

Antiplatelets Activity of Vitamin E in Relation to Dose and Duration of Therapy

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Abstract

Vitamin E, having the well known antioxidant activity through scavenging free radicals and occurs in several isomeric forms, these isomers have relatively different functions. One of these actions is related to its ability to inhibit platelets aggregation and hence thrombosis. The present study included a total number of apparently healthy 62 males. 11 of them served as standard group, treated with 100 mg aspirin /day for more than one month. Another 31 subjects were randomly grouped into 5 groups that received different daily doses of α -tocopherol: 400 IU, 800 IU and 1200 IU for 2-6 months. The remainder (20) subjects served as a control group (received no therapy). Platelets function was assessed based on measuring bleeding time and Slide Platelets Aggregation Time (SPAT) Meanwhile, thiobarbituric acid reactive substances (TBARS) were measured as a marker for oxidative stress. The results showed that the commercially available vitamin E preparations (α -tocopherol) could exert anti-coagulant effect, such effect is more dependant on duration of therapy, rather than dose related action. In addition to its antioxidant effect, which seems to be significantly correlated to its antiplatelets effect ($r=0.994$, $p<0.05$). Hence, long term administration of high doses of vitamin E could be effective in decreasing the incidence of thrombosis, which in turn depends on platelets function. Such effect might not affect bleeding time obviously, but it could reduce chances for platelets recruitment, which might represent an additional advantageous action for vitamin E over other antioxidants.

Key Words: Vitamin E, α Tocopherol, Antiplatelets.

الخلاصة

ان فيتامين أي معروف بخاصيته المضادة للاكسدة من خلال اقتناصه للجذور الحرة المتكونة. ان هذه الجزيئة توجد في عدة صور جزيئية وهذه الاشكال المختلفة للجزيئة تتميز كل منها بخصائص ووظائف مختلفة. احدها يرتبط باحباط قابلية الصفائح الدموية للتكتل وتكوين الخثرة الدموية تضمنت هذه الدراسة 62 ذكرا سليما، اعتبر احد عشر منهم مجموعة قياسية للمقارنة حيث تم علاجها بالاسبرين 100 ملغم يوميا ولمدة اكثر من شهر واحد. وتم تقسيم 31 شخصا اخر وبصورة عشوائية الى مجاميع تتناول الفا -توكوفيرول بجرع مختلفة (400 و 800 و 1200 وحدة دولية يوميا) ولمدد مختلفة تراوحت بين الشهرين والستة أشهر. اما باقي المشاركين بالدراسة فقد تم اعتبارهم مجموعة مقارنة لم يتناولوا أي علاج. تم اعتماد الفحوصات المختبرية الخاصة بفعالية الصفائح الدموية مثل قياس مدة النزف و الوقت اللازم للصفائح الدموية للتجمع على السلايد إضافة الى قياس مستوى المواد المتفاعلة مع حامض الثايوباربيجورك كدليل لدرجة الاجهاد التاكسدي. اظهرت النتائج ان النوع المتوفر في اسواق العراق من الفيتامين E وهو في الاغلب يتكون من نوع الفا توكوفيرول فقط يمكن ان يحدث تأثيرات على عملية تجلط الدم. وان هذا التأثير فيما يبدو يعتمد بصورة اكبر على مدة استمرار العلاج اكثر من ارتباطه بالجرعة المعطاة من هذا الفيتامين. لذلك ان تناول فيتامين أي -الفا توكوفيرول- و بجرع كبيرة نسبيا ولمدة طويلة -اكثر من خمسة أشهر- يمكن ان يعطي تأثيرا مفيدا من خلال تقليل احتمالية الاصابة بتخثرات الدم من خلال تأثيرها على فعالية الصفائح الدموية وهذا التأثير الايجابي يمكن ان يكون تأثيرا اضافيا لتفضيل فيتامين أي على غيره من المواد المضادة للاكسدة.

