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Research Paper

Effect of Long - Acting Phosphodiesterase Type-5 Inhibitor - Tadalafil on Human Platelet Aggregation

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ABSTRACT

Introduction: *Erectile dysfunction is predominantly a vascular disease and may even be a marker for occult-cardiovascular disease.There are reports that PDE5 inhibitors inhibit platelet aggregation in animal models and only few studies presenting in vitro data of human platelet modulation by PDE type-5 inhibitor Sildenafil. The present study therefore, was planned to evaluate effect of long-acting phosphodiesterase type-5 inhibitor, tadalafil on human platelet aggregation.*

Methods: *The study was conducted on 30 healthy male volunteers between the age of 30 to 50 years. Tadalafil 10 mg and 20 mg was given to 15 patients in each group. Blood samples were collected after four and twenty-four hours of drug administration. All blood samples were subjected for the estimation of platelet aggregation on ELVI-840 aggregometer and Omni scribe chart recorded.*

Results: *Administration of Tadalafil has decreased platelet aggregation after 4 hrs and 24 hrs; which in both the cases was statistically significant. However, the decrease in platelet aggregation at the end of 24 hrs as compared to 4 hrs was not significant.*

Conclusion: *Tadalafil is effective inhibitor of platelet aggregation induced by ADP and collagen. Collagen induced aggregation is more significantly blocked by Tadalafil. The dose of 20 mg is more effective in inhibition of platelet aggregation at 24 hours as compared to 10 mg.*

KEYWORDS: Phosphodiesterases; cGMP; cAMP; NO; Erectile dysfunction

INTRODUCTION

Erectile dysfunction (ED) is a common condition and studies predict that it will become even more common in future. There is increasing evidence to suggest that it is predominantly a vascular disease and may even be a marker for occult-cardiovascular disease. The common pathological process is at the level of endothelium. The Massachusetts Male Aging Study (MMAS), a large population based random sample, confirmed that ED is highly correlated with vascular disease such as hypertension, heart disease and diabetes[1].

Several families of phosphodiesterase (PDE) enzyme have been identified and characterized[2]. Since selective pharmacological inhibitors of isoforms-5 (cGMP-specific PDE), such as sildenafil, vardenafil, tadalafil, have become available, the physiological function and interaction of different PDE isoforms, their tissue distribution and therapeutic potential of PDE 5 inhibition have attracted increasing interest[3]. The differential distribution of PDE isoforms in various tissues as well as selectivity of pharmacological agents is the basis for potential tissue specific effects of PDE inhibitors[4]. PDE5 is found in high concentration in smooth muscle cells of corpora cavernosa. Physiologically, nitric oxide (NO), which is released during sexual stimulation in the corpora cavernosum, increases concentration of cGMP by activating soluble guanylate cyclase, this in turn mediates vasorelaxation and subsequent filling of corpora cavernosa with blood. The cGMP is degraded by PDE5 and once PDE5 is inhibited by a phosphodiesterase inhibitor such as sildenafil, vardenafil or tadalafil, effects of cGMP are enhanced and erection is supported (Figure 1). PDE5 is also expressed in various other tissues, such as arterial vasculature, including pulmonary and coronary arteries, venous vasculature, skeletal muscles, visceral and tracheobronchial muscles and platelets.

Human platelets reported to contain three isomers of phosphodiesterase's (type 1,3,5)[5]. The activation of human platelets can be inhibited by two intracellular pathways, regulated by either cGMP or cAMP. However, nitric oxide causes the activation of cGMP dependent protein kinases, which prevents the agonist induced myosin light chain kinase and protein kinase C and inhibits the agonist induced calcium mobilization from intracellular stores without any major effect on ADP regulated cation channel [6]. Additionally, cGMP causes an increase of cAMP by inhibition of cAMP phosphodiesterase. Increased cGMP level inhibits agonist induced platelet aggregation [7].

There are reports that PDE5 inhibitors inhibit platelet aggregation in animal models and only few studies presenting *in vitro* data of human platelet modulation by PDE type-5 inhibitor Sildenafil[8-10]. The present study therefore, was planned to evaluate effect of long-acting phosphodiesterase type-5 inhibitor, tadalafil on human platelet aggregation.

MATERIAL AND METHODS

The present study was conducted on apparently 30 healthy male volunteers between the ages of 30 to 50 years. After obtaining institutional ethical approval and informed consent, the study subjects were randomly divided into two groups. Group I (n = 15) was administered Tadalafil 10 mg and Group II (n = 15) was administered Tadalafil 20 mg.

