ASSESSMENT OF ANTICOAGULANT EFFECT OF EVENING PRIMROSE OIL

AZRA RIAZ, RAFEEQ ALAM KHAN* AND SHAHIDA PARVEEN AHMED
Department of Pharmacology, University of Karachi, Karachi-75270, Pakistan

ABSTRACT
Effect of evening primrose oil (EPO) was assessed on coagulation parameters following 30 and 60 days administration of 90, 180 and 360 µl/kg oil to healthy rabbits of either sex. There was significant increase in all assays except Fibrinogen time. These effects might be due to inactivation or inhibition of factors affecting coagulation. The intake of evening primrose oil also significantly decreased platelet count. Results of this study suggest that evening primrose oil shows considerable anti-anticoagulant and anti-platelet activity in animals and has potential to reduce cardiovascular morbidity and mortality.

Keywords: Evening primrose oil, Thrombin time, Prothrombin time, activated partial thromboplastin time, Fibrinogen time.

INTRODUCTION

Essential oils are volatile substances extracted from plants. They are used for various therapeutic purposes (Burns, 2000). Evening primrose, borage and black currant oils have similarity of one unsaturated fatty acid i.e. gamma-linolenic acid (Setty *et al.,* 2005; Peschel *et al.,* 2007). But evening primrose oil may be most effective among other, due to its simple composition and high level of linoleic acid that may facilitate the absorption of gamma-linolenic acid (Dines *et al.,* 1995). Evening primrose oil (EPO) provides direct supply of gamma-linolenic acid (GLA) in disease states where conversion of the dietary precursor linoleic acid to GLA is attenuated (Fieldsend *et al.,* 2000; Hassig et al; 2000; Senanayake et al; 2004) such as cardiovascular diseases (Balasinska, 1998) and diabetes mellitus (Fang *et al.,* 1997; Kuruvilla *et al.,* 1998).

Blood coagulation, itself is a complex set of physical, cellular and biochemical events leading to thrombus formation (Brummel *et al.,* 2002). Thrombus plays an important role in the pathogenesis and progression of atherosclerosis and cardiovascular disease (Chandler *et al.,* 2003; Colman, 2006). Possible mechanisms to prevent and control the coagulation abnormalities as well as thrombogenic state in human or animals have been widely studied (Brummel *et al.,* 1999). There are evidences that EPO caused remarkable improvement in clotting time; severity of atherosclerotic lesion as well significantly decreased thrombin induced platelets aggregation (Renaud *et al.,* 1982). Platelets plays critical role in hemostasis, both for the formation of clot and activation of coagulation proteins (Colman, 2006). EPO inhibits platelet aggregation in hyperlipidemic rabbits through multiple mechanisms and could be considered as antithrombotic (Cruz *et al.,* 1997). These evidences show that EPO may have an effective role on coagulation parameters hence an in vivo study was designed to examine the effect of EPO on prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), fibrinogen time (Fg) and platelet count.

**Corresponding author:* e-mail: rkhan1959@gmail.com

MATERIALS AND METHODS

Drug treatment
EPO was obtained from Evecure capsules a product of local pharma with following composition i.e. Linoleic acid 73% , γ-linolenic acid 9% , oleic acid 8.6%, palmitic acid 6.3% , stearic acid 1.9% and vitamin E 10 IU, warfarin sodium was used as standard and both drugs were administered through oral route. The normal recommended dose of EPO is two capsules three times daily each capsule contains 500 µl of EPO.

Animal selection
The study was carried out on fifty healthy white rabbits of either sex weighing from 1000-1500 grams. Animals were housed individually in cages, under controlled condition of temperature (23±2°C), and humidity (50-60%). Diet and water was provided ad labitum.