Introduction

Vitamin E has been known as an essential nutrient to maintain normal reproduction since 1922⁽¹⁾. The large scale studies have shown an inverse correlation between its high dietary intake and the incidence of coronary heart diseases^(2,3). However, these studies did not provide sufficient evidences, that vitamin E administration can prevent cardiovascular events, nor the subsequent cardiac deaths⁽⁴⁾. Such confliction could be attributed to the fact that, clinical studies usually utilizes the commercially available vitamin E preparations, which almost contain α -tocopherol

alone, whereas vitamin E in food occurs in several other forms. Thus, the absence of other tocopherols – than α -tocopherol – in the pharmaceutical preparations (utilized in the previously mentioned studies) may account for such unexplained results (5,6). Naturally vitamin E occurs as a family of eight members, four of them are referred to as tocopherols and the other four members are known as trienols. Both tocopherols and tocotrienols are subdivided into 4 types: alpha (α), beta (β), gamma (γ) and delta (δ), according to their substitutions on the molecule⁽⁷⁾, as shown in figure (1) and table 1.

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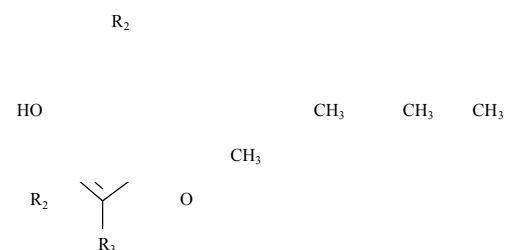
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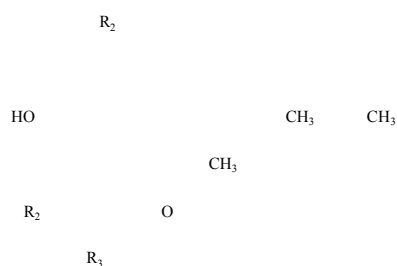
Table - 1-
Different types of vitamin E

	R 1	R 2	R 3
Alpha	CH ₃	CH ₃	CH ₃
Beta	CH ₃	H	CH ₃
Gamma	H	CH	CH ₃
Delta	H	H	CH ₃

R = Substituted groups in the general structure of Tocotrienols



General structure of Tocopherole



General structure of Tocotrienols

Figure 1

The most abundant forms of vitamin E in nature are alpha and gamma – tocopherols⁽⁸⁾. Hence, some members of vitamin E was shown to exert specific functions, that may not be found in others⁽⁹⁾. Considering vitamin E action, it can act as scavenger of free radicals, thereby it can provide protection from free radicals – produced damage⁽¹⁰⁾. Also, it had been reported that vitamin E could exert an antiinflammatory effects through inhibiting lipooxygenase action, thus inhibiting leucotrienes release (a powerful mediator of inflammation)⁽¹¹⁾. Meanwhile, vitamin E can decrease the cyclooxygenase cascade in leukocytes which interferes with inflammatory process⁽¹²⁾. Some observations by *Chan and Leith* (1981) and *Gilber et al* (1983), demonstrated that vitamin E enhances the release of prostacyclin – a potent vasodilator and inhibitor of platelets aggregation – in a dose-dependent manner. This study was designed to evaluate the

antiplatelets action of α –tocopherol in the commercially available vitamin E preparations in Iraqi market, in relation to dose and duration of therapy.

Subjects and Methods :

The study included 62 male subjects with age ranged between 32 and 55 years old (45 ± 4.2). The contributing subjects were selected to have no past history of cardiovascular disease or thrombotic disorder, from those attended a private clinic for infertility at Al-Sadoon Street /Baghdad, under supervision of a senior physician for the period July-November 2005. Twenty of them served as a control group

(received no therapy). Another group of 11 subjects were treated with a daily dose of 100 mg aspirin for more than one month (1-3 months). The remainder (31 subjects) were subdivided into 5 groups to be treated with vitamin E (α -tocopherol) as follows:

Group A : included 6 subjects treated with 400 IU /day for less than 5 months (2-4 months).

Group B : included 7 subjects treated with 400 IU / day for more than 5 month (5-6 months).

Group C : included 6 subjects treated with 800 IU/ day for less than 5 months (2-4 months).

Group D : included 6 subjects treated with 800 IU / day for more than 5 months (5-6 months).

Group E : included 6 subjects treated with 1200 IU / day for less than 5 months (2-4 months).

The treatment in all groups did not exceed six months. Blood specimens were obtained by venipuncture, to perform the platelets assessments anticoagulant(EDTA-K2) was added, whereas those aliquots used to assess serum TBARS were obtained by centrifugating blood specimens after clotting.

Platelet function was evaluated by measuring **Slide platelet aggregation time** SPAT TM, based on measuring time required by platelets to aggregate on a slide in the presence of a potent soluble aggregating agent (30 micromol propylgallate), purchased from Analytical Control System ACS Inc⁽¹⁵⁾.

Bleeding time was measured for each subject at the end of treatment period according to **TVY method**⁽¹⁶⁾.

Oxidative stress was assessed by measuring **thiobarbituric acid reactive substances TBARS** in serum according to Beuge and Auest method(1978).

Results was expressed as mean \pm SD , student t-test (unpaired) were used considering P values less than 0.05 to be significant⁽¹⁸⁾.

Results

Effects of α -tocopherol therapy on bleeding time :

Bleeding time values showed no significant change in subjects treated with daily dose of 400 IU of α -tocopherol (group A), even in those continued therapy for more than five months (group B) figure (2) . Whereas , the effect of a dose of 800 IU /day was time-dependent , as shown by results of the group treated for more than 5 months (group D).Higher doses (1200 IU/day) of α -tocopherol for the same period (less than 5 month) also failed to produce significant change in bleeding time values (group E) . While , the standard therapy with antiplatelet agent(aspirin) produced a significant elevation .

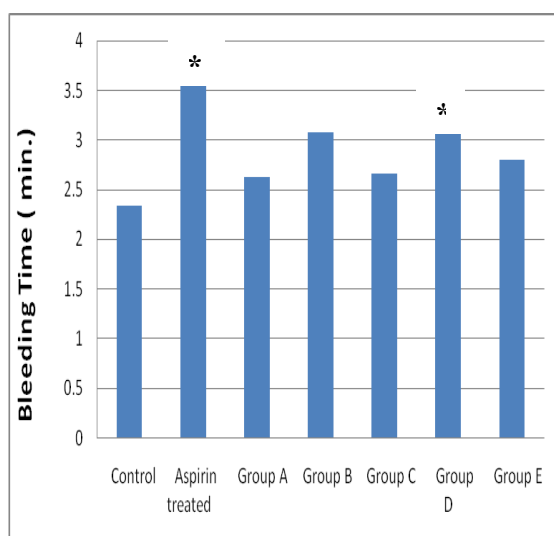


Figure 2 . Effects of Alpha – Tocopherol on Bleeding Time

Group A : included subjects treated with 400 IU /day less than 5 months (2-4 months) .

Group B : included subjects treated with 400 IU / day more than 5 month (5-6 months) .

Group C : included subjects treated with 800 IU/ day less than 5 months (2-4 months) .

Group D : included subjects treated with 800 IU / day more than 5 months (5-6 months) .

Group E : included subjects treated with 1200 IU / day less than 5 months (2-4 months).

Aspirin group : included subjects treated with 100 mg / day (1-3months) .

* = significantly different from control (< 0.05)

Effects of α -tocopherol therapy on SPAT values :

The results of SPAT test for the studied groups are illustrated in figure (3) . Significant alterations in SPAT values were observed in those subjected to therapy that continued more than 5 months , by either doses : 400 or 800 IU α -tocopherol /day – i.e. groups Band D , respectively .

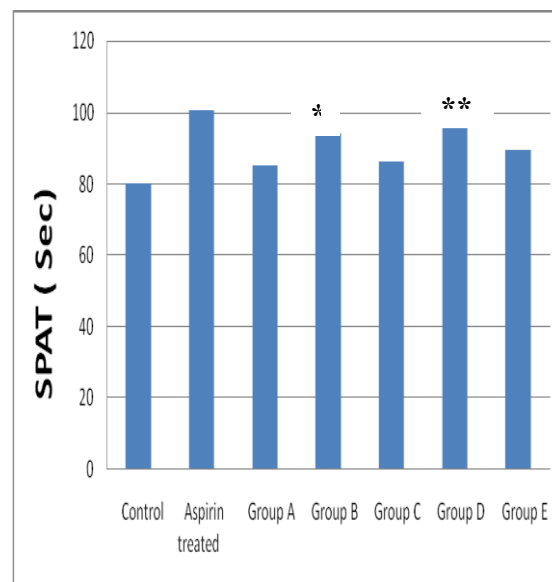


Figure 3 . Effects of alpha –tocopherol on Slide Platelet Aggregation Test (SPAT) values

Group A : included subjects treated with 400 IU /day less than 5 months (2-4 months) .

Group B : included subjects treated with 400 IU / day more than 5 month (5-6 months) .

Group C : included subjects treated with 800 IU/ day less than 5 months (2-4 months) .

Group D : included subjects treated with 800 IU / day more than 5 months (5-6 months) .

