



## THE EFFECT OF PDE-5 INHIBITORS ON BLOOD HOMEOSTASIS IN RELATION TO THE TYPE & DURATION OF THERAPY

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### ABSTRACT

The probability of erectile dysfunction increases with ageing and the presence of Diabetes mellitus, hypertension, hypercholesterolemia, ischemic cardiac disease, depression, and obesity. Phosphodiesterase type-5 inhibitors (PDE5-i) are currently used in the treatment of male erectile dysfunction. PDE5 selective inhibitors : Sildenafil, tadalafil, vardenafil, and the newer types are selectively inhibit PDE5, which is cGMP-specific and responsible for the degradation of cGMP in the corpus cavernosum. But growing evidence supports important roles for the enzyme in both the vasculature and heart physiology, hence in related disorders such as cardiac failure. This study is aimed to investigate the biochemical changes associated with administration of PDE-5 inhibitors (Sildanafile or Tadalafile) on some haematological parameters: (Prothrombin time (PT), Activated Partial Thromboplastin Time (APTT), Platelete Function Assay (Collagen/Epinephrine and Collagen/Adenosine diphosphate), Fibrinogen weight and D-dimer titer, compared to the controls. This study included seventy male patients with erectile dysfunction. In addition to 70 control subjects, all the participants were with range of age (20-50 years) and apparently had no other diseases. Thirty- eight subjects with erectile dysfunction ED were treated with Sildenafil tablet of 100 mg, and thirty-two subjects with ED treated with Tadalafile tablet of 20 mg. Venous blood specimens were utilized to perform hematological analysis. Results revealed that Sildenafil produced significant alterations in prothrombine time, platelets function analysis (cephalin/Epinephrine & cephalin /ADP), fibrinogen weight analysis, D-Dimer values after 6 weeks of starting treatment. Whereas, tadalafil produced more pronounced alterations after 4 weeks of treatment on the same parameters. As conclusions; PDE-5 inhibitors (sildenafil & tadalafil) increase platelets activity and activate their aggregation. Sildenafil have less aggregatory effects on platelet than that produced by tadalafil when used for the same duration of time.

**KEYWORDS :** PDE-5 inhibitors, Sildenafil, Tadalafil, Platelet function, D-dimer, prothrombin time.

### INTRODUCTION

Erectile dysfunction (ED) is the persistent inability to achieve and maintain an erection adequate for satisfactory sexual performance<sup>[1]</sup>. Its prevalence is underestimated because the patients treated (less than 20% out of total) are considered as only the 'tip of the iceberg'<sup>[2,3]</sup>. The probability of erectile dysfunction increases with ageing<sup>[4]</sup> and the presence of diabetes mellitus<sup>[5]</sup>, hypertension<sup>[6]</sup>, hypercholesterolemia<sup>[7]</sup>, ischemic cardiac disease<sup>[8]</sup>, depression<sup>[9]</sup> and obesity<sup>[10]</sup>. Although cigarette smoking is not a direct causative factor, it may increase the risk of presenting with peripheral vascular disease and hypertension<sup>[11]</sup>. Drugs and alcohol abuse may also increase the risk of erectile dysfunction<sup>[12]</sup>. More than 70% of the male population affected by moderate to severe erectile dysfunction is complaining of concomitant diseases<sup>[13]</sup>. The modification of associated risk factors may contribute to improve erectile dysfunction in internal medicine patients<sup>[14]</sup>. Phosphodiesterase type-5 inhibitors (PDE5-i) are currently used in the treatment of male erectile dysfunction<sup>[15]</sup>. Sildenafil, vardenafil and tadalafil all inhibit PDE5 at the level of the corpus cavernosum with different onset of action<sup>[16]</sup>. A phosphodiesterase inhibitor is a drug that blocks one or more of the subtypes of the enzyme phosphodiesterase (PDE)<sup>[17]</sup>, thereby preventing the inactivation of the intracellular second messengers cyclic adenosine monophosphate (cAMP) and cyclic

guanosine monophosphate (cGMP) by the respective PDE subtype(s)<sup>[18]</sup>. PDE5 selective inhibitors: Sildenafil, tadalafil, vardenafil, and the newer types are selectively inhibit PDE5, which is cGMP-specific and responsible for the degradation of cGMP in the corpus cavernosum. These phosphodiesterase inhibitors are used primarily as remedies for erectile dysfunction, as well as having some other medical applications such as treatment of pulmonary hypertension<sup>[19,20]</sup>. But growing evidence supports important roles for the enzyme in both the vasculature and heart in disorders such as cardiac failure. PDE-5A up regulation may contribute to a decline in cGMP and protein kinase G signaling, exacerbating dysfunction. PDE5A plays an important role in the pulmonary vasculature where its inhibition benefits patients with pulmonary hypertension. In the heart, PDE5A signaling appears compartmentalized, and its inhibition is cardio protective against ischemia-reperfusion and antracycline toxicity, blunts acute adrenergic contractile stimulation, and can suppress chronic hypertrophy and dysfunction attributable to pressure-overload<sup>[21,22]</sup>. Phosphodiesterase type-5 inhibitor (PDE5-i) drugs were first marketed in 1998 (sildenafil) for 'on-demand' treatment of male erectile dysfunction (ED) of any origin<sup>[23]</sup>. They selectively inhibit intrapenile PDE5 isoenzyme which in turn increases intracellular cyclic guanosine monophosphate levels, thus resulting in prolonged relaxation of cavernosum smooth

muscle cells and facilitating the erection process<sup>[16]</sup>. Since 2003, two new molecules (tadalafil and vardenafil) have been introduced, resulting in greater interest in these compounds and leading patients to ask for more prescriptions from their doctors. The vast use of PDE5-i in diabetic and cardiovascular ED patients led researchers to investigate their possible extra sexual effects <sup>[24]</sup>. This study is aimed to investigate the biochemical changes associated with administration of PDE-5 inhibitors (Sildanafil or Tadalafil) on some haematological parameters: (Prothrombin time (PT), Activated Partial Thromboplastin Time (APTT), Platelete Function Assay (Collagen/ Epinephrine and Collagen/ Adenosine diphosphate), Fibrinogen weight and D-dimer titer, compared to the controls.

**MATERIALS & METHODS**

**Patients Selection**

This study included seventy out patients (all of them are males) from Baghdad city, where the samples were collected and brought to the laboratory in the Hematology Center of Al Mustansyria University for period from the first of February/ 2014 to the end of April/2014. In addition to 70 control subjects, all the participants were with range of age (20-50 years) and apparently had no other diseases. All participants were well informed about the study and gave their consent to participate prior to having blood samples taken .Those subjects were divided into three groups:

**1. Group C:** composed of seventy subjects as a control (mean age 35.257 ±7.692 yrs).

**2. Group S:** included thirty- eight subjects with erectile dysfunction ED (mean of age 39.710 ± 6.559 yrs) those intake Sildenafil tablet of 100 mg.

**3. Group T:** included thirty-two subjects with ED (mean of age 29.968±5.214 yrs) those intake Tadalafil tablet of 20 mg.

The subjects in the groups S&T started taking the drug two tablets weekly for four weeks and then one tablet daily for the next four weeks. Table -1 summarizes demographic data of subjects included in the study.

**Specimen Analysis**

Venous blood specimens were withdrawn from each subject initially 2 ml of the specimen was placed into EDTA tube for complete blood count performance by automated blood analyzer (Hemolyzer 5 /Analyticon Biotechnologies AG, Germany)<sup>[25]</sup>, for complete blood count, when the results were normal (the platelet count and Hct)<sup>[26]</sup>. Later on another specimen was obtained after an overnight fasting (4.5 ml) of blood that to be placed in plane tube contain 0.5 ml sodium citrate (3.8%) to determine Prothrombin time (PT), Kaolin-Activated Partial Thromboplastin Time (APTT) and Quantitative Determination of Fibrinogen (FIB) were performed by (Diagnostica Stago for measuring PT, APTT and FIB/ Junior Instrument, France)<sup>[27]</sup>, blood flow by platelet function analyzer (PFA-100/ Dade Behring, Düringen, Switzer-Land) <sup>[28]</sup> and Latex Agglutination Slide Test for the Qualitative and Semi-Quantitative Determination of D-dimer using specific Kit <sup>[29]</sup>.