Experimental design
Animals were divided in five groups, each containing 10 animals. Three groups were treated as test animals and were administered normal, moderate and high doses of EPO i.e. 90, 180, and 360 µl /kg daily. Animals treated as standard were given 0.54 mg/kg warfarin (Zacchiga et al 2004). While controlled animals were administered water equivalent to the corresponding dose of EPO in µl / kg of the body weight. Body weights of the animals were measured weekly. 5 ml Blood samples were collected twice, once at 30 days and other at the end of dosing period i.e. 60 days from ear vein of the animals.

Hematological examination
Humacount plus a fully automated hematology analyzer (Human Germany) was used to examine the hematological parameters, red blood cell (RBC), white blood cell (WBC), Platelet (PLT) and hemoglobin (Hb).

Measurement for coagulation parameters
Blood samples were collected in coagulation tubes, plasma was separated by centrifugation at 1500 rpm for 15 min in 14K Humax centrifuge. TT, PT, aPTT and Fg were measured by Humaclot duo, using standard reagent kits of Merck (Chan *et al.,* 2007).

STATISTICAL ANALYSIS

All values were compared with control by taking mean and standard error to the mean using student t-test, values of $P<0.05$ were considered as significant and $P<0.005$ as highly significant. All statistical procedures were performed according to the method of Alcaraz and Jimenez (1989).

RESULTS

Table 1 shows the effect of EPO on coagulation parameters at 30 days. There was significant increase in PT and TT at normal and high doses and highly significantly increase was observed at moderate dose and standard drug warfarin. There was significant increase in aPTT at normal dose and highly significantly increased was observed with moderate and high dose of EPO and standard drug warfarin. Fibrinogen time was not effected significantly at any dose.

Table 2 shows the effect of EPO on coagulation parameters at 60 days. There was highly significant increased in TT and significant increased in PT at all doses. aPTT was significantly increased at normal and high doses while highly significant increased was observed at moderate dose and warfarin. Fibrinogen time was not effected significantly at any dose.

Table 1: Comparative Effect of EPO and warfarin on coagulation parameters at 30 days

Parameters (seconds)	Animal groups				
	Control	EPO µl/kg Doses			Warfarin 0.5 mg/kg
		90	180	360	
TT	9.36±0.93	*12.44±0.90	**13.58±0.78	*12.51±0.78	**15.08±1.4
PT	5.20±0.12	*5.90±0.22	**6.10±0.24	*5.95±0.31	**8.72±0.72
aPTT	8.05±0.69	*12.69 ± 1.40	**13.82±1.50	**12.47±0.83	**14.53±1.90
Fg	49.30±17	51.60±15	32.60±06	33.8±4.9	23.3±3.2

n = 10
mean ± SEM
*p < 0.05, **p < 0.005, as compared to control

Table 2: Comparative Effect of EPO and warfarin on coagulation parameters at 60 day

Parameters (seconds)	Animal groups				
	Control	EPO µl/kg Doses			Warfarin 0.5 mg/kg
		90	180	360	
TT	9.36±0.93	**13.05±0.62	**15.06±0.77	**14.33±0.72	**16.58±1.70
PT	5.20±0.12	*6.08±0.37	*7.08±0.74	*6.72±0.63	*10.79±1.70
aPTT	8.05±0.69	*14.53 ±1.90	**14.70±1.70	*14.10±1.90	**14.71±2.20
Fg	49.30±17	35.0±8.50	32.20±5.80	26.1±5.50	23.9±5.60

n = 10
mean ± SEM
*p < 0.05, **p < 0.005 as compared to control.

Table 3 shows significant decrease in platelets at moderate dose, at 30 days, while table 4 shows significant decrease in platelets at all doses, at 60 days. There were no significant changes in other hematological parameters i.e. RBC, WBC and hemoglobin (Data not shown).

Table 3: Effect of EPO on platelets count at 30 days

Parameters	Animal groups			
	Control	EPO µl/kg Doses		
		90µl	180µl	360µl
PLT ($x10^3$/µl)	410.0 ± 50	275.0 ± 47	*270.7 ± 30	317.2 ± 29

n = 10.
mean ± SEM
*p < 0.05, as compared to control.

