Effect of evening primrose oil on primeralary blood coagulation investigation in male rabbits

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الخلاصة

هدفت هذه الدراسة لمعرفة تاثير زيت زهرالربيع على معايير التخثربعد 30 و60 يوم من اعطاء 90 ملغم\كغم من زيت زهرالربيع لذكور الارانب السليمة .التي نتجت عن زيادة بالغة الاهمية في وقت البروثرومبين ووقت البارشيال ثرومبوبلاستين (p<0.01) وعدد الصفيحات الدموية قل بنسبة بالغة الاهمية (p<0.01) في كلا الوقتين بينما تركيز الفايبرنوجين قل بنسبة غير مهمة . هذه التاثيرات ربما تكون نتيجة هبوط في بعض عوامل التخثر.

Abstract

This study was performed to determine the effects of Evening primrose oil (EPO) on haemostatic parameters following 30 and 60 days administration of 90 mg/kg (EPO) to healthy male rabbits. The laboratory resulting in significant increase in Prothrombin time (PT), activated partial thromboplastin time (aPTT) assays ($p<0.01$), platelets count significantly decreased ($p<0.01$) at both times. While fibrinogen concentration are insignificantly decreased. These effects might be due to inactivation or inhibition of factors affecting coagulation.

Introduction

Atherosclerosis is the major cause of morbidity and mortality in the developing and developed countries[1], as it is the most frequent underlying cause of coronary artery disease, carotid artery disease, and peripheral arterial disease which are resulted from superimposed thrombosis[2]. So if thrombosis could be averted, atherosclerosis would be a much more benign disease and rarely fatal.[3]Because that rupture or ulceration of an atherosclerotic plaque may precipitate the growth of platelet and fibrinaceous elements in an already narrowed lumen,[4] growth of the fibrous plaque results in vascular remodeling, progressive luminal narrowing, blood-flow abnormalities, and compromised oxygen supply to the target organs.[5]

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Essential fatty acids are fatty acids that cannot be manufactured within the body and must be supplied by the diet.[6,7]. Evening primrose oil (EPO) provides direct and rappid supply of gamma-linolenic acid (GLA) in disease states where conversion of the dietary precursor linoleic acid to GLA is attenuated[8,9,10] such as cardiovascular diseases[11] and diabetes mellitus[12,13]. GLA (*via* DGLA which is the precursor of the prostaglandin PGH_1, which in turn forms PGE_1 and the thromboxane TXA_1 by the action of COX has anti-inflammatory, vasodilatory, and anti-aggregatory actions.[14] Alternative anticoagulants which target clotting factors, like omega-3 oils from fatty fish or plant oils such as flax or canola oils have proven to be helpful in clinical trials[15].

There are evidences that EPO caused remarkable improvement in clotting time; severity of atherosclerotic lesion as well significantly decreased thrombin induced platelets aggregation[17]. Platelets plays critical role in homeostasis, both for the formation of clot and activation of coagulation proteins[18]. EPO inhibits platelet aggregation in hyperlipidemic rabbits through multiple mechanisms and could be considered as antithrombotic[19]. These evidences show that EPO may have an effective role on haemostatic parameters hence an in vivo study was designed to examine the effect of EPO on prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen time (Fg) and platelet count.

Materials and methods

Drug treatment

EPO was obtained from vitane pharmaceutical inc., California, USA. And was taken from local pharmacy with following composition: Linoleic acid 730 mg, γ-linolenic acid 90 mg, drug were administered through oral route. The normal recommended dose of EPO is one capsule three times daily each capsule contains 1000 mg of EPO.

Animal selection

The study was carried out on twelve domestic healthy rabbits of male sex weighing from 1250-1500 grams. Animals were housed individually in cages, under controlled condition of temperature (23±2°C), and humidity (50-60%). Animals were freely access water.