**TABLE -1** Demographic Data of Subjects (Mean value ± SD)

Characters/Groups	Group C N=70	Group S N=38	Group T N=32	P
Gender				
Female	0 (0%)	0 (0%)	0 (0%)	
Male	70 (100%)	38 (100%)	32 (100%)	
Residence				
Village	0 (0%)	0 (0%)	0 (0%)	
City	70 (100%)	38 (100%)	32 (100%)	
Smoking habit				
Smoker	0 (0%)	0 (0%)	0 (0%)	
Non-smoker	70 (100%)	38 (100%)	32 (100%)	
Age (Mean ± SD)	35.257±7.692	39.710±6.559	29.968±5.214	P 0.05
Body Mass Index (Mean ± SD)	26.900±2.11	27.122±1.633	26.83±3.990	

**RESULTS**

**Prothrombine Time**

Table -2 shows that prothrombine time for all studied groups. The subjects treated with sildenafil show no significantly variation in PT values after 4 weeks and 6 weeks as compared to the controls. However, 8 weeks of treatment with Sildenafil (S4) expressed significantly

lower PT values (12.94%) as compared to the pretreatment values. Considering Tadalafil treatment , lowered PT value after 4 weeks of treatment ( by 5%), which was further lowered after 8 weeks of treatment as compared to baseline value T1 (18.27%) and to control values. Furthermore, Tadalafil treatment resulted in lower values of PT as compared to sildenafil treatment for the same periods .

**TABLE 2:** Prothrombine Time for all Groups at Different Periods (Mean value  $\pm$  SD)

Groups	C	S1	T1	S2	T2	S3	T3	S4	T4	P
Parameter										
PT(sec)	13.581 $\pm$ 0.23 A	12.98 $\pm$ 0.14 B	12.48 $\pm$ 0.12 B	12.94 $\pm$ 0.12 B	11.85 $\pm$ 0.20 C	12.00 $\pm$ 0.12 B	11.10 $\pm$ 0.23 C	11.30 $\pm$ 0.12 C	10.20 $\pm$ 0.20 D	0.05

Mean values with the same letter are not significantly different. Mean values with the different letter are significantly different. C : Control group, T1 : T group before Tadalafil therapy, T2 : T group after 4 weeks of therapy , T3: T group after 6 weeks of therapy, T4: T group after 8 weeks of therapy, S1: S group before Sildenafil treatment, S2: S group after 4 weeks of therapy, S3: S group after 6 weeks of therapy, S4 :S group after 8 weeks of therapy (single dose daily of 100 mg of seldinafile ), P: probability.

### Activated Partial Thromboplastin Time (APTT)

Table -3 shows that treatment with sildenafil produced no significant change in APTT independent of duration of therapy (4-8 weeks) as compared to control & pre-

treatment values. Whereas, Tadalafil treatment caused a significant reduction in APTT values after 6 weeks of starting treatment & caused (9.21%) reduction after 8 weeks.

**TABLE 3:** APTT for all groups at different periods of treatment (Mean value  $\pm$  SD)

Groups	C	S1	T1	S2	T2	S3	T3	S4	T4	P
Parameter										
APTT(sec)	31.17 $\pm$ 0.14 A	31.30 $\pm$ 0.15 A	31.16 $\pm$ 0.14 A	30.36 $\pm$ 0.76 A	30.01 $\pm$ 0.17 A	30.26 $\pm$ 0.19 A	29.53 $\pm$ 0.39 B	30.22 $\pm$ 0.19 A	28.29 $\pm$ 0.45 C	0.05

Mean values with the same letter are not significantly different. Mean values with the different letter are significantly different. C : Control group, T1 : T group before Tadalafil therapy, T2 : T group after 4 weeks of therapy , T3: T group after 6 weeks of therapy, T4: T group after 8 weeks of therapy, S1: S group before Sildenafil treatment, S2: S group after 4 weeks of therapy, S3: S group after 6 weeks of therapy, S4 :S group after 8 weeks of therapy (single dose daily of 100 mg of seldinafile ), P: probability.