Exclusion criteria

- Diabetes, hypertension, hyperlipidaemia, ischemic heart disease
- Hepatic and liver dysfunction
- Smokers or consuming tobacco in any form.
- Use of drugs – NSAIDs, Antiplatelet, Nitrates, CYP inhibitors (erythromycin, ketoconazole, Cimetidine).

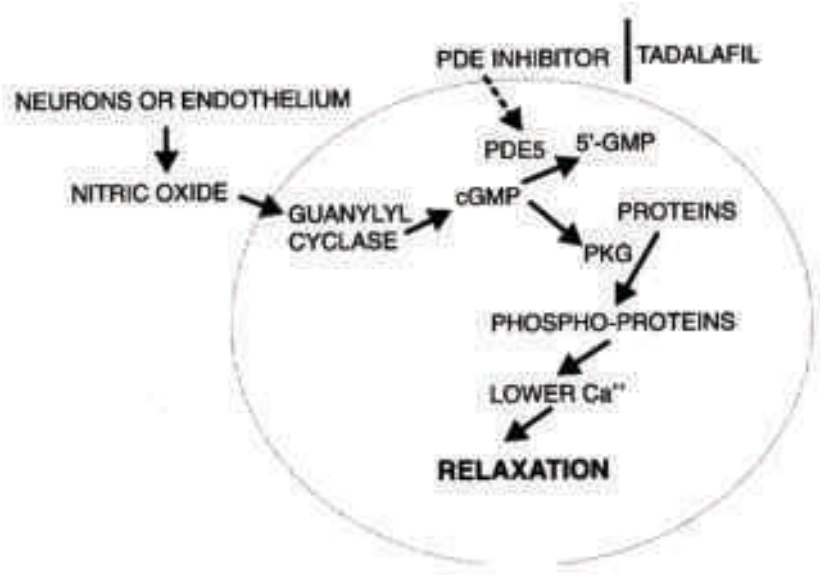

Figure 1: Regulation of penile corpus cavernosum smooth muscle relaxation and effect of PDE 5 inhibitor. (PDE, phosphodiesterases)

Study protocol

After an overnight fast, 4.5 ml of venous blood was collected

without undue pressure and a single dose of Tadalafil (10/20 mg) was administered. Subsequent blood samples were collected after four and twenty-four hours of drug administration. All blood samples were subjected for the estimation of platelet aggregation on ELVI-840 aggregometer and Omni scribe chart recorded.

Platelet aggregation

Most important function of platelets is their role in haemostasis i.e. adhesion to the damaged tissue surfaces and cohesion to one another. This cohesion phenomenon is known as aggregation and may be initiated by variety of substances including collagen, adenosine 5' diphosphate (ADP), epinephrine, serotonin and ristocetin. Aggregation is one of the numerous in vitro tests performed as a measure of platelet function. The described procedure is a turbidimetric method of measuring the effect of collagen, ADP and epinephrine on platelets.

Reagents

- 3.8 per cent citric acid (Trisodium salt dehydrate): Prepared by dissolving 3.8 gm citric acid in 100 ml of deionized water.
- Tris buffer: Tris (hydroxyl methyl), methylamine, 1.21 gm (0.01 M), disodium ethylene diamine tetra acetic acid 0.372 gm (0.001 M), sodium chloride 8.76 gm (0.15 M), dissolved in distilled water adjusted to pH 7.5 with hydrochloric acid and made up to one litre distilled water.
- ADP reagent: Adenosine 5'-diphosphate lyophilized with buffer salts (supplied by Sigma Diagnostics). ADP solution was prepared by reconstituting ADP reagent with 1.0 ml deionized water to yield solution containing ADP 2x10-4 mol/l. It was swirled to mix and allow to stand at room temperature (19-26°C) for 15 minutes before use. It was kept at room temperature only for duration of testing. The reconstituted reagent is stable for one month if stored in refrigerator (2 to 6°C).
- Collagen reagent: Collagen (calfskin) acid soluble, approximately 2 mg lyophilized with buffer salts. Collagen solution was prepared by reconstituting a vial of collagen reagent with 1.0 ml deionized water.