Group E : included subjects treated with 1200 IU / day less than 5 months (2-4 months).

Aspirin group : included subjects treated with 100 mg / day (1-3months) .

*=significantly different from control($p < 0.05$)

** =significantly different from control ($p < 0.01$) .

Effects of α -tocopherol on serum TBARS levels :

Figure (4) summarizes the changes in serum TBARS levels in response to tested therapy regimens .A significant reduction was detected in groups treated with aspirin and those given α -tocopherol in doses of 400 or 800 IU/day for more than five months.

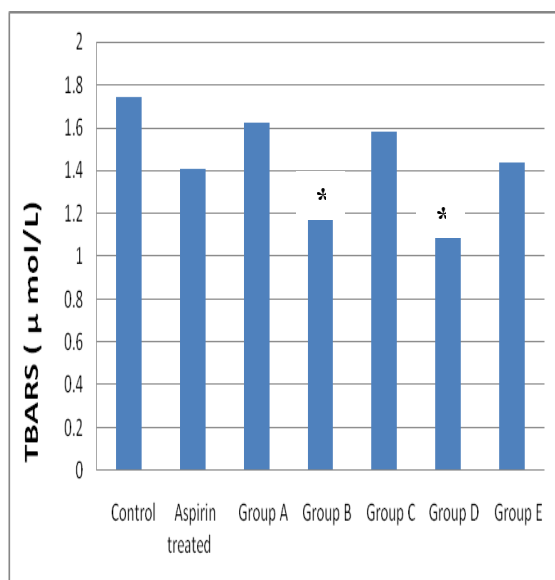


Figure 4 . Serum thiobarbituric acid reactive substances (TBARS) concentration in various groups

Group A : included subjects treated with 400 IU /day less than 5 months (2-4 months) .

Group B : included subjects treated with 400 IU / day more than 5 month (5-6 months) .

Group C : included subjects treated with 800 IU/ day less than 5 months (2-4 months) .

Group D : included subjects treated with 800 IU / day more than 5 months (5-6 months) .

Group E : included subjects treated with 1200 IU / day less than 5 months (2-4 months)

Aspirin group : included subjects treated with 100 mg / day (1-3months) .

* = significantly different from control ($p < 0.05$) .

Discussion

One of the reported actions for vitamin E is the anti-coagulant and antiplatelets activity. Such effect is mainly presented by the gamma (γ) form of tocopherols , as prolongation of bleeding time and abnormal platelet aggregability results after ingesting high doses of vitamin E for long period of time^(19,20). In vitro , alpha (α) , gamma (γ) and delta (δ) – tocopherols have similar effects on human platelet aggregation and a combination of these tocopherols has a synergistic platelets inhibitory effect over the α –tocopherol alone^(21,22) . One of the proposed mechanisms for vitamin E antiplatelet activity could be through its interference with vitamin K activity with subsequent disturbance of the cascade of reactions for clot formation ,that predispose to thrombosis . However, such interruption depends on the isomer of vitamin E used , dose and period of administration⁽²³⁾ . Another proposed mechanism to explain the platelet antiaggregability effect of vitamin E is related

to NO bioactivity^(24,25) . Decreased bioavailability of NO is a characteristic feature in patients with coronary artery disease and impaired platelet NO production which predicts acute coronary syndrome⁽²⁶⁾ . Platelets -derived NO has been found to inhibit platelets aggregation and to reduce recruitment to grow to thrombus⁽²⁷⁾ . Incorporation of α – tocopherol might increase NO production in platelets by its free radicals scavenging activity and by preventing NO quenching by peroxy radicals^(28,29) . The results of the present study shows that α –tocopherol when administered alone could exert significant modifications in platelets function as presented by changes in SPAT values (figure -3 -).Such effect seems to be related to duration of therapy rather than to dose administered . Although , such effect was less obvious in bleeding time values (figure -2-) .However , the concomitant changes in serum TBARS in the studied groups (figure 4)could strongly suggest a relationship to exist between antioxidant activity of vitamin E with it's antiplatelets activity⁽³⁰⁾ , indicated by a significant correlation between TBARS and SPAT values ($r=0.994, p<0.05$) . Although some reported that antiplatelets activity of vitamin E is independent on it's antioxidant effect⁽³¹⁾ .The lowering effect of TBARS by vitamin E may represent an index for delivering vitamin E to membrane structures of different cells including the platelets, which is reflected by a decrease in platelets aggregability upon longer time of exposure to these doses of α –tocopherol , through increasing the amount of α –tocopherol inside body with possible participation of it's antioxidant activity to affect SPAT values. Aspirin administration for more than one month could lower TBARS levels (figure -3-) through increasing the apoferritin level , whose duty is to quench free iron in plasma , since free iron catalyses free radicals generation through Fenton's reaction^(32,33) . Long-term ingestion of α –tocopherol (more than 5 month) is needed to exert it's antiplatelets activity which may be explained on the bases of its pharmacokinetic behavior , because it is stored initially in adipose tissues before its action appears in circulation^(34,35) . Thus to get greater benefit from vitamin E administration , it may be preferable to take other forms of tocopherols (i.e. γ –tocopherol) with a pharmacokinetic behavior that does not require to build up a concentration after accumulation in adipose tissues^(36,37) . However , similar studies including larger number of subjects and longer duration of therapy could provide more clear picture about such effects of different isomeric forms of tocopherols. In conclusion ,

vitamin E administration can produce significant effects in those patients with high risk of thrombus formation to be preferred over other antioxidants like vitamin C .

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References

- 1- Evans H. M. and Bishop K.S. :On the existence of a hitherto unrecognized dietary factor essential for reproduction . Science 1922,56 : 650-651.
- 2- Rimm E.B.,Stampfer M. J.,Ascherio A., et al :Vitamin E consumption and the risk of coronary heart disease in men .N Engl J Med 1993; 328 : 1450- 1456 .
- 3- Stampfer M.J.,Hennekens C. H.,Manson J.E.,et al :Vitamin E consumption and the risk of coronary disease in women .N Engl J Med 1993; 328 : 1444-1449.
- 4- Yusuf S., Dagenais G.,Pogue J., et al : The Heart Outcomes Prevention Evaluation Study Investigators . N Engl J Med 2000; 342:154-160.
- 5- Wolf G. : γ - Tocopherol : an efficient protector of lipids against nitricinitiated peroxidative damage . Nutr.Rev 1997; 55 : 376 – 378.
- 6- Ohrvall M.,Sundlof G.and Vessby B. :Gamma but not alpha tocopherol levels in serum are reduced in coronary heart disease patients .J Intern. Med 1996 ; 239: 111-117 .
- 7- Delgado J.N.and Degado W.M:Text of organic medical andpharmaceutical chemistry ,2004, 9 th edition ,Lippencot Comp. , Chapter- 4.
- 8- Goby S. K.and Machlin L.J. ; Vitamin E intake and health an overview . J Clin Nutr 1998 ; 3 : 445 – 452 .
- 9- Fourell P.M., Levin S.L.,Muphy M.D., et al : Plasma tocopherol lipid relations in normal population .Am J Clin Nutr 1998; 13 :1720-1726 .
- 10- Burton G.W.,Joyce A., Ingold K. U. : Is vitamin E the only lipid soluble chain – breaking antioxidant in human blood plasma and erythrocytes membranes ? Arch. Biochem. Biophys 1983; 221 : 281-290 .
- 11- Rood L.and Canary J.J. :Vitamin and relations among tocopherols in human plasma platelets, lymphocytes and RBCs : Am J Clin Nutr 2000 ;47: 470-475.
- 12- Klein J.,Reutter F.,Schweer H., et al : Generation of the isoprostane 8-epi-prostaglandine F2- alpha in vitro and in vivo via the cyclooxygenase J.Pharmacol. Exp Ther 1997; 282:1656 –1658.
- 13- Chan A.C. and Leith M.K. : Decreased prostacyclin synthesis in vitamin E deficient rabbit aorta . Am.J clin Nutr 1981;34:2341-2347.
- 14- Gilber V.A., Zebrowski E. J. and Chan A.C. :Differential effects of mega- vitamin E on prostacyclin and thromboxane synthesis in streptozotocin- induced diabetic rats . Horm Metab Res 1983;15:320-325.
- 15- Analytical Control System ACS reagent kit literature. ACS Inc. 90589 Technology drive Fishers IN 46038 –2005.
- 16- Lewis S.M.and Bain B.J.(Ed) : Dacie andLewis ; Practical heamatology , thrombolytic and platelet therapy –9 th ed. 2001,Chuchill Livingston, London . chapter 18 .pp:215-263.
- 17- Beuge J.A. andAuest S.D. Meth Enzy 1978;51 :302 –305 .
- 18- Duncan R. C. ,Knapp R.G.,Miller M.C.(Ed) : Introductory Biostatistics for The Health Sciences .2 nd ed. 1983.Awily Medical Publication , Newyork. pp: 85-90.
- 19- Steiner M.andAnastasia J. : Vitamin E an inhibitor of the platelet release reaction .J Clin Invest 1976 ;57 :732-737.
- 20- Srivastava K. C. :Vitamin E exerts antiaggregatory effects without inhibiting the enzymes of the arachidonic acid cascade in platelets . Prostagl Leukot Med 1986 ;21 :177-185 .
- 21- Mabile L.,Bruckdovfer K. R., Rico-Evans C.: Moderate supplementation with natural alpha – tocopherol decreases platelet aggregation and low-density lipoprotein oxidation .Atherosclerosis 1999 ; 147 :177-185.
- 22- Liu M.,Wallmen A. , Olsson-Mortlock C. et al :Mixed tocopherols inhibit platelet aggregation in humans; potential mechanisms .Am J Clin Nutr 2003, 77 (33) :700 –706.
- 23- Freedman J.E.and Keaney J.F. : Vitamin E inhibition of platelet aggregatration is independent of antioxidant activity .J Nutr 2001 ; 131 : 3745-37775.
- 24- Li D.,Saldeen T., Romeo F., et al : Different isoforms of tocopherols enhance nitric oxide synthase phosphorylation and inhibit human platelet aggregation and lipid peroxidation ; implications in therapy with vitamin E. J Cardiovasc Pharmacol Ther 2001 ; 6 : 155- 161 .
- 25- Freedman J.E.,Sauter R., Battinelli E.M., et al : Deficient platelet –derived nitic oxide and enhanced hemostasis in mice