### Collagen/Epinephrine Test

Table -4 shows the values of C/EPI values after 4 weeks of sildenafil treatment was no significantly difference from the baseline, while after 6 weeks of treatment there was significant decrease (20.46%) from the baseline. Furthermore after 8 weeks of treatment (S4) was lowered

significantly difference from baseline (by 20.54%). While, Tadalafil treatment after 4 weeks (T2) produced significant decrease from the baseline that decreased by (9.9%), while 8 weeks treatment (T4) was significantly different from T1, T2, T3 by (43.5% & 37.28% & 30.24%, respectively).

**TABLE 4:** C/EPI for all groups at different periods of treatment (Mean value  $\pm$  SD)

Groups	C	S1	T1	S2	T2	S3	T3	S4	T4	P
Parameter										
C/EPI (sec)	135.11 $\pm$ 4.42 A	131.289 $\pm$ 5.13 A	110.65 $\pm$ 6.7 A	125.23 $\pm$ 5.79 A	99.68 $\pm$ 4.74 B	104.31 $\pm$ 5.53 B	89.62 $\pm$ 6.78 B	104.31 $\pm$ 5.53 B	62.51 $\pm$ 6.67 C	0.05

Mean values with the same letter are not significantly different. Mean values with the different letter are significantly different. C: Control group, T1: T group before Tadalafil therapy, T2: T group after 4 weeks of therapy, T3: T group after 6 weeks of therapy, T4: T group after 8 weeks of therapy, S1: S group before Sildenafil treatment, S2: S group after 4 weeks of therapy, S3: S group after 6 weeks of therapy, S4: S group after 8 weeks of therapy (single dose daily of 100 mg of seldinafile), P: probability.

### Collagen/Adenosine Diphosphate Test

Table -5 shows the control and S & T groups in the baseline and after 4weeks of treatment all were not-significantly difference. Even after 6 weeks and 8 weeks of sildenafil treatment no- significant difference baseline

value (only 3.7% changes). Whereas, Tadalafil treatment after 6 weeks & 8 weeks produced significant difference from the baseline and decreased by 23.57% & 48.43%, respectively.

**TABLE 5:** C/ADP for all groups at different periods of treatment (Mean value  $\pm$  SD)

Groups	C	S1	T1	S2	T2	S3	T3	S4	T4	P
Parameter										
C/ADP (sec)	96.0 $\pm$ 0.0 A	96.0 $\pm$ 0.0 A	96.0 $\pm$ 0.0 A	96.0 $\pm$ 0.0 A	96.0 $\pm$ 0.0 A	92.36 $\pm$ 2.53 B	73.37 $\pm$ 4.94 D	92.36 $\pm$ 2.53 B	49.50 $\pm$ 6.02 C	0.0001

Mean values with the same letter are not significantly different. Mean values with the different letter are significantly different. C : Control group, T1 : T group before Tadalafil therapy, T2 : T group after 4 weeks of therapy, T3: T group after 6 weeks of therapy, T4: T group after 8 weeks of therapy, S1: S group before Sildenafil treatment, S2: S group after 4 weeks of therapy, S3: S group after 6 weeks of therapy, S4 :S group after 8 weeks of therapy (single dose daily of 100 mg of seldinafile ), P: probability.

**Fibrinogen Weight**

Treatment with sildenafil produced no significant change throughout the study period (8 weeks) as presented in figure-1. Whereas Tadalafil treatment caused significant

elevation in fibrinogen values after 4 weeks (by 20.7%), but decreased after (8 weeks) of Tadalafil therapy (by 42.6%).