Experimental design

Animals were divided in two groups, each containing six animals. a group were treated as test animals and were administered 30 mg / kg of the body weight of EPO. While controlled animals were administered water equivalent to the corresponding dose of EPO in mg/kg of the body weight. 3 ml Blood samples were collected three times, once as baseline after two week of adaptation, and the other at 30 days and last one at the end of dosing period i.e. 60 days by heart puncture of the animals.

Hematological examination:

Blood sample collection

Three ml of blood is obtained from direct heart puncture by mean of disposable plastic syringe with wide poor needle, then 1.8ml of blood but in a test tube containing 0.2ml tri-sodium citrate (3.8 %), mixture containing tube are centrifuged at 3000 rpm for 15 minute in bench centrifuge at room temperature to prepare platelet poor plasma for(pt , aptt, and fibrinogen estimation). In EDTA containing tube immediately transferring 1ml of blood (1.2 mg for 1ml blood) for platelet count.[20]

Prothrombin time (pt)

0.1ml of prepared plasma is delivered to another glass tube placed in water bath at 37C˚, and then 0.1ml of thromboplastin is added to the glass tube, then is allowed to warm for 1-3 min , then 0.1ml of pre wormed $cacl_2$ at 37C˚ is added and stopwatch is started to record end point time (clot formation).[20]

Partial thromboplastin time (ptt)

Mixed equal volumes of phospholipid reagent and kaolin suspension were delivered in a glass tube and left in water bath37C˚. In another glass tube 0.1ml of prepared platelet poor plasma is mixed with 0.2ml of thromboplastin- kaolin solution and left for 3 min in 37C˚ water bath with occasional shaking. Then at exactly 3min 0.1ml of pre warmed cacl2 is added to the mixture and stopwatch is started to record end point time (clot formation). [20]

Platelet count

Estimation of platelet count has been carried out using Neubaur improved chamber slide and ammonium oxalate 10 mg/l as a diluents in about 1:20 ratio of dilution.[20]

Fibrinogen Assay

Dilute plasma as 1/10 in dilution buffer. Pre warm the thrombin in water bath at 37C˚. 0.2 ml of dilution incubated for 2 minutes at 37C˚, then mixed with 0.2 ml of thrombin. Simultaneously start a timer and record the clotting time. Fibrinogen concentration calculated according to the calibration curve plotted on a regular graph the clotting time measured for the dilution (1/d) of tested plasma on the Y-axes. Read on the X-axis the corresponding value (a) and calculate the result as follow: Fibrinogen (mg/dl) =F*d/a (F: concentration of fibrinogen in the reference plasma ,d: reciprocal dilution of the tested plasma =10 if diluted 1/10, a: X-axis value read on calibration curve.[21]

Statistical Analysis

All data analyzed by ANOVA followed by a least significant difference (LSD).

Results

In this study there was significant increase in prothrombine time, partial thromboplastin time, in treated groups as compared with the control group, ($p<0.01$), as in table (1) with significantly decrease in platelets count,($p<0.01$), and insignificant decrease in fibrinogen concentration in treated groups as compared with the control group, as in table (2).

Table (1) Effect of evening primrose oil on prothrombin time and activated partial thromboplastin time in compare with control group in male rabbits.

Time(day)	groups	PT in sec.	aPTT in sec.
baseline	EPO 90mg/kg	7.7500±.79183	15.717±2.0904
	control	8.1167±.79352	15.867±1.8683
30day	EPO 90mg/kg	9.9667±.78145*	24.1833±1.70695*
	control	7.9333±.79415	16.1000±1.93804
60day	EPO 90mg/kg	10.650±.7765*	25.317±1.5289*
	control	8.100±.4427	16.233±.7062

*($p<0.01$) The values is Mean± Std. Deviation.

Table (2) Effect of evening primrose oil on platelets count and fibrinogen concentration in compare with control group in male rabbits.