Table 4: Effect of EPO on Platelet count at 60 days.

Parameters	Animal groups			
	Control	EPO µl/kg Doses		
		90	180	360
PLT ($x10^3$/µl)	410.0 ±50	*247.0 ± 41	*265.0 ± 44	*264.0 ± 33

n = 10.
mean ± SEM
*p < 0.05, as compared to control.

DISCUSSION

The process of blood coagulation plays a central role in an organism's response to vascular injury on one hand and in cardiovascular diseases (CVD) or thrombosis on the other hand (Orfao *et al.,* 2008). The treatment with any oral anticoagulants must be monitored to ensure that the dose is providing the required effects (Zacchigna *et al.,* 2004).

Coagulation tests like PT, aPTT, Fg and TT can better define the risk of bleeding. They monitor the influence of evening primrose oil on blood coagulation process. In addition to that effect of evening primrose oil on platelets count was also evaluated.

Evening primrose oil caused significant increase in PT and aPTT at all doses. Response of moderate dose was almost equivalent to the response of warfarin. Prolongation in PT may be due to decrease in coagulation factors like V, VII and X involved in extrinsic pathway. While prolongation of aPTT may be due to decrease in coagulation factors such as VIII, IX, XI, XII and vWF (Chan *et al.,* 2007) involve in intrinsic pathway. PT and aPTT are often used to assess variation in coagulation factors (Yuan *et al.,* 2007). Evening primrose oil has hypocholesterolemic effect (Balasinska, 1998). It may be postulated that decrease in coagulation factors may be due to hypocholesterolemic effect of evening primrose oil. Since high cholesterol diet has been reported to significantly increase plasma concentrations of clotting factors II, VII and X in rabbits (Mitropoulos *et al.,* 1987). Increase catabolic rate of prothrombin stimulate hepatic synthesis of clotting factors, resulting in increased plasma concentration (Miller, 2005). Therefore we can postulate that if increased concentration of cholesterol can cause increase plasma concentration of coagulation factors then decrease concentration of cholesterol may decrease concentration of coagulation factors.

Vitamin K deficiency cause decrease in synthesis of factors II, VII, IX and X in liver that in turn results in hypocoagulable state (Krupiczojc *et al.,* 2008; Shearer, 2009). There is evidence that decrease absorption of lipids from gastrointestinal tract results in vitamin K deficiency (Kamali, *et al.,* 2001), hence reduced total cholesterol index by evening primrose oil may leads to vitamin K deficiency, which ultimately reduces synthesis of clotting factors resulting in prolongation of the PT. Warfarin inhibits the action of vitamin K by inhibiting vitamin K cycle and causes prolongation of PT (Gage *et al.,* 2000). Therefore it can be assumed that EPO produces the effect in a way similar to warfarin.

Heparin forms a complex with antithrombin-III and removes many activated coagulation factors that could be measured by prolonged aPTT (Allford *et al.,* 2007). Present study reveals prolonged aPTT caused by evening primrose oil, hence it may be postulated that evening primrose oil may produces anticoagulant effect in the manner similar to heparin.

Present study revealed significant increase in TT that indicates deficiency of fibrinogen or inhibition of thrombin (Lane *et al.,* 2005). Hence prolonged TT may be the results of reduced activity of coagulation factors because some factors such as IX and X (Di Cera, 2008) XI and XII are essentially required for thrombin generation (Gailani *et al.,* 2007). Decrease platelets count may be another reason for prolong TT (Di Cera, 2008) since platelet generated thrombin is necessary to produce a stable fibrin plug (Wolberg, 2007). Studies suggest that evening primrose oil attenuates atherogenesis and thrombogenesis probably by inhibiting both cyclooxygenase and nitric oxide synthase in lipid-fed rabbits (Cartledge *et al.,* 2000, Alison *et al.,* 2002).