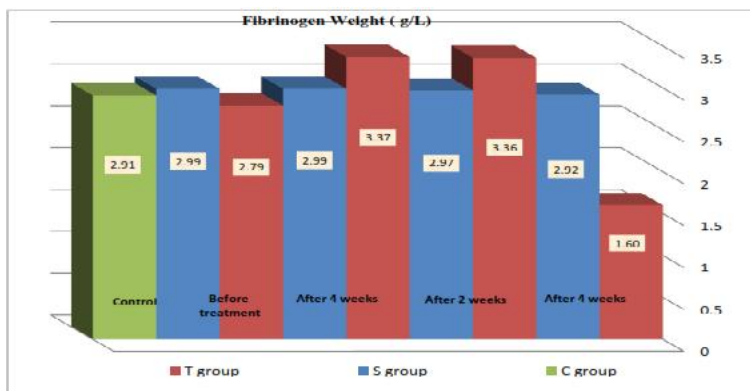


FIGURE 1: Mean value of fibrinogen weight (g/L) for all groups at different periods of treatment.

**Titer of D-Dimer**

Figure-2 showed that the Sildenafil treatment resulted in significant increase after 6 weeks & 8 weeks from the baseline (by 40% & 67.5% respectively), but they were not significantly difference from each other. After 4 weeks of Tadalafil treatment produced an increase in D-dimer

values by 80% and continues to increase after 6 weeks and 8 weeks by 200% & 210%, respectively. Furthermore, Sildenafil treatment resulted in lower values of titer as compared to Tadalafil treatment which produced higher values for the same periods as shown in figure -2.

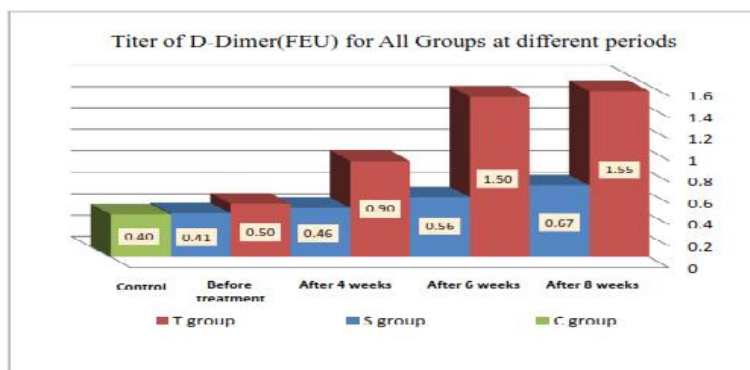


FIGURE 2: Mean value for Titer of D-Dimer (FEU) for all groups at different periods of treatment

**DISCUSSION**

**Effect of Sildenafil or Tadalafil on Prothrombin Time (PT) and Activied Partial Thromboplastin Time (APPT)**

In spite of the statistical differences that shown in table (2), the changes produced by either drugs the values are still within the reference range (11.5-15sec)<sup>[30]</sup> as compared to controls. Seldinafil treatment on prothrombin time begins to decrease values mildly. Whereas, tadalafil treatment produced continues decreasing until PT became out of the normal range at (T4) 8weeks of treatment. Such effect seen at this period began the chronic effect of platelet activity due to agents action. Many patterns of prothrombin time are possible because these tests are classically associated with the coagulation mechanism<sup>[31]</sup>. However, PT results can also be decreased by some disorders of fibrinolysis, because of coagulation mechanism. When PT values decreased in the third phase of homeostasis (after vasoconstriction and platelet plug formation)<sup>[32]</sup>. That means increased platelet activity in high percent of patients

in T-group and less percent in S-group because of chronic use of drugs. Such decrease means that there are some homeostasis events leading to increased platelets activity, may be due to decreased intra-platelet cGMP level that occur because of late increased of PDEs activity. Similarly table -3 shows results of APTT values which continued to decrease clearly until it became out of the normal range at the last period of therapy T4 (8 weeks of treatment) as compare with control and basal line values. Although the values near from normal reference, but that don't mean there are no changes in the homeostasis state because the sensitivity of the assay to factor deficiencies, inhibitors, and heparin also varies with the reagents used in the assay, because of these variables, a normal APTT result does not exclude a mild coagulation events. This test like PT test where several patterns are possible, this test is classically associated with the coagulation mechanism<sup>[33]</sup>. This might be explained by that first, PDE-5 inhibitors have been shown to enhance nitric oxide (NO)-driven cGMP. An important function of NO is the activation of NO-sensitive