- lacking the NOS III gene . Circ Res 1999 ; 84 : 1416-1421 .
- 26-** Freedman J.E., Ting B., Hankin B., et al : Impaired platelet production of nitric oxide predicts presence of acute coronary syndromes. Circulation 1998; 98 : 1481-1486.
- 27-** Freedman J.E., Loscalzo J., Barnard M.D., et al : Nitric oxide released from activated platelet recruitment . J. Clin. Invest. 1997, 100 : 350-356.
- 28-** Huie R. E. and Padmaja S. : The reaction of NO with superoxide .Free Radic Res Commun 1993; 18 : 195-199
- 29-** Rubbo H., Radi R., Trujillo M., et al : Nitric oxide regulation of superoxide and peroxynitrite- dependent lipid peroxidation; formation of novel nitrogen – containing oxidized lipid derivatives . J Biol Chem 1994; 269 : 26606-26075 .
- 30-** Muller M. and Sorrell T.C. : Oxidative stress and the metabolism of arachidonic acid in stimulated human platelets ; role of hydroxyl radical . Prostagl. 1997; 54 : 493-509 .
- 31-** Jane E Freedman and John F Keaney Jr : Vitamin E inhibition of platelets aggregation is independent of antioxidant activity . J Nut 2002;131:374S -377S.
- 32-** Buczynski A., Wachowicz B., Kedziora-Kornatowska K. et al : Changes in antioxidant enzymes activities . aggregability and malondialdehyde concentration in blood platelets from patients with coronary heart disease. Atheroscler 1993; 100 : 223-228.
- 33-** Okuma M., Steiner M., Baldini G., et al : Studies on lipid peroxidation in platelets II ; Effect of aggregating agents and platelets antibody . Lab.Clin. Med 1971;77:728-742 .
- 34-** Brigelius-Flohe R. and Traber M. G. : Vitamin E ; function and metabolism. FASEB J 1999 ; 13 : 1145-1155.
- 35-** Mehta J., Li D. and Mehta J . L. : Vitamins C and E prolong time to arterial thrombosis in rats . J Nutr 1999 ; 129 : 109-112.
- 36-** Li D., Suldeen T., Romeo F., et al : Relative effects of alpha and gamma tocopherol on low density lipoprotein oxidation and superoxide dismutase nitric oxide synthase activity and protein expression in rats . J Cardiovas Pharmcol Ther 1999 ; 4 : 219- 226 .
- 37-** Unchern S, Laohruangpanya N, Phumala N, et al : The effects of vitamin E on platelets activity in beta thalassaemia patients . Br J Haem 2003;123(4) :738-744.