Present study reveals insignificant decrease in fibrinogen level that may conversely favor anti-coagulation. There is evidence that increased plasma fibrinogen level has been recognized as an independent risk factor for vascular diseases (Ford *et al.,* 2001). In recent years, it has become evident that low thrombin concentration produces turbid fibrin clot composed of thick, loosely woven fibrin

strands. While higher concentration produces fibrin clot composed of relatively thinner, more tightly packed fibrin strands (Wolberg, 2007). These finding suggests that inhibition of thrombin may only affect fibrin clot structure but not fibrinogen time. Factor XIII (transglutaminase) increases the stability of the fibrin clot (Raut *et al.,* 1994). Hence its decrease concentration may affect fibrin clot structure rather its formation time.

Present study did not reveal significant reduction in platelets. There is evidence of inhibiting platelet function by evening primrose oil (Matsuo *et al.,* 1996), this effect may be due to GLA that stimulates PGE_1 and inhibits thromboxane A_2 synthesis (Cruz *et al.,* 1997).

Hence it may be assumed that decrease platelet count may be due to inhibition affect of evening primrose oil at initiation phase. In response to vascular injury recruitment of platelets and interaction between platelet GPIb-V-IX and vWF takes place (Hamilton, 2008). Hence evening primrose oil may by decreasing coagulation factors produce this effect by inhibiting the above interaction between platelets receptors and coagulation factors V, IX and vWF resulting in platelets inhibition at their initiation phase.

Thrombin and platelets are interdependent; since decrease platelets count by evening primrose oil may leads to decrease number of platelet receptors for thrombin, termed protease-activated receptors (PARs), since thrombin is essentially required for activation of platelets. This suggests that thrombin-induced platelet activation is likely to be as important as platelets availability for thrombus formation in vivo (Hamilton, 2008).

In recent years a dual role of thrombin has been revealed. It is not only involved in blood coagulation, but also associated with inflammatory response, cell-mediated immunity and cell death. This shows a relationship between coagulation and inflammation (Krupiczojc *et al.,* 2008). Since coagulation and inflammation has been reported as biological mediators of cardiovascular disease (Rallidis *et al.,* 2004; Hamer *et al.,* 2008). Furthermore GLA is metabolized to dihomogammalinolenic acid (DGLA), the immediate precursor of prostaglandin E1, an eicosanoid with various physiological functions in the human body with known anti-inflammatory and immunoregulatory properties (Dirks *et al.,* 1998; Peterson *et al.,* 1999; Puri, 2004).

CONCLUSION

It may be concluded that EPO has anticoagulant properties and its anticoagulant activity is supported by its anti-inflammatory effect. These effects along with antiplatelet activity suggest that EPO may be of value in cardiovascular diseases.

REFERENCES

Alcaraz MJ and Jimenez MJ (1989). Anti-inflammatory compound from sideritis javalambrensis n-hexane extract. *Journal of Natural Products*, **52**: 1088-1091.

Alison MJ, Keegan A, Cotter MA and Cameron NE (2002). Effects of diabetes and evening primrose oil treatment on responses of aorta, corpus cavernosum and mesenteric vasculature in rats. *Life Sciences*, **71**: 1863-1877.

Allford SL and Machin SJ (2007). Haemostasis. *Surgery,* **25**: 241-244.

Balasinska B (1998). Hypocholesterolemic effect of dietary evening primrose oil (Oenothera paradoxa) cake extract in rats. *Food Chemistry,* **63**: 453-459.

Brummel KE, Butenas S and Mann KG (1999). An integrated study of Fibrinogen during Blood Coagulation. *J. Biological Chemistry,* **274**: 22862-22870.

Brummel KE, Paradis SG, Butenas S and Mann KG (2002). Thrombin functions during tissue factor-induced blood coagulation. *Blood,* **100**: 148-152.

Burns MM (2000). Alternative medicine: Herbal preparation. *Clin. Ped. Emerg. Med.*, **1**: 186-190.

Cartledge JJ, Eardley Y and Morrison JFB (2000). Impairment of corpus cavernosal smooth muscle relaxation by glycosylated human haemoglobin. *British Journal of Urology International*, **85**: 734-735.

Chan Kung-chi, Yin Mei-chin and Chao Wan-ju (2007). Effect of diallyl trisulfide-rich garlic oil on blood coagulation and plasma activity of anticoagulation factors in rats. *Food and Chemical Toxicology*, **45**: 502-507.

Chandler WL and Velan T (2003). Estimating the rate of thrombin and fibrin generation in vivo during cardiopulmonary bypass. Hemostasis, thrombosis and vascular biology. *Blood,* **101**: 4355-4362.

Colman RW (2006). Are hemostasis and thrombosis two sides of the same coin? *J. Exp. M.,* **203**: 493-495.

Cruz JPDL, Romero MM, Carmona JA and Villalobos MA (1997). Effect of evening primrose oil on platelet aggregation in rabbits fed an atherogenic diet. *Thrombosis Research,* **87**: 141-149.

Di Cera E (2008). Thrombin. Review. *Molecular Aspects of Medicine*, **29**: 203-254.

Dines KC, Cotter MA and Cameron NE (1995). Comparison of the effect of Evening primrose oil and triglycerides containing gamma linolenic acid on nerve conduction and blood flow in diabetic rats. *J. Pharmaccol. Exp. Ther.,* **273**: 49-55.

Dirks J, Aswegen CHV and Plessis DJ (1998). Cytokine levels affected by gamma-linolenic Acid. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, **59**: 273-277.

Fang C, Jiang Z and Tomlinson DR (1997). Expression of constitutive cyclooxygenases (COX.l) in rats with streptozotocin-induced diabetes; effects of treatment

with evening primrose oil or an aldose reductase inhibitor on COX-1 mRNA levels. *Prostaglandins, Leukotrienes and Essential Fatty Acid,* **56**: 157-163.

Fielsend AF and Morison JIL (2000). Climatic conditions during seed growth significantly influence oil content and quality in winter and spring evening primrose crops (oenothera spp.). *Industrial Crops and Products*, **12**: 137-147.

Ford I, Cotter MA, Cameron NE and Greaves M (2001). The effects of treatment with α-lipoic acid or evening primrose oil on vascular haemostatic and lipid risk factors, blood flow, and peripheral nerve conduction in the streptozotocin-diabetic rat. *Metabolism*, **50**: 868-875.

Gage BF, Fihn SD and White RH (2000). Management and dosing of warfarin therapy. *Am. J. Med.,* **109**: 481-488.

Gailani D and Renne T (2007). Intrinsic pathway of coagulation and arterial thrombosis. *Arteriosclerosis Thrombosis and Vascular Biology*, **27**: 2507-2513.

Hamer M and Emmanual S (2008). The accumulative effects of modifiable risk factors on inflammation and Haemostasis. *Brain, Behavior, and Immunity*, **22**: 1041-1043.

Hamilton JR (2008). Protease-activated receptors as targets for antiplatelet therapy. *Blood Reviews*, **06**: 1-5.

Hassig A, Liang WX and Stampfli K (2000). Bronchial asthma: information on phytotherapy with essential fatty acids. *Medical Hypotheses,* **54**: 72-74.

Kamali F, Edwards C, Wood P, Wynne HA and Kesteven P (2001). Temporal variation in plasma vitamin K and lipid concentration and clotting factor activity in humans. *American Journal of Hematology,* **68**: 159-163.

Krupiczojc MA, Scotton CJ and Chambers RC (2008). Coagulation signaling following tissue injury: Focus on the role of factor Xa, *The International Journal of Biochemistry & Cell Biology,* **40**: 1228-1237.

Kuruvilla R, Peterson RG, Kincaid JC and Eichberg J (1998). Evening primrose oil treatment corrects reduced conduction velocity but not depletion of arachidonic acid in nerve from streptozotocin-induced diabetic rats. *Prostaglandins, Leukotrienes and Essential Fatty Acids,* **59**: 195-202.

Lane DA, Philippou H and Huntington JA (2005). Directing Thrombin. *Blood*, **06**: 2605-2612.

Matsuo N, Osada K, Kodama T, Lim BO, Nakao A, Yamada K and Sugano M (1996). Effects of γ-1inolenic acid and its positional isomer pinolenic acid on immune parameters of Brown-Norway rats, Prostaglandins, *Leukotrienes and Essential Fatty Acids.* **55**: 223-229.

Miller GJ (2005). Dietary fatty acids and the haemostatic system. Review: *Atherosclerosis,* **179**: 213-222.

Mitropoulos KA, Esnouf MP and Meade TW (1987). Increased factor VII coagulant activity in the rabbit following diet-induced hypercholesterolaemia. *Atherosclerosis,* **63:** 43-52.

Orfao SC, Jank G, Mottaghy K, Walcher S and Zerz E (2008). Qualitative properties and stabilizability of a model for blood thrombin formation. *Journal of Mathematical Analysis and Applications,* **346**: 218-226.

Peschel W, Dieckmann W, Sonnenschein M and Plescher A (2007). High antioxidant potential of pressing residues from evening primrose in comparison to other oilseed cakes and plant antioxidants. *Industrial Crops and Products,* **25**: 44-54.

Peterson LD, Thies F and Calder PC (1999). Dose-dependent effects of dietary γ-linolenic acid on rat spleen lymphocyte functions. *Prostaglandins, Leukotrienes and Essential Fatty Acids,* **61**: 19-24.

Puri BK (2004). The clinical advantages of cold-pressed non-raffinated evening primrose oil over refined preparations. *Medical Hypotheses*, **62**: 116-118.

Rallidis LS, Georgios P, Papaioannou ML, Liakos GK, Panagiotakos DB, Anastasiadis G and Zampelas A (2004). The effect of diet enriched with α-linolenic acid on soluble cellular adhesion molecules in dyslipidaemic patients. *Atherosclerosis,* **174**: 127-132.

Raut S, Belgrave D, Merton RE and Barrowcliffe TW (2004). Proposed 1st International Standard for Factor XIII, Plasma (02/206). Final report and recommendations. World Health Organization. Expert Committee on Biological Standardarization Geneva, 15 to 19 November 2004. WHO ORGANISATION MONDIALE DE LA SANTE.

Renaud S, Gregor LM, Morazain R, Thevenon C, Benoit C, Dumont E and Mendy F (1982). Comparitive beneficial effects on platelets functions and atherosclerosis of dietary linoleic and γ-linolenic acids in the rabbit, *Atherosclerosi,* **45**: 43-51.

Senanayake Namal SPJ and Shahidi F (2004). Incorporation of docosahexaenoic acid (DHA) into evening primrose oil (Oenothera biennis L.) oil via lipase-catalyzed transesterification. *Food Chemistry,* **85**: 489-496.

Setty AR and Sigal LH (2005). Herbal medications commonly used in the practice of rheumatology: Mechanisms of action, efficacy, and side effects. *Seminars in Arthritis and Rheumatism,* **34**: 773-784.

Shearer MJ (2009). Vitamin K deficiency bleeding (VKDB) in early infancy. *Blood Reviews,* **23**(2): 49-59.

Wolberg AS (2007). Thrombin generation and fibrin clot structure. *Blood Reviews*, **21**: 131-142.

Yuan S, Ferrell C and Chandler WL (2007). Comparing the prothrombin time INR versus the APTT to evaluate the coagulopathy of acute trauma. *Thrombosis Research,* **120**: 29-37.

Zacchigna M, Luca GD, Cateni F and Maurich V (2004). Improvement of warfarin bio pharmaceutics by conjugation with poly (ethylene glycol). *European Journal of Pharmaceutical Sciences*, **23**: 379-384.