{inhs} because fibrinogen is a protein and its synthesis in the liver increases during the acute phase of inflammation and injury.

As a result of PGI₂-induced vasodilatation, inhibition of platelet aggregation, and PDE5_{inhs} inhibition of thrombi formation, the blood pressured decreased and blood flow increased. This suggestion is supported by Kloner *et al.* [46] and Weinsaft *et al.* [47], who showed that tadalafil resulted in small changes in blood pressure, and in normal regions during increased workload, it increases myocardial blood flow significantly, while in diabetic ED patients with hypertension, sildenafil alone produces significant modest decreases in blood pressure of 8-10 mm Hg and not accompanied by any change in heart rate [48].

In the current study, the PDE5_{inhs} studied groups showed a slightly different pattern to each other. This difference could be due to a difference in their selectivity, efficacy, side effects and pharmacokinetics [8] and they are dose-dependent drugs, which inhibit different PDE isoforms via many sec-

ondary messengers resulting in new physiological action [13].

In conclusion, PDE5_{inhs} has time-dependent mild to moderate endothelial cyto-protective, thrombo-resistance, anti-inflammatory, and anti-nociception effects. The prolonged use of PDE5_{inhs} activates eNOS, increases PGI₂ synthesis and decreases both TXA2 and fibrinogen, with a significant increase in PT and TT.

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Conflict of interest

The authors declare no conflict of interest.

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