guanylyl cyclase through its binding to the prosthetic heme group of the enzyme. The resulting activation of guanylyl cyclase leads to an enhanced conversion of GTP to the second messenger cGMP<sup>[34]</sup>. In platelets, an important regulator of cGMP levels is the cGMP-binding cGMP-specific PDE (PDE5). The enzyme is a homodimer with two regulatory, putative cGMP-binding GAF domains and one catalytic site per monomer<sup>[35]</sup>. The components of the NO/cGMP signaling pathway can mediate PDE5 activation and phosphorylation in response to NO in intact platelets. NO-induced cGMP response in human platelets, cause the activation and phosphorylation of phosphodiesterase type 5 (PDE5) where, cyclic GMP-dependent protein kinase I as the kinase responsible for the NO-induced PDE5 phosphorylation. However, cGMP can directly activate PDE5 without phosphorylation in platelet cytosol, most likely via binding to the regulatory GAF domains. These effects may be seen firstly in equilibrium of increased and decreased of cGMP, but reduced cGMP response after 3 min of pre-incubation with NO. Phosphorylation enhanced the cGMP-induced activation, allowing it to occur at lower cGMP concentrations<sup>[36]</sup>. The reversal of activation was slow and was not completed after 60 min because of proposed long-term desensitization of the cGMP response induced by NO, we looked at the cGMP response in intact platelets; long period of activation PDE-5 and low intra-platelet level of cGMP<sup>[35,36]</sup>. Second Platelets possess three PDE isoforms (PDE2, PDE3 and PDE5), with different selectivity for cAMP and cGMP. Platelets possess three PDE isoforms (PDE2, PDE3 and PDE5), with different selectivity for cAMP and cGMP<sup>[37]</sup>. Hidaka and Asano<sup>[38]</sup> were the first to resolve the PDE activity of platelets into three distinct peaks, as follows: the first prefers cGMP as a substrate, with a  $K_m$  of about 1  $\mu$ m, and is selectively inhibited by PDE5 inhibitors; the second hydrolyses cAMP and cGMP equally well and is selectively inhibited by PDE2 inhibitors; and Third the high affinity for both cAMP and cGMP, but hydrolyses cAMP much more rapidly than cGMP, and is selectively inhibited by PDE3 inhibitors. Thus, in platelets cAMP is hydrolysed by PDE3 and PDE2, and cGMP is hydrolysed by PDE5 and PDE2<sup>[39]</sup>. Since these drugs (sildenafil & tadalafil) will inhibit PDE-5, lead to increased intra-platelet level of cGMP, so increase cGMP because of inhibition PDE-5 that will cause stimulation of PDE-2, leading to hydrolysis both cAMP and cGMP and stimulate platelet activity<sup>[37]</sup>. Low selectivity of sildenafil for PDE5 (with a  $K_m$  of about 1  $\mu$ m), other more selective PDE5 inhibitors have been developed: vardenafil ( $K_D$  of 0.6–0.7 nm; selectivity over PDE6, approximately 16-fold) and tadalafil ( $K_D$  range of 0.9–6.7 nm; 200–700 times more selective for PDE5 than PDE6)<sup>[38]</sup>. By above we see that PDE-5 inhibitors leading to change in PT values due to change in platelet activity by decreased intra-platelet cGMP level.

**Effect The treatment with the Sildenafil and Tadalafil on Platelet Function Assay; Collagen/Epinephrine Test (C/EPI) and Collagen/ Adenosine diephosphate Test (C/ADP)**

Table-4 indicated to stable decreased in closure time of using C/EPI in different periods of both groups (S-group & T-group) as compared with control. This continues and fast lowering from the normal values in the S1& T1 of two