The vial was allowed to stand undisturbed for at least 15 minutes at room temperature before use. Warming to 37°C may be necessary to complete dissolution. It was swirled to mix prior to use and kept at room temperature only for the duration of testing. It is usually stable for at least 2 weeks, refrigerated (2-6°C) stability may be extended by freezing.

Specimen collection

Blood was collected by avoiding stasis and contamination with tissue fluids into plastic tubes containing 0.1 ml buffer and 3.8 per cent sodium citrate in a ratio of blood to anticoagulant in a ratio of 9:1.

Preparation of platelets rich plasma (PRP)

The anticoagulant sample was centrifuged at 400 rpm for 10 minutes. PRP was removed carefully using a plastic transfer pipette and was expelled into a plastic tube and covered it and kept at room temperature for duration of the test.

Preparation of platelets poor plasma (PPP)

It was prepared by again centrifuging the PRP at 6000 rpm for 10 minutes. Supernatant was transferred to a labelled PPP tube, covered and kept at room temperature for the duration of test.

The platelet count of PRP was adjusted to the range of 4 to 5 lac/mm3 by addition of autologous PPP to PRP sample. The caution should be taken to assay platelet aggregation within 30 minutes of collection of test samples.

Aggregating agents

ADP induced aggregation may occur in one or two phases and it may be followed by rapid disaggregation. Collagen induced platelet aggregation may occur in an irreversible single-phase curve or a reversible single-phase curve depending on the collagen concentration in the PRP.

Procedure

Cuvette with PRP was introduced into the aggregometer. The electromagnetic agitation was started by means of stirred control after having introduced a small stirring bar into the sample. Agitation speed was maintained at 1000 rpm. Baseline of the recorder was adjusted by means of the zero control. The cuvette with PRP was removed and cuvette with PPP was inserted. By means of gain control the maximum excursion of the pen on the recorded was adjusted. Cuvette with PPP was removed and cuvette with PRP was reinserted and it was readjusted, if necessary, by means of the zero control. The sliding of the recorded paper was started. The aggregating agents (ADP and collagen) were added to the PRP by means of micropipette. The aggregation was recorded for a minimum of five minutes and results were expressed as percentage aggregation.

Statistical Methods

Mean, percentage changes, standard deviation and standard errors of the mean were obtained. P values were calculated to determine the statistical significance of effect of Tadalafil administration to healthy humans (n=30) in doses (10 and 20 mg) on mean value of platelet aggregation induced by ADP and collagen. A $P < 0.05$ was considered significant.

RESULTS

The effect of Tadalafil on ADP induced platelet aggregation (%) in healthy volunteers has been shown in table 1. Administration of 10 mg of Tadalafil has decreased platelet aggregation by approx. 17 per cent and 13 per cent at 4 hrs and 24 hrs respectively which in both the cases was significant. However, the decrease in platelet aggregation at the end of 24 hrs as compared to 4 hrs was not significant (Figure 2).

Table 1: Effect of Tadalafil on ADP (2 × 10-4 mol/L) induced Platelet Aggregation (%) in Healthy Volunteers (n = 15)

Dose	Initial	After 4 hours	After 24 hours
10 mg	44.08 ± 3.13	36.58 ± 2.83 *P < 0.01	38.50 ± 2.77 *P < 0.02 **P = NS
20 mg	40.08 ± 3.66	36.08 ± 2.89 *P < 0.01	36.33 ± 3.56 *P < 0.05 **P = NS

All values are expressed as Mean ± Standard Error of Mean (SE)
P values:
*As compared to Initial
**As compared to 4 hr
NS = Not significant

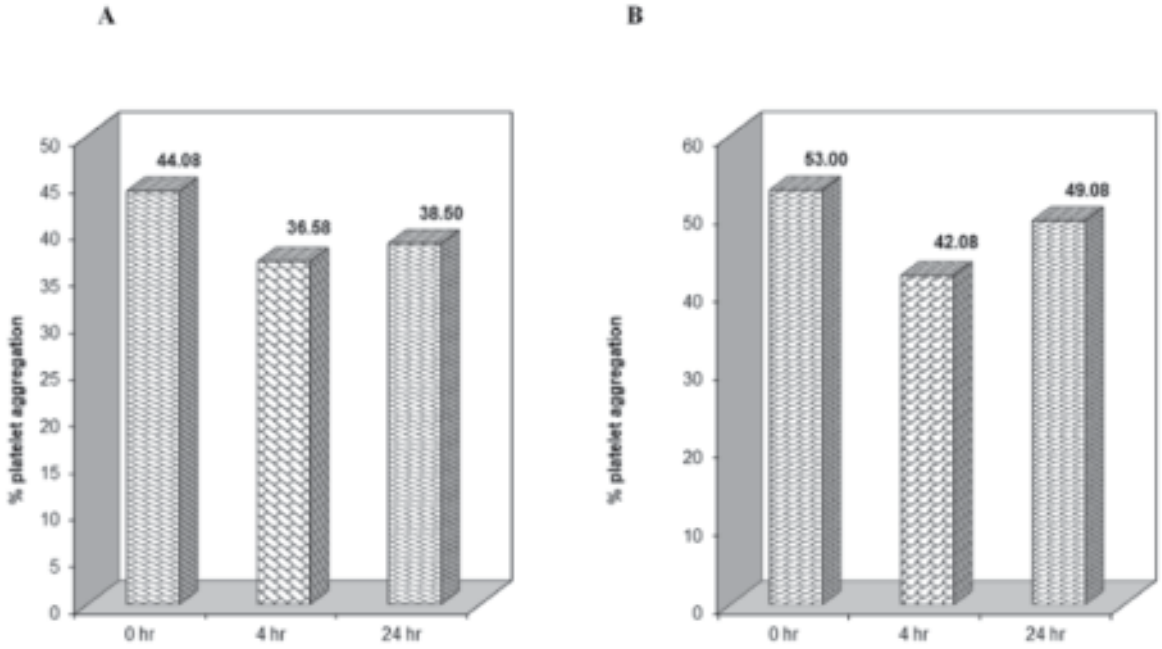

Figure 2: Effect of 10 mg Tadalafil on ADP (Panel A) and Collagen (Panel B) induced Platelet Aggregation in Healthy Volunteers

The effect of Tadalafil (20 mg) on ADP induced platelet aggregation in healthy volunteers is shown in table. Administration of 20 mg of Tadalafil has decreased platelet aggregation by about 10 per cent at the end of 4 hrs and about 9 per cent at the end of 24 hrs. This effect was seen in almost all individuals. The mean value of platelet aggregation has decreased from 40.08 ± 3.66 to 36.08 ± 2.89 at the end of 4 hrs and 36.33 ± 3.56 at the end of 24 hrs, which is statistically significant in both cases. However, difference in platelet aggregation at the end of 24 hrs compared to aggregation at the end of 4 hrs was not significant (Figure 3).

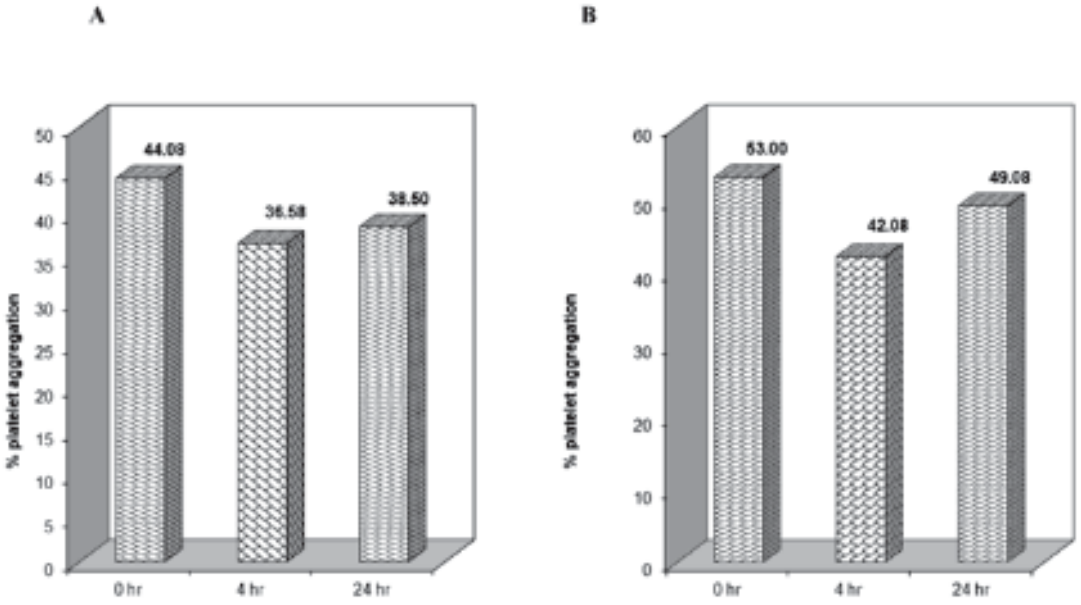

Figure 3: Effect of 20 mg Tadalafil on ADP (Panel A) and Collagen (Panel B) induced Platelet Aggregation in Healthy Volunteers