Time(day)	groups	Platelets count (cell/l)	Fibrinogen conc. (mg/dl)
baseline	EPO 90mg/kg	230.33±19.044	214.3333 ±3.88158
	control	227.17±14.743	213.8333 ±6.61564
30day	EPO90 mg/kg	203.00±5.099*	211.16667±9.304121
	control	222.33±15.591	213.00000±3.741657
60day	EPO 90mg/kg	160.83±27.088*	205.167±6.1779
	control	218.50±14.612	214.000±8.4617

($p<0.01$) * The values is Mean± Std. Deviation.

Discussion

As coagulation tests like PT, aPTT, Fg are often used to assess variation in coagulation factors[22].So they can better monitor the influence of evening primrose oil on blood coagulation process. Thus we evaluate them and the effect of evening primrose oil on platelets count was also evaluated.

Prolongation in PT may be due to decrease in coagulation factors like, VII, X and V involved in extrinsic pathway. While prolongation of aPTT may be due to decrease in coagulation factors such as VIII, IX, XI, XII.[23] involve in intrinsic pathway. Present study reveals that evening primrose oil caused significant increase in PT and aPTT. Which resulted from that evening primrose oil has hypocholesterolemic effect,[24] and the decrease in the concentration of cholesterol may decrease the concentration of coagulation factors[16].As in hypercholesterolemia, increased catabolic rate of prothrombin will stimulate hepatic synthesis of clotting factors, resulting in increased plasma concentration of clotting factors.[25] Also decrease absorption of lipids from gastrointestinal tract results in vitamin K deficiency,[26] Vitamin K deficiency cause decrease in synthesis of factors II, VII, IX and X in liver that in turn results in hypocoagulable state.[27,28] So reduced total cholesterol by evening primrose oil may leads to vitamin K deficiency, which ultimately reduces synthesis of clotting factors resulting in prolongation of the PT. Study that asses the anticoagulant effect of EPO agree with this result and found the same prolongation in PT and PTT

also found that EPO produces it's effect in a way similar to warfarin as both of them prolong the pt of the same levels. Also the study suggest that evening primrose oil may produces anticoagulant effect in the manner similar to heparin as both of them prolonged aPTT.[16]

Present study reveals insignificant decrease in fibrinogen level. There is evidence that increased plasma fibrinogen level has been recognized as an independent risk factor for vascular diseases.[29] As 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.[30] Factor XIII (transglutaminase) increases the stability of the fibrin clot.[31] Inflammation and platelet aggregation will increase fibrinogen production in the liver. On the other hand, Fibrinogen level increases in response to interleukins 1 and 6 (cytokines produced in arterial disorders).[32] GLA has anti-inflammatory and immune-regulatory properties, [33,34] so fibrinogen production in the liver will be decreased. Study that asses the anticoagulant effect of EPO found that fibrinogen time are insignificantly decreased, as that decreased factor XIII concentration and thrombin concentration may affect fibrin clot structure rather its formation time.[16]

Present study reveals significant reduction in platelets count. There is evidence of inhibiting platelet function by evening primrose oil, [35] this effect may be due to GLA that stimulates PGE1 and inhibits thromboxane A2 synthesis.[19] This results are disagree with other study that suggest that decrease platelet count only after 60 days with same dose (90mg/dl) and at 30 days with higher dose only, and suggest that decrease platelet count may be due to inhibition effect of evening primrose oil at initiation phase.[19]

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. In response to vascular injury recruitment of platelets and interaction between platelet GPIb-V-IX and vWF takes place, [36]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.[36] There is a relationship between coagulation and inflammation.[27] Since coagulation and inflammation has been reported as biological mediators of cardiovascular disease.[37, 38] So EPO may be of value in cardiovascular diseases, as it has anticoagulant properties that is supported by its anti-inflammatory effect, along with it's anti platelet activity.[16]

Conclusion

EPO has anticoagulant effect that may be as effective as other anticoagulant and anti platelets drugs that it may interact with these drugs. In addition to that it can be used as preventive measures of atherosclerosis.

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