group till it reach out of reference ranges in the T4 period (78-199 sec)<sup>[40]</sup>. Since platelet function analyzer was introduced to mimic in vivo haemostatic plug formation. This simple, rapid, in vitro method aids in the detection of platelet dysfunction. B4170-00, PFA-100 Analyzer for platelet function assay (C\EPI, C/ADP) have technical feature, that if the C/EPI value was normal then will cancel C/ADP value, therefore used mid value between the normal reference range (55-137 sec)<sup>[41]</sup> which was (96 sec) for statistic necessity. From that any 96 value that mean all subjects with normal C\EPI value and normal C/ADP of the same subjects as shown in table -5. This simple, rapid, in vitro method aids in the detection of platelet dysfunction. Where citrated whole blood is aspirated at a high shear rate (High-shear force dynamic flow system) from a sample reservoir through a 150  $\mu$ m aperture in a membrane coated with collagen and ADP (C/ADP): Collagen, epinephrine and ADP are, under in vivo physiological conditions, substances that favor platelet adhesion and aggregation<sup>[42]</sup>. Platelets adhere at the aperture where they are activated previously by the drugs (sildenafil & tadalafil), and more activated by the collagen and then aggregate. The two agonist's epinephrine and ADP enhance aggregation to the normal level but because of high activity of platelet due to these drugs, a platelet plug occludes the aperture and blood flow stops with abnormal closure time<sup>[43]</sup>. The continuous lowering in closure time of using C/ADP in different periods of both groups (S-group & T-group) as compared with control. This continues and fast lowering from the normal values in the S1& T1 of two group till it reach out of reference ranges in the T4 period (55-137 sec). Closure time provides a measure of overall platelet-associated primary homeostasis, with shorter closure time indicative of higher platelet function<sup>[44]</sup>. Since there is a good correlation between the bleeding time and the PFA-100 in certain patient populations, therefore, there is a trend to replace the bleeding time with the PFA-100 for a first-line screening test for platelet dysfunction in patients undergoing preoperative evaluation<sup>[45]</sup>. Other clinical applications include the evaluation of coagulation or anticoagulation effect of drugs<sup>[46]</sup>. Low selectivity of sildenafil for PDE5 (with a  $K_m$  of about 1  $\mu$ m) where it inhibited both PDE-5 & PDE-2. By inhibition of PDE-5 lead to increase cGMP that will stimulate PDE-2<sup>[38]</sup> that will inhibition by low selective effect of sildenafil leading to increase both cAMP & cGMP. The cGMP in turn will activate PDE-5 but cAMP will decreased platelet activity. While the more selective PDE5 tadalafil ( $K_D$  range of 0.9–6.7 nm; 200–700 times more selective for PDE5 than PDE6), inhibit PDE-5 only leading to increased the intra-platelet cGMP that cause stimulation of PDE-2, leading to hydrolysis both cAMP and cGMP and stimulate platelet activity<sup>[39]</sup>. That means the activation effect of platelet function because of the two agents. But tadalafil have thrombogenic and platelet activation effect stronger than sildenafil.

**Effect the treatment with Sildenafil and Tadalafil on titer of D-dimer**

The fibrin monomers that are produced from breakdown of fibrinogen molecules into fragments then aggregate to form fibrin, which is subsequently stabilized by factor XIIIa. Which represent the origin of D-dimer, the

degradation product that is specific of fibrin. The values of D-dimer titer it is for the positive results only (the positive results occur when the values equal to or more than 0.5  $\mu\text{g/ml}$ , while the negative results don't have any value, but for statistical necessity considered the negative result any value smaller than 0.5  $\mu\text{g/ml}$  like 0.4  $\mu\text{g/ml}$  [47,48].

Figure-1 showed that the control value of 0.4  $\mu\text{g/ml}$  that mean all subjects were of negative values, while S1 value of 0.41  $\mu\text{g/ml}$  that mean there are some subjects have positive values (base line values) before started the treatment of present study that lead to elevated S1 value by 0.01. Whereas T1 value was 0.5  $\mu\text{g/ml}$  that mean high number of subjects were having positive values; the number of subjects of positive values among the users of tadalafil was more than those uses sildenafil [49]. Since this test reflects the specific degradation of fibrin (*i.e.*, fibrinolysis) which is the reactive mechanism responding to the formation of fibrin and D-dimer is the terminal fragment in the degradation process of fibrin. Its appearance in the plasma compartment is thus proof that the fibrinolytic system is in action in response to coagulation activation [50]. Rapid increased in the values of D-dimer titer in different periods of both groups as compared to the control, mean that these drugs increased coagulation mechanism as indicated by stimulating fibrinolytic mechanism, its appearance in the plasma compartment is thus proof that the fibrinolytic system is in action in response to coagulation activation [51]. That was activated because of increased fibrin presence due to thrombotic events induced by these drugs (sildenafil and tadalafil) that may occur by a mechanism of indirectly decreasing the intra-platelet cGMP because of inhibiting of PDE-5 leading to increased cGMP that re-activated PDE-5 as feedback mechanism. By compared the different periods of both group (S&T) with each other see that increased in the T-dimer titer values of T-group it very high if it is compared with previous period of the same group or with corresponding period. S-group may reach some time a double or triple values as comparing T4 by T1 whereas (300%). While these high differences can't be seen in the S-group at different periods where the difference between S1& S4 was only 61% of baseline value.

When the fibrinolytic system is activated and therefore the D-dimer level increases, hence D-dimer assays can help in the diagnosis of DIC, because D-dimer level increases during the activation states of coagulation because such states induce the production of thrombin which is followed by the formation of fibrin and leads to fibrinolysis, and thus increases D-dimer following coagulation activation [50]. Low selectivity of sildenafil for PDE5 (with a  $K_m$  of about  $1\mu\text{m}$ ) where it inhibited both PDE-5 & PDE-2 by inhibition of PDE-5 lead to increase cGMP that will stimulate PDE-2 that will inhibition by low selective effect of sildenafil leading to increase both cAMP & cGMP. cGMP will activate PDE-5 but cAMP will decreased platelet activity [37], while tadalafil ( $K_D$  range of 0.9–6.7nm; 200–700 times more selective for PDE5 than PDE6), inhibits PDE-5 only leading to increased the intra-platelet cGMP that cause stimulation of PDE-2 and to hydrolyse both cAMP and cGMP [52], hence stimulating platelet activity.

### The effect of Sildenafil and Tadalafil treatment on fibrinogen Weight (FIB)

Fibrinogen is an essential protein contribute in the homeostasis process produce by the liver about (3-5) g/l daily increase this value or decrease mean there is a defect. Elevated the value mean increase the production of fibrinogen as reflex for its consumption, and low value of fibrinogen mean either decrease production of fibrinogen because liver diseases or increase consumption of fibrinogen because some diseases or conditions like thrombus formation that ended by fibrinolysis [53]. Figure-2 shown continuous decreased values for fibrinogen weight at different periods in S-group all values within the normal range. While fibrinogen weight at different periods for T-group was: T1 where before began the study and irregular treatment within the normal range, but after two dose weekly the values of fibrinogen weight were elevated because increased the production as a reflex for increased consumption by fibrinolysis. An increased levels of fibrinogen can be found in cases of diabetes, inflammatory syndrome and obesity [54]. Furthermore, fibrinogen seems to be involved in the pathogenicity of thrombotic cardiovascular events. Because fibrinogen can be degraded by plasmin [55], at T3 & T4 decreased the values of fibrinogen because the consumption more than production, that mean firstly increase the thrombogenic effect of tadalafil in T1 & T2 led to increased production of fibrinogen by liver and then increase the consumption by fibrinolysis led to decrease fibrinogen values. Regular decline in fibrinogen weight in the different periods of S-group but remind within normal reference because the consumption is less than production due to low severity of fibrinolysis to thrombogenesis effect of sildenafil. The thrombogenic effect of tadalafil is more than that of sildenafil, since sildenafil is less selective for PDE5 where it inhibit both PDE-5 & PDE-2. By inhibition of PDE-5 an increase in cGMP that will stimulate PDE-2 that will be inhibited by low selective effect of sildenafil [26] leading to increase both cAMP & cGMP, thus cGMP will activate PDE-5 but cAMP will decreased platelet activity [37,38]. While the more selective PDE5 tadalafil ( $K_D$  range of 0.9–6.7 nm; 200–700 times more as conclusion; PDE-5 inhibitors (sildenafil & tadalafil) increase platelets activity and activate their aggregation. Sildenafil have less aggregatory effects on platelet than that produced by tadalafil when used for the same duration of time.

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