Table 2 shows the effect of Tadalafil on collagen induced platelet aggregation in healthy volunteers. Administration of 10 mg Tadalafil has decreased platelet aggregation by about 21% and 7% at the end of 4 hrs and 24 hrs. At the end of 4 hrs, the mean value of platelet aggregation has decreased from 53.00 ± 1.37 to 42.08 ± 2.17, which is statistically highly significant (P < 0.001). Similarly, at the end of 24 hrs, the mean value of platelet aggregation has decreased to 49.08 ± 1.14, which again is statistically highly significant (P < 0.001) (Figure 2). However, when platelet aggregation at the end of 24 hrs was compared to aggregation at the end of 4 hrs, the aggregation inhibition effect of tadalafil was no more seen and there was a significant (P < 0.001) rise in platelet aggregation.

Table 2: Effect of Tadalafil on Collagen (0.2 µg/ml) induced Platelet Aggregation (%) in Healthy Volunteers (n = 15)

Dose	Initial	After 4 hours	After 24 hours
10 mg	53.00 ± 1.37	42.08 ± 2.17 *P < 0.001	49.08 ± 1.14 *P < 0.001 **P < 0.001
20 mg	53.91 ± 1.83	46.66 ± 1.96 *P < 0.001	49.75 ± 2.06 *P < 0.002 **P = NS

All values are expressed as Mean ± Standard Error of Mean (SE)
P values:
*As compared to Initial
**As compared to 4 hr
NS = Not significant.

Administration of 20 mg Tadalafil has decreased platelet aggregation by 13 per cent and 8 per cent at the end of 4 hrs and 24 hrs respectively. The mean of platelet aggregation has decreased from 53.91 ± 1.83 to 46.66 ± 1.96 and 49.75 ± 2.06 at the end of 4 hrs and 24 hrs respectively (Figure 3). This effect was statistically significant at both the levels. When platelet aggregation at the end of 24 hrs was compared to 4 hrs, the difference was not significant.

DISCUSSION

It has long been accepted that elevation of cGMP has an inhibitory effect on platelet aggregation and PDE-5 inhibitor inhibits platelet aggregation and adhesion in animal models. There are only few reports of the effect of PDE 5 inhibitors on human platelet aggregation. Human platelets have been reported to contain 3 isomers of phosphodiesterase (Type I, II and V). The activation of human platelets can be inhibited by the intracellular pathways, regulated by either cGMP or cAMP. However, nitric oxide causes the activation of cGMP-dependent protein kinase. Additionally, cGMP causes an increase in cAMP by inhibition of cAMP phosphodiesterase. Increased cGMP levels inhibit agonist induced platelet aggregation. Tadalafil, is an orally administered phospho-diesterase-5 inhibitor, used for the treatment of erectile dysfunction. It acts by producing, elevation of cAMP in the presence of nitric oxide, and activation of guanyl cyclase that is released during sexual stimulation.

The present study was envisaged to observe the effect of longacting phosphodiesterase type-5 inhibitor, Tadalafil, on human platelet aggregation ex vivo. Administration of 10 mg Tadalafil significantly inhibited platelet aggregation induced by ADP at 4 hrs ($P < 0.01$) and 24 hrs ($P < 0.02$). The platelet aggregation inhibition was maximum at 4 hours and thereafter the inhibition was less. At 24 hrs, there was still significant ($P < 0.02$) inhibition of platelet aggregation as compared to baseline values, however, it was not significant as compared to 4 hours value. Collagen induced platelet aggregation inhibition, on the other hand, was more significant at 4 hours ($P < 0.001$) and 24 hours ($P < 0.001$) when compared to initial value. The effect did last for 24 hours, but when compared to 4 hours level, the aggregation was significantly ($P < 0.001$) more, suggesting thereby that the inhibition is rapidly reverting back. Increasing the dose of Tadalafil to 20 mg, the platelet aggregation induced by ADP was again inhibited significantly at 4 hours ($P < 0.01$) and 24 hours ($P < 0.05$), but importantly, both 4 hours and 24 hours values were essentially same, meaning thereby that the inhibition of platelet aggregation which was maximum at 4 hours was persistent to 24 hours. Likewise, the collagen induced platelet aggregation was very significantly decreased at 4 hours ($P < 0.001$) and 24 hours ($P < 0.002$) and the difference between 4- and 24-hours level was statistically not significant.

Wallis and colleagues3 have published their work showing *in vitro* data of human platelets that have been incubated with sildenafil or sodium nitroprusside or both. Berkels and associates[10] have demonstrated modulation of human platelet aggregation by sildenafil. The study revealed that sildenafil (50 mg and 100 mg) did not inhibit ADP induced platelet aggregation, whereas the collagen-induced aggregation was markedly reduced after 1 hr and significantly inhibited after 4 hrs of 100 mg of sildenafil administration. Verma and Jain observed the effect of PDE 5 inhibitor sildenafil on platelet aggregation ex-vivo in 30 healthy volunteers[11]. Sildenafil, in a single dose of 100 mg, significantly inhibited

collagen-induced platelet aggregation at 2 hrs ($P < 0.05$) and 4 hrs ($P < 0.001$). 50 mg sildenafil did inhibit platelet aggregation induced by collagen at 2 hrs and 4 hrs after its administration, but significantly only at 4 hrs ($P < 0.05$). ADP induced platelet aggregation, however, was not significantly inhibited by sildenafil in either dose. The effect of another PDE 5 inhibitor Zaprinast, which is 10 times less potent, has been reported[12]. Addition of Zaprinast to a nitric oxide solution resulted in potent increase of the inhibitory effect of nitric oxide. Li and associate studied the effect of Sildenafil on human platelet aggregation induced by restocetin or thombin[13]. They suggested that Sildenafil may have a biphasic effect on platelets, initially potentiating platelet aggregation and then inhibiting the response. They also observed that sildenafil sensitizes human platelets to the pro-aggregatory effects of ristocetin in a concentration dependent fashion (0.05- 1.1 μM). Similar observations were made with thrombin, wherein the pro-aggregatory effect was blocked by a protein kinase G (PKG) inhibition. The point to be seriously considered is that the effects of cGMP on platelet sensitization was biphasic; at relatively low concentration/short incubation time (<5 min), cGMP promoted platelet aggregation while at higher concentration and longer incubation times (5-10 min) the more customary inhibitory effects were observed. From a clinical perspective, the author speculate that sildenafil may potentiate platelet aggregation in patients with pro-thrombotic conditions and that this may explain the thrombotic complications in a small number of patients taking sildenafil. However, their clinical extrapolations are not supported by clinical data for Tadalafil, nor for the PDE 5 inhibitor class generally.

The present study conclusively demonstrates that Tadalafil in both dosage schedules inhibit platelet aggregation induced by ADP and collagen and the effect lasts up to 24 hours. However, increasing the dose from 10mg to 20 mg, the effectivity increases at 24 hours. The significant ($P < 0.001$) rise in aggregation induced by collagen observed at 24 hours with 10 mg Tadalafil group was checked when 20 mg was administered. Not only this, the ADP induced platelet aggregation was also significantly blocked by Tadalafil, the effect not observed by sildenafil. No adverse effects were observed in both dosage schedule in any of the volunteers.

The present observation is important in view of Tadalafil administration to subjects receiving other antiplatelet or anticoagulant medications. It might further enhance the antiaggregatory response and cause bleeding. However, no published data are available suggesting that bleeding time of patients treated with anticoagulants and antiplatelets is

increased by Tadalafil or any other PDE 5 inhibitors.

Erectile dysfunction is now been considered as vascular endothelial dysfunction, usually associated with other conditions such as hypertension and coronary artery disease. Administration of PDE-5 inhibitor Tadalafil will not only improve the erectile function for 48-72 hours but also check the thrombotic predisposition by favourably affecting platelet aggregation. This preposition however, needs further evaluation.

CONCLUSION

Tadalafil is an effective inhibitor of platelet aggregation induced by ADP and collagen. Collagen induced aggregation is more significantly blocked by Tadalafil. Twenty mg dose of Tadalafil is more effective in inhibition of platelet aggregation at 24 hours as compared to 10 mg dose. The possibility of interaction with other antiplatelet and anticoagulant medication needs further studies.

CONFLICT OF INTEREST: None

FINANCIAL SUPPORT: None

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