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Original Article

Evening primrose oil ameliorates platelet aggregation and improves cardiac recovery in myocardial-infarct hypercholesterolemic rats

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Abstract: Omega-6 polyunsaturated fatty acids (n-6 PUFA) are well known for their role in cardiovascular disease (CVD). We proposed that Evening prime rose oil (EPO) can improve the outcome of a heart with myocardial infarction (MI) in the presence of diet-induced hyperaggregability. This study was designed to examine its cholesterol lowering, antithrombotic and anti-inflammatory effects. High fat diet was administered for 4 weeks then MI was induced by isoproterenol (85 mg/kg/s.c./24 h). Treatment with EPO (5 or 10 gm/kg/day) for 6 weeks improved the electrocardiographic pattern, serum lipid profile, cardiac biomarkers as well as Platelet aggregation percent. We reported decreased serum level of TNF-α, IL-6 and COX-2 with attenuation of TNF-α and TGF-β in the cardiac homogenate. Moreover, histopathology revealed marked amelioration. Finally, we provide evidence that EPO improve cardiac recovery in hypercholesterolemic myocardial infarct rats. These effects are attributed to direct hypocholesterolemic effect and indirect effect on the synthesis of eicosanoids (prostaglandins, cytokines).

Keywords: Hypercholesterolemia, high fat diet, myocardial infarction, evening primrose oil

Introduction

The relation of dietary fat to risk of cardiovascular disease (CVD) has been studied extensively using different approaches. Elevated levels of serum total or low-density lipoprotein cholesterol have been related to increased risk of CVD [1]. Changes in plasma lipoproteins affecting platelet function have been found in hyperlipidemia [2]. High LDL-C levels could trigger spontaneous platelet aggregation [3] via intraplatelet calcium mobilization [4] and increasing platelet function and sensitivity [5, 6]. The increase in transfatty acids in our diet interfere with the desaturation and elongation of omega-6 fatty acids (n-6 PUFA) [7]. n-6 PUFAs have a critical role in CVD risk reduction mainly by lowering LDL-C levels [8]. In addition to blood pressure, inflammatory markers, and hemostatic parameters reduction [9]. About 10% of total energy from n-6 PUFA appears justified for the prevention of ischemic heart disease according to the American heart association recommendation [10-12] mainly through reducing inflammatory states and preventing atherogenesis, even in a pro-inflammatory condition [13-15]. Cyclooxygenase (COX) is considered as one of the major enzyme families that catalyze the rate-limiting step in the formation of prostaglandins (PGs) and (TXA_2) [16]. COX-2 is an inducible form that is expressed during inflammation as a result of stimulation by cytokines, nitric oxide and growth factors [17, 18]. A dietary supplement that proved useful in cardiovascular protection is EPO. EPO supplies (LA) and γ-linolenic acid (GLA), a product that increases the production of series 1 prostaglandins. These in turn have antithrombotic activity, enhances smooth muscle relaxation and vasodilation. In accordance, EPO reduces platelet hyperaggregability in rabbits fed an atherogenic diet [19]. Based on above information, we proposed that EPO can improve the outcome of a heart with myocardial infarction in the presence of diet-induced hyperaggregability. Therefore, this study was designed to examine the choles-

terol lowering, antithrombotic and anti-inflammatory effects of EPO in the current model of combined hypercholesterolemic myocardial infarct rats.

Materials and methods

Animals

All animal care and experimental procedures were approved by the Institutional Laboratory Animal Care and Use Committee in The Faculty of medicine, Suez Canal University. Sixty adult male Wistar rats (with a weight range of 280-300 g) were purchased from the Egyptian Organization for Biological Products and Vaccines (Cairo, Egypt). Before experiments, animals were allowed to adapt to the new environment for one week. Rats were housed in groups of four in well-ventilated clean stainless steel cages under controlled environmental conditions (21-25°C) and normal dark/light cycle). Food and water were provided *ad libitum*.

Drugs and chemicals

Cholesterol was obtained from GFS chemicals & reagents (Texas, USA). Bile salts were purchased from SAS Chemicals Co. (Mumbai, India). Pork fat was purchased from the market and used to prepare the high fat diet (HFD). Isoproterenol hydrochloride was purchased from Sigma-Aldrich (MO, USA) and was freshly prepared by dissolution in sterile saline. EPO containing 9% GLA was purchased from Now Foods Co. (Illinois, USA). All the other chemicals were of analytical grade from commercial suppliers.

Experimental design

Rats were randomly divided into the following groups; 10 rats each.

Group I: served as a normal group and was maintained on normal palatable diet (NPD) throughout the experiment (10 weeks).

The remaining four groups were maintained on a HFD containing 87.7% standard diet (w/w), 10% pork fat (w/w), 2% cholesterol (w/w) and 0.3% bile salt (w/w) throughout the experiment [11].

Group II (HFD control group): rats were injected with two successive doses of saline (vehicle of isoproterenol, 2 ml/kg/day, s.c.) at the end of week 4.

Group III (HFD-isoproterenol group): rats were injected with two successive doses of isoproterenol (85 mg/kg/day, s.c.) at the end of week 4 for induction of acute myocardial infarction (AMI) [20].

Group IV and V: rats were injected with isoproterenol at the same above-mentioned schedule at week 4. At this time, rats of group IV and V started a therapeutic regimen with EPO (5 or 10 gm/kg/day), respectively that continued for six weeks. In general, EPO was administered daily by gastric gavage for six weeks.

Another group of rats (NPD-isoproterenol group) were fed NPD for four weeks followed by 2 injections of isoproterenol (85 mg/kg/day) followed by six weeks of NPD feeding; this group was used to determine the difference in sensitivity to isoproterenol between rats fed with a NPD and those fed a HFD (group III). However, this group was not demonstrated in illustrations to focus on the effect of EPO on the measured parameters compared to HFD-fed rats.

ECG monitoring

At the end of week 4 and 10, all rats were anesthetized using thiopental sodium (50 mg/kg i.p.) [21]. Then, ECG was recorded using research Biopac data acquisition device mp150. The electrodes were placed subcutaneously in the gently extended limbs of the supine animal. ECG amplifier was adjusted to channel (I). Data were obtained from lead II. V+ needle was inserted to the left leg of the rat and V- to the right arm and ground to the right leg of the rat. For each ECG tracing ST segment displacement was recorded and pathological Q wave was monitored [22].

Blood sampling

After ECG recording, the body weight of rats was registered. Then, blood samples were collected by cardiac puncture. Half of this amount was used for platelet aggregation assay and the other half was processed by centrifugation at 2000 × *g* for 15 min within 30 min of collection. After that, serum samples were separat-

ed, collected in clean tubes and stored at -80°C until used for different assays. A midline incision were made and the hearts were removed, trimmed of connecting tissue and rinsed with ice-cold phosphate buffered saline. The heart as well as the left ventricles were weighed to calculate the ratio of the heart weight to left ventricular weight to the body weight. These ratios were determined as indices for cardiac hypertrophy. Ventricular myocardium was divided into two parts. The first part (0.2 g) was kept at -80°C), whereas, the remaining parts were fixed using 10% phosphate-buffered paraformaldehyde solution (PH1/4 7.4) for 18 h and then embedded with paraffin. After that, all tissues were re-sectioned at 4 mm thickness at the cardiac apex and left to dry over night at 37°C. Sections were then subjected to deparaffinization, rehydration and prepared for histopathological staining.

Determination of serum cardiac biomarkers

Serum lactate dehydrogenase activity was estimated using an enzymatic kit [23]. Creatine phosphokinase activity was estimated according to the method developed previously [24]. All the previous methods were performed using an ultraviolet- visible spectrophotometer (UV-160 1PC Schimadzu, Japan). The activity of serum creatine kinase-MB isoenzyme was measured using immune- inhibition method [25]. All the previous methods were performed using commercial kits purchased from Stanbios (Texas, USA). All procedures followed the manufacturer's instructions.

Platelet aggregation assay

Half the amount of blood was collected from anesthetized rats by cardiac puncture was processed into 3.8% sodium citrate solution (9:1 V/V). To prepare platelet-rich plasma (PRP), samples were centrifuged immediately at 160 × *g* for 15 min at room temperature, then PRP was transferred into plastic tubes and the remaining blood was centrifuged at 3000 × *g* for 10 min to obtain platelet-poor-plasma (PPP). Platelet count in PRP was adjusted to (5×10^8/ml) with PPP. Platelet aggregation was measured by addition of 5 µg/ml collagen (Chrono-Log corp.) using a dual channel aggregometer (Clot 2, SEAC- Radium Company, Italy). Results were expressed as a percentage of aggregation, extent of aggregation was estimated by change in light transmission [12].

Measurement of serum lipid profile

Serum triglycerides (TGs), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) were assayed colorimetrically using commercially available kits (Biodignostic®, Cairo, Egypt). The absorbance was measured using a UV-visible spectrophotometer (UV-1601-PC, Shimadzu, Japan).

Determination of serum levels of TNF-α, TGF-β, IL-2 and COX-2 using ELISA kits

Serum levels of tumor necrosis factor-α (TNF-α) and transforming growth factor-β (TGF-β) were determined, as well as, serum levels of interlukien-2 (IL-2) and cyclooxygenase enzyme-2 (COX-2) were assessed. Rat ELISA kits (Ray Biotech Inc., Norcross, USA) were used according to the instructions of the manufacturer using an automated ELISA reader (Europe S.A. Belgium).

Determination of cardiac levels of TNF-α and TGF-β using ELISA kits

Each frozen cardiac sample (0.2 g) were homogenized using a teflon homogenizer (Glascol homogenizer system, Vernon hills, USA). Homogenization was carried out in 1 ml phosphate-buffered saline solution that contains a protease inhibitor and centrifuged at 3000 g for 15 min at 4 C. The supernatant was collected and divided into 2 dry tubes. ELISA kits for tumor necrosis factor-α (TNF-α) (Ray Biotech Inc., Norcross, USA), transforming growth factor-β (TGF-β) (Boster Biological technology, Wuhan, China) were used for determination of tissue level of these markers. The assays were recarried out following the instructions of the manufacturer using an automated ELISA reader (Europe S.A., Belgium).

Histopathological examination of the heart

The prepared sections were assessed for myocardial morphology by hematoxylin and eosin staining was done under a light microscope (Olympus, CX21, Japan). Also, the separate set of paraffin-embedded tissue sections at 4 µm were stained using Masson's trichrome (Sigma-

Table 1. Effect of treatment with EPO (5 or 10 gm/kg) for six weeks on % survival and ECG findings in HFD-myocardial infarct rats

Groups	Percentage survival	ST segment elevation (mm)	% Pathological Q waves
NPD	100	0	0
HFD	100	0	0
HFD + Isoproterenol	50*,†	3.2 ± 0.31*,†	92.4 ± 5.1*,†
HFD + Isoproterenol + EPO (5 gm/kg)	50*,†	1.8 ± 0.15*,†,‡	45 ± 2.9*,†,‡
HFD + Isoproterenol + EPO (10 gm/kg)	40*,†	1.1 ± 0.08*,†,‡	26 ± 1.8$^{*,\dagger,\ddagger,\$}$

Rats were fed with a HFD for four weeks and then injected with two doses of isoproterenol (85 mg/kg/24 h, s.c.) to induce acute myocardial infarction. Rats were treated with EPO (5 or 10 gm/kg/rat/day) for another six weeks. NPD: normal palatable diet, HFD: high-fat diet, ECG: electrocardiography, ST segment elevation is represented by the number of small squares shifted from the base line. Values are expressed as mean ± S.E.M. and analyzed using one-way ANOVA followed by the Bonferroni's test for multiple comparisons. *Compared to NPD group at $P < 0.05$. †Compared to HFD group at $P < 0.05$. ‡Compared to HFD + isoproterenol group at $P < 0.05$. $^{\$}$Compared to HFD + isoproterenol + EPO (5 gm/kg) group at $P < 0.05$, n = 6-10.

Table 2. Effect of treatment with EPO (5 or 10 gm/kg) for six weeks on body weight and heart weight ratios in HFD myocardial infarct rats

Groups	Baseline Bwt (g)	Final Bwt (g)	LV wt to Ht W Ratio (g/g)	LV wt to Bwt ratio (g/g xE-03)
NPD	230.2 ± 4.2	288.4 ± 1.8	0.315 ± 0.001	3.05 ± 0.17
HFD	236.2 ± 4.6	364.43 ± 5.2*	0.341 ± 0.04	3.45 ± 0.19
HFD + Isoproterenol	212.3 ± 4.8	359.4 ± 4.3*	0.403 ± 0.01*,†	3.98 ± 0.20*
HFD + Isoproterenol + EPO (5 gm/kg)	227.1 ± 5.4	320.3 ± 1.9†,‡	0.352 ± 0.02	3.51 ± 0.22
HFD + Isoproterenol + EPO (10 gm/kg)	239.7 ± 4.4	324.5 ± 2.6†,‡	0.345 ± 0.006	3.16 ± 0.17†

Rats were fed with a HFD for four weeks and then injected with two doses of isoproterenol (85 mg/kg/24 h, s.c.) to induce acute myocardial infarction. Rats were treated with EPO (5 or 10 gm/kg/rat/day) for another six weeks. NPD: normal palatable diet, HFD: high-fat diet, Bwt: body weight, LV: left ventricle, Ht wt: heart weight. Values are expressed as mean ± S.E.M. and analyzed using one-way ANOVA followed by the Bonferroni's test for multiple comparisons. *Compared to NPD group at $P < 0.05$. †Compared to HFD group at $P < 0.05$. ‡Compared to HFD + isoproterenol group at $P < 0.05$. n = 6-10.

Aldrich Co., St. Louis, MO, USA). Tissues stained with Masson's Trichome were quantified sterologically on ten regularly spaced sections covering the entire surface of the heart. Each section was first viewed at low power (× 10 magnification) whereas; the scoring was performed at high power (× 40 magnification). Myocardial fibrosis and necrosis was evaluated in each section of the heart tissue using a morphometric point counting procedure [26]. Two pathologists grade the histopathological changes as 1, 2, 3, 4, for low, moderate, high and intensive pathological changes, respectively.

Statistical analysis

All results are expressed as mean ± standard error of the mean. Results were assessed by one-way repeated measures analysis of variance (ANOVA) followed by Bonferroni's *post-hoc* test. The difference between the HFD-isoproterenol rats and NPD-isoproterenol rats was determined using unpaired student t test. Data were analyzed using The Statistical Package for the Social Sciences, version 17 (SPSS Software, SPSS Inc., Chicago, USA). Differences between means were considered to be statistically significant when $P < 0.05$.

Results

Effect of treatment with EPO on percent survival and ECG pattern

In the present study, changes in ST segment elevation and % abnormal Q waves as well as % survival are shown in **Table 1**. NPD and HFD groups showed normal ECG pattern whereas HFD + isoproterenol treated group showed an elevated ST segment as well as significant increase in the % abnormal Q waves when compared to either NPD or HFD groups ($P < 0.05$). Oral treatment with EPO (5 and 10 gm/kg/rat) in HFD + isoproterenol treated rats induced a

Table 3. Effect of treatment with EPO (5 or 10 gm) for six weeks on serum lipid profile in HFD myocardial infarct rats

Groups	*Total cholesterol (mg/dl)*	*Triglycerides (mg/dl)*	*LDL-C (mg/dl)*	*HDL-C (mg/dl)*
NPD	213.2 ± 17.2	21.73 ± 1.8	121.2 ± 9.2	98.3 ± 8.7
HFD	365.2 ± 22.6*	64.43 ± 5.2*	239.5 ± 18.4*	61.4 ± 5.9*
HFD + Isoproterenol	358.3 ± 26.8*	59.4 ± 4.3*	247.1 ± 17.6*	54.3 ± 4.2*
HFD + Isoproterenol + EPO (5 gm/kg)	263.1 ± 21.4†,‡	27.3 ± 1.9†,‡	232.9 ± 19.2*	67.1 ± 4.8*
HFD + Isoproterenol + EPO (10 gm/kg)	273.7 ± 20.4†,‡	24.5 ± 2.6†,‡	189.5 ± 21.6†,‡	62.6 ± 5.7*

Rats were fed with a HFD for four weeks and then injected with two doses of isoproterenol (85 mg/kg/24 h, s.c.) to induce acute myocardial infarction. Rats were treated with EPO (5 or 10 gm/kg/day) for another six weeks. NPD: normal palatable diet, HFD: high-fat diet, LDL-C: low density lipoprotein, HDL-C: high density lipoprotein. Values are expressed as mean ± S.E.M. and analyzed using one-way ANOVA followed by the Bonferroni's test for multiple comparisons. *Compared to NPD group at $P < 0.05$. †Compared to HFD group at $P < 0.05$. ‡Compared to HFD + isoproterenol group at $P < 0.05$. n = 6-10.

Table 4. Effect of treatment with EPO (5 or 10 gm/kg) for six weeks on serum LDH activity, CK activity and CK-MB activity in HFD myocardial infarct rats

Groups	LDH (U/L)	CK (U/L)	CK-MB (U/L)	Cardiac CK-MB index (%)
NPD	582.1 ± 45.3	276.5 ± 19.2	54.2 ± 3.2	19.5 ± 1.6
HFD	573.2 ± 55.2	255.3 ± 21.3	48.3 ± 3.7	18.9 ± 1.4
HFD + Isoproterenol	692.7 ± 41.3*,†	487.5 ± 38.5*,†	178.9 ± 25.5*,†	36.7 ± 4.3*,†
HFD + Isoproterenol + EPO (5 gm/kg)	643.3 ± 58.2*,†,‡	396.5 ± 34.2*,†,‡	162.3 ± 17.2*,†	40.9 ± 4.1*,†
HFD + Isoproterenol + EPO (10 gm/kg)	611.6 ± 53.1†,‡,$	355.4 ± 31.7*,†,‡	103.7 ± 9.4*,†,‡,$	29.4 ± 3.2*,†,‡,$

Rats were fed with a HFD for six weeks and then treated with two doses of isoproterenol (85 mg/kg/24 h, s.c.) to induce acute myocardial infarction. NPD: normal palatable diet, HFD: high fat diet, LDH: lactate dehydrogenase, CK: creatinine kinase, CK-MB: creatinine kinase isoenzyme MB. Values are expressed as mean ± S.E.M. and analyzed using one-way ANOVA followed by the Bonferroni's test for multiple comparisons. *Compared to NPD group at $P < 0.05$. †Compared to HFD group at $P < 0.05$. ‡Compared to HFD + isoproterenol group at $P < 0.05$. $Compared to HFD + isoproterenol + EPO (5 gm/kg/rat) group at $P < 0.05$, *n* = 6-10.

significant decrease in ST segment as well % Q waves when compared to HFD + isoproterenol treated group ($P < 0.05$). The percentage of survival decreased significantly in the HFD + isoproterenol group when compared to the NPD or HFD groups ($P < 0.05$). Even when the dose of EPO was increased to 10 gm/kg the percentage of survival was decreased significantly when compared to the NPD and HFD groups ($P < 0.05$).

Effect of treatment with EPO (5 or 10 gm/kg/rat) on LV weight, Ht weight and body weight

In the current study, treatment with EPO (5 or 10 gm/kg/rat) for 6 weeks significantly ameliorated the LV to BWt ratio compared to HFD + isoproterenol group. Further, EPO (10 gm/kg/rat) decreased the Ht W to BWt ratio compared to HFD + isoproterenol group. Remarkably, the effect of the high dose of EPO (10 gm/kg) on the LV to Bwt ratio and Ht Wt to Bwt ratio was different from that produced by the low dose (5 gm/kg/rat) ($P < 0.05$, **Table 2**).

Effect of treatment with EPO (5 or 10 gm/kg/rat) for six weeks on serum lipid profile in HFD myocardial infarct rats

It is clear that there is a significant increase in total cholesterol level when comparing NPD group with the other 4 groups ($P < 0.05$, **Table 3**). Remarkably, total cholesterol and triglycerides level increased in the HFD and the HFD + Isoproterenol in comparison to the NPD ($P < 0.05$, **Table 3**). The effect of the treatment with EPO (5 or 10 gm/kg) on total cholesterol and triglycerides level was significant on comparison with either the HFD or HFD + isoproterenol group ($P < 0.05$, **Table 3**). There is also a significant increase in LDL-C when comparing HFD or HFD + isoproterenol group or even the 5 gm/kg

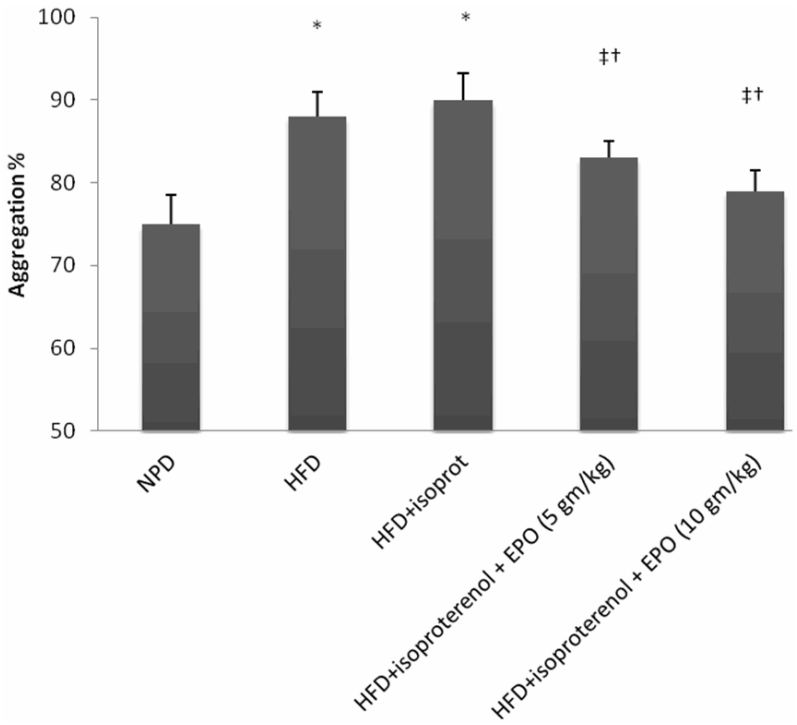

Figure 1. Platelet aggregation in the experimental groups. NPD: normal palatable diet, HFD: high-fat diet, EPO: evening primrose oil. Rats were injected twice with isoproterenol (85 mg/kg, s.c.) to induce acute myocardial infarction. Values are expressed as mean ± S.E.M. and analyzed using one-way ANOVA followed by the Bonferroni's test for multiple comparisons. *Compared to NPD group at $P < 0.05$. †Compared to HFD group at $P < 0.05$. ‡Compared to HFD + isoproterenol group at $P < 0.05$. $Compared to HFD + isoproterenol + EPO (5 gm/kg) group at $P < 0.05$, n = 4-10.

EPO treated group with NPD group. Fortunately, there is a significant decrease in the LDL-C level in the 10 gm/kg EPO group when comparing with HFD and HFD + isoproterenol groups ($P < 0.05$, **Table 3**). In the current study, HDL-C level was decreased significantly when comparing the NPD group to the remaining four groups ($P < 0.05$, **Table 3**).

Effect of treatment with EPO (5 or 10 gm/kg) for six weeks on cardiac enzymes in HFD myocardial infarct rats

Increased levels of cardiac markers such as LDH, CK, CK-MB as well as cardiac index were observed in the HFD + isoproterenol as well as the 5 gm EPO treated groups on comparing with either NPD or HFD groups. The HFD + isoproterenol + EPO (5 gm/kg) group showed significant amelioration in the levels of LDH as well as CK in comparison with HFD + isoproterenol group. A remarkable decrease in all cardiac markers were observed in the HFD + isoproterenol + 10 gm EPO in comparison to either HFD or HFD + Isoproterenol group. Moreover, High dose treated EPO group showed a significant decrease in all LDH, CK-MB and cardiac index on comparison with low dose treated EPO group ($P < 0.05$, **Table 4**).

Effect of treatment with EPO (5 or 10 gm/kg) for six weeks on the percentage of platelet aggregation

It was evident that the platelet aggregation percentage increased significantly on comparing the HFD + isoproterenol group as well HFD group with the NPD. But the 2 doses of EPO treatment revealed a significant decrease in the platelet aggregation percentage when compared to either HFD or HFD + isoproterenol groups ($P < 0.05$, **Figure 1**).

Effect of treatment with EPO (5 or 10 gm/kg/rat) for six weeks on serum level of TGF-β1, TNF-α, IL-6 and COX-2 and cardiac tissue level of TGF-β1 and TNF-α in the experimental groups

The serum level of TNF-α, IL-6, TGF-β1 and COX-2 were increased significantly in the HFD + isoproterenol group in comparison to the NPD and the HFD. Also, serum level of TNF-α, IL-6 and COX-2 were decreased significantly in the HFD + isoproterenol + EPO 5 gm/kg and 10 gm/kg groups in comparison to the HFD + isoproterenol group. Moreover, serum level of TNF-α was decreased significantly in the HFD + isoproterenol + EPO 10 gm/kg group in comparison to the HFD + isoproterenol + EPO 5 gm/kg group ($P < 0.05$, **Figure 2**). In addition, EPO (5 or 10) decreased the level of TNF-α in the cardiac homogenate significantly when comparing with the HFD + isoproterenol group ($P < 0.05$, **Figure 3**).

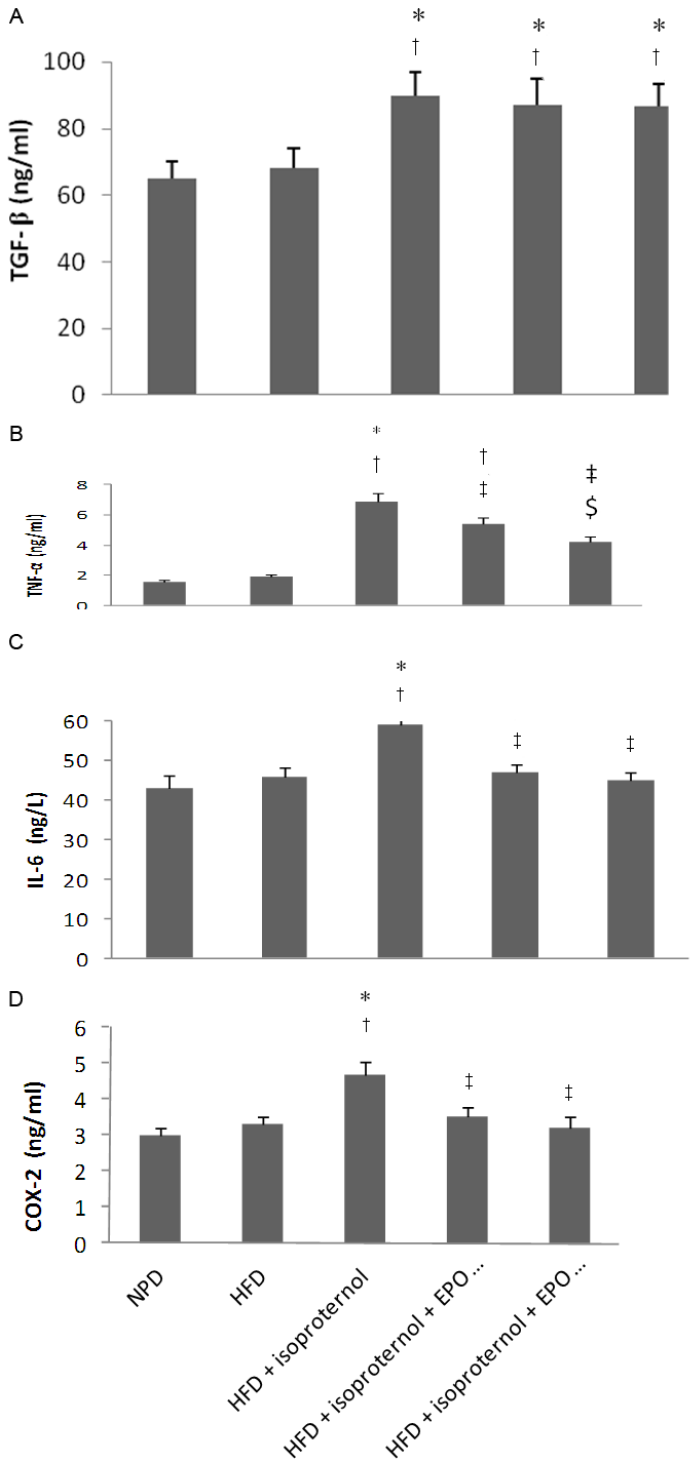

Figure 2. Serum level of TGF-β1 (A), TNF-α (B), IL-6 (C), and COX-2 (D) in the experimental groups. NPD: normal palatable diet, HFD: high-fat diet, EPO: evening primrose oil. Rats were injected twice with isoproterenol (85 mg/kg, s.c.) to induce acute myocardial infarction. Values are expressed as mean ± S.E.M. and analyzed using one-way ANOVA followed by the Bonferroni's test for multiple comparisons. *Compared to NPD group at $P < 0.05$. †Compared to HFD group at $P < 0.05$. ‡Compared to HFD + isoproterenol group at $P < 0.05$. $Compared to HFD + isoproterenol + EPO (5 gm/kg) group at $P < 0.05$, n = 4-10.

Effect of treatment with EPO on Platelet aggregation % when correlated to LDL-C, IL-6 and COX-2 respectively

Person correlation coefficient derived from the linear regression equation described a strong correlation between Platelet aggregation % and LDL-c. Moreover, there is a positive correlation between platelet aggregation % and IL-6 as well as Cox-2 serum levels (**Figure 4**).

Histopathological examination of the cardiac tissues

The myocardial fibers were arranged regularly with clear striations with no evident inflammation, degeneration, necrosis or even fibrosis in both NPD and HFD in contrast to isoproterenol + HFD group (**Figure 5**). The 2 doses of EPO (5 and 10 gm/kg/rat) significantly ameliorated the widespread subendocardial necrosis, hypertrophia and fibroplastic hyperplasia which appeared apparent in the HFD + isoproterenol group (**Figure 5**). A significant difference in the mean fibrosis grade was observed on comparing the isoproterenol treated groups with either NPD or HFD. Whereas the two doses of EPO significantly decreased the mean fibrosis grade ($P < 0.05$, **Table 5**).

Discussion

In this study, our combined hypercholesterolemic myocardial infarct rat model was treated with EPO (5 or 10 gm/kg/rat). Hence, the rats were injected with isoproterenol on two subsequent days to induce myocardial ischemia with a concomitant HFD to ensure the presence of thrombotic, hyperlipidemic as well as hyperaggregable condition all through the study.

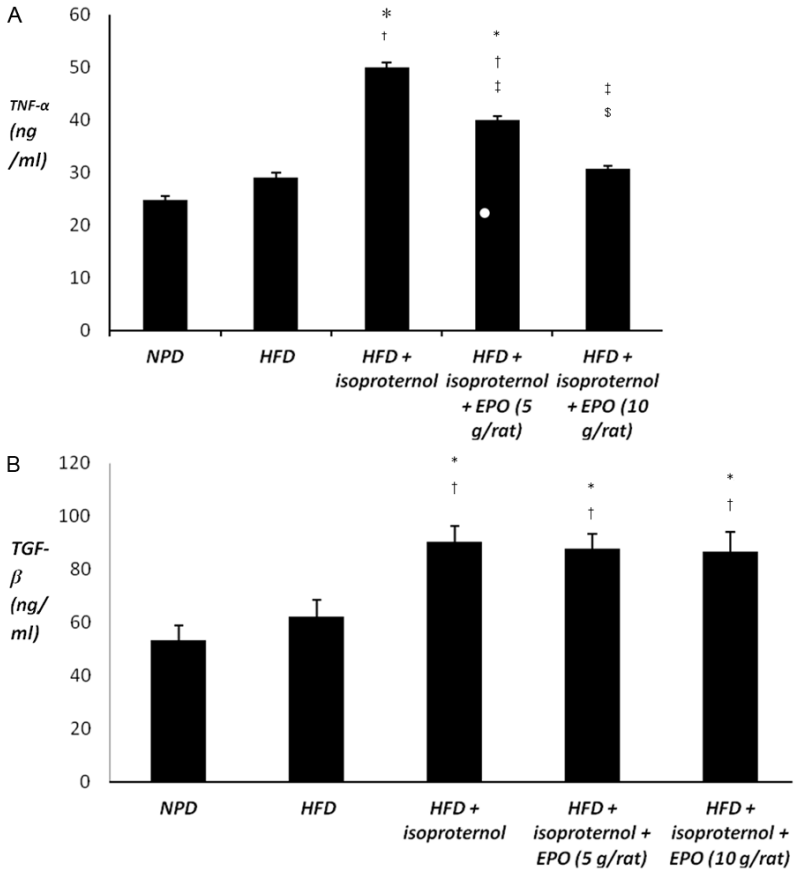

Figure 3. The level of TGF-β1 (A), TNF-α (B) in the cardiac homogenate of the experimental groups. NPD: normal palatable diet, HFD: high-fat diet, EPO: evening primrose oil. Rats were injected twice with isoproterenol (85 mg/kg, s.c.) to induce acute myocardial infarction. Values are expressed as mean ± S.E.M. and analyzed using one-way ANOVA followed by the Bonferroni's test for multiple comparisons. *Compared to NPD group at $P < 0.05$. †Compared to HFD group at $P < 0.05$. ‡Compared to HFD + isoproterenol group at $P < 0.05$. $Compared to HFD + isoproterenol + EPO (5 gm/kg) group at $P < 0.05$, n = 4-10.

EPO is rich in n-6 essential fatty acids, including (LA) and (GLA) in high levels [27, 28]. GLA represents 7-14% of total fatty acids [29] and needed to increase the tissue content of GLA [30]. So, we selected EPO as a natural source to increase the GLA content as well as LA in the tissue. Isoproterenol induced significant ECG changes in HFD group when compared to either the NPD or HFD rats; this may be attributed to the reduced mechanical capacity of the ventricles. The ST-segment elevation is the most sensitive parameter for MI as it reflects myocardial necrosis as well, the parallel loss of the cell membrane integrity in the injured myocardium [31]. Q wave reflects the extent of myocardial damage present in the isoproterenol treated group. The generation of highly cytotoxic free radicals with the subsequent disturbance in the physiological balance between production of free radicals and the antioxidant defense mechanism [32, 33] have been implicated as an important possibility in the loss of myocardial membrane integrity as well as function. Administration of isoproterenol generates the free radicals in the myocardium through oxidative stress with subsequent loss of cell membranes leading to myocardial necrosis [34].

Oral treatment with EPO (5 or 10 gm/kg/rat) significantly improved the pathological alterations. EPO may counteract the postulated oxidative stress hypothesis by the fact that GLA is rapidly elongated to DGLA then converted to arachidonic acid (AA) very slowly, approximately 0.2% [35-37]. Moreover, DGLA in the macrophages converted into PGE1 by the COX activity [38] this reduces the oxidative stress [30]. Excess DGLA inhibits the synthesis of AA-derived eicosanoids [38] so it may reduce the inflammatory condition within the ischemic heart.

Treatment with EPO (5 or 10 gm/kg/rat) significantly decreased the serum levels of cardiac markers revealing an ameliorating effect on the myocardium. Isoproterenol is cardiotoxic agent due to its ability to destroy myocardial cells [39]. This amelioration may be due to restricting the leakage of these enzymes from the myocardium. EPO resulted in the accumulation of DGLA in tissue phospholipids [38, 40, 41]. GLA enhances the integrity and fluidity of the membranes [41]. Collectively, this reflects suppression of membrane damage. The previous reported amelioration in cardiovascular mechanical and biochemical parameters in our study

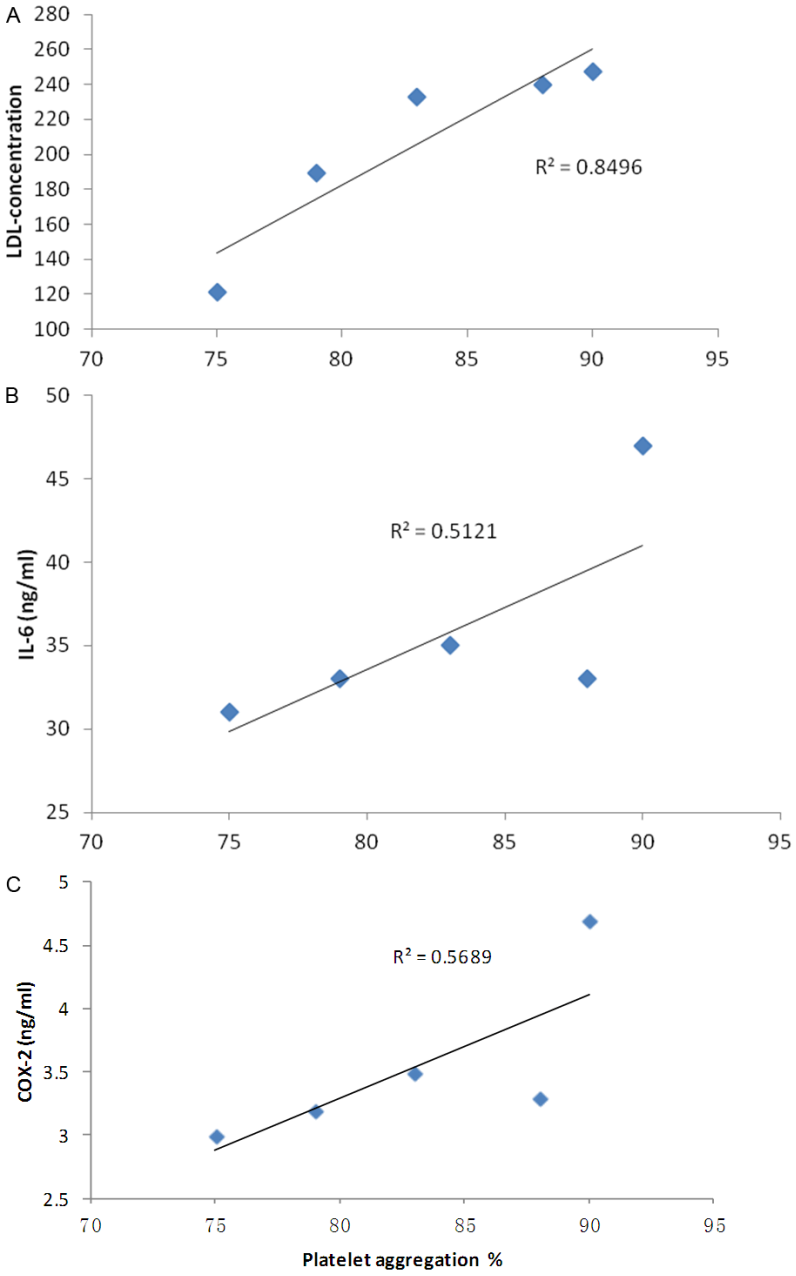

Figure 4. Linear regression lines correlate Platelet aggregation % and LDL-c (A), IL-6 concentration (B), COX-2 concentration (C) in the experimental groups. The correlation coefficient (r^2) indicates positive correlation between platelet aggregation % and the different parameters.

came on line with the results of some studies [42-44].

In our study, feeding with HFD led to significant mixed hyperlipemia: hypercholesterolemia and hypertriglyceridemia. The total cholesterol level showed a significant reduction and so the triglycerides did after the treatment with both doses of EPO. We found that LDL-C level was decreased significantly in EPO 10 gm/kg/rat treated group. These findings were similar to other studies [45-49]. This may be explained by the findings that PUFAs are potent inhibitors of 3-hydroxy 3-methyl glutaryl coenzyme A reductase (HMG-CoA reductase) enzyme [46, 50] which is the rate limiting step in the mevalonate pathway for cholesterol biosynthesis [51]. Its action mimics those of statins and this could be one mechanism by which they lower cholesterol levels.

Platelet aggregation is one of the main determining factors in MI prognosis especially in concomitance with HFD. Our findings showed that platelet hyperactivity caused by feeding with a HFD was reduced when EPO was added to the diet. Platelet hyperaggregability in hyperlipemic states has been documented in several studies [52, 53] by increased thromboxane synthesis [52], increased the production of PGE-1 or increased oxidized LDL [2, 53]; the latter mechanism may be applied to our study as LDL levels were elevated as shown previously. So, EPO may inhibit platelet hyperaggregability by reducing hyperlipemia [19, 54]. To test the influence of EPO consumption on inflammation, we measured local and plasma levels of TNF-α and IL-6. TNF-α plays a key role in activation of other inflammatory cytokines. TGF-β as anti-inflammatory marker was also monitored. We found that the local and plasma levels of TNF-α and IL-6 were decreased significantly in the hypercholesterolemic infracted

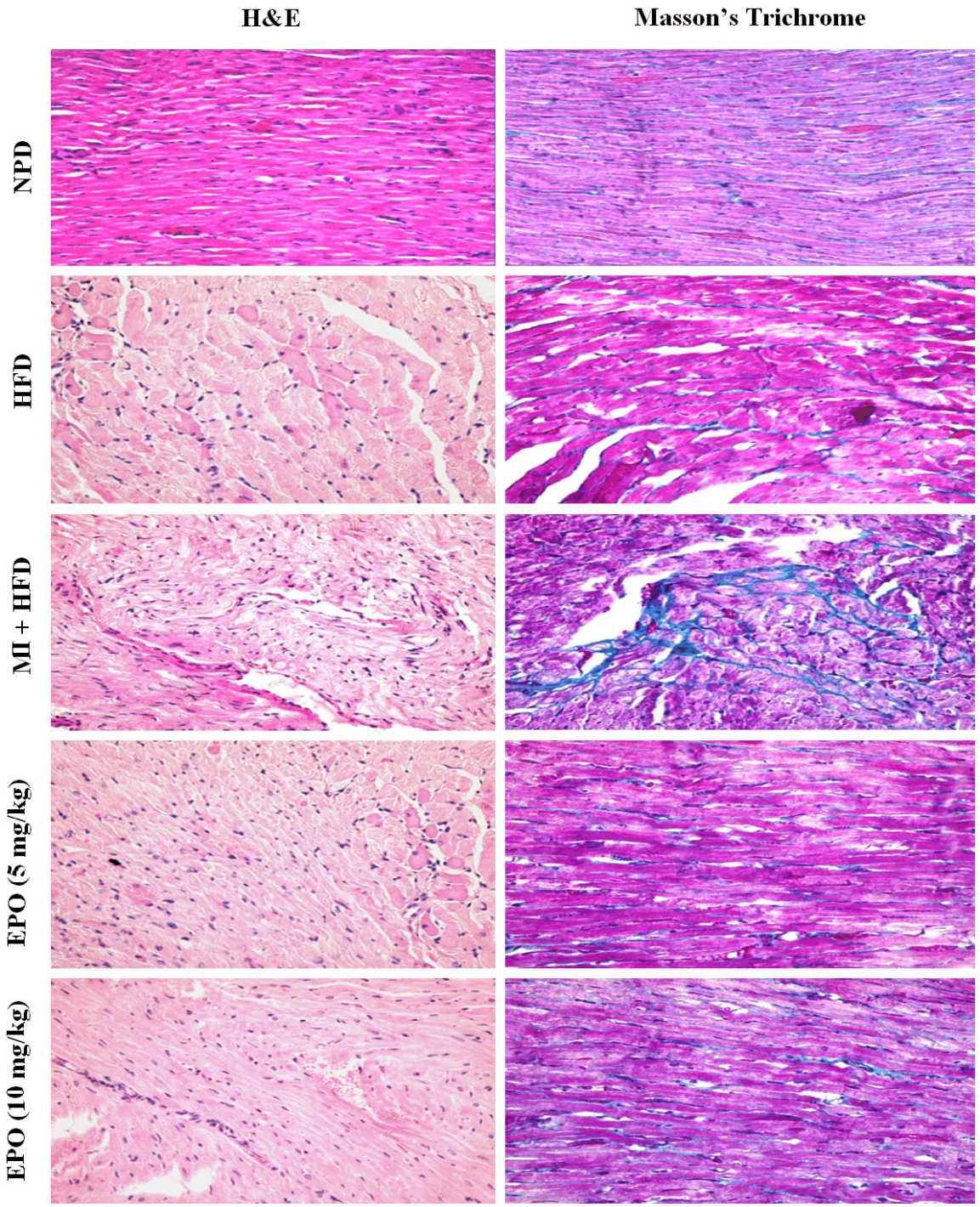

Figure 5. Histopathologic examination of the heart muscles from the experimental groups. NPD: normal palatable diet, HFD: high-fat diet, EPO: evening primrose oil. The left side represents photomicrographs of the cardiac muscles stained with hematoxylin & eosin (× 40). The right side represents photomicrographs of the cardiac muscles stained with Masson's trichrome stain (× 40). Heart tissue from the saline treated rats showed normal appearance of the cardiac tissue whereas rats treated with the 2 doses of isoproterenol (85 mg/kg/s.c) showed intensive fibrosis. Treatment with EPO alleviated the extent of fibrosis.

rats treated with EPO 5 gm/kg/rat and 10 gm/kg/rat groups in comparison to the diseased group. Our findings may be supported by many studies [14, 55, 56].

Table 5. Fibrosis grades in the cardiac tissues from the different experimental groups

Groups	Grades					Mean fibrosis grade
	0	1	2	3	4	
NPD	*10*	-	-	-	-	*1 ± 0*
HFD	*7*	*2*	*1*	-	-	*0.4 ± 0.22*
HFD + isoproterenol	-	-	-	*2*	*3*	*3.6 ± 0.22**,†
HFD + isoproterenol + EPO (5 gm/kg)	-	-	-	*5*	-	*3 ± 0**,†,‡
HFD + isoproterenol + EPO (10 gm/kg)	-	-	*3*	*1*	-	*2.25 ± .19**,†,‡

Rats were fed with a HFD for four weeks and then injected with two doses of isoproterenol (85 mg/kg/24 h, s.c.) to induce acute myocardial infarction. Rats were treated with EPO (5 or 10 gm/kg/rat/day) for another six weeks. NPD: normal palatable diet, HFD: high-fat diet. The histopathological changes were assigned as 1, 2, 3, and 4 for low, moderate, high, and intensive pathological changes, respectively. Mean values are expressed as mean ± S.E.M. *Compared to saline group at $P < 0.05$. †Compared to HFD group at $P < 0.05$. ‡Compared to HFD-isoproterenol group at $P < 0.05$. $^{\$}$Compared to EPO (5 g/rat) group at $P < 0.05$, n = 6-10. Values are expressed as mean ± S.E.M. and analyzed using one-way ANOVA followed by the Bonferroni's test for multiple comparisons. *Compared to NPD group at $P < 0.05$. †Compared to HFD group at $P < 0.05$. ‡Compared to HFD + isoproterenol group at $P < 0.05$. n = 6-10.

Fortunately, we can explain the ameliorating effect of EPO. High cholesterol level implies proinflammatory actions and HFD directly activate leukocytes and macrophages to produce pro-inflammatory cytokines: IL-6 and TNF-α [50], moreover, local activation of neutrophils in the ischemic myocardium [14], So, EPO hypocholesterolemic effect as reported previously may be the corner stone in explaining this amelioration in the inflammatory markers level. Mimicking statins in inhibition of IL-6 and TNF-α production through down-regulation of nuclear factor-κB and activation of peroxisome proliferator activated receptor-γ [57] may be the claimed mechanism but our study design did not allow us to test or confirm this mechanism.

We reported a significant reduction in the serum COX-2 levels in EPO treated groups and this may enhance EPO anti-inflammatory effect. COX-2 is undetectable in most normal tissues becoming abundant in activated macrophages and other cells at sites of inflammation [58-60]. COX-2 oxygenate DGLA (ω-6) to give the series-1 prostanoids, which are less inflammatory [30]. DGLA is competitive inhibitors with AA for the COX pathways. This inhibition is a major mode of action in the way that dietary sources of DGLA (e.g. EPO) reduce inflammation [61].

Histopathological examination revealed higher degree of fibrosis in isoproterenol treated rats with evident amelioration in EPO treated groups. This amelioration could be attributed to the reported attenuation in the inflammatory markers and platelet aggregation % in this combined model. Besides, the increase in DGLA exerts an anti-inflammatory effect [35] and hence, cardiac inflammation and consequent fibrosis showed this significant amelioration with evident cardiac remodeling and decreased extent of fibrosis.

We found that there is a strong positive correlation between platelet aggregation % and LDL-C. Also, there is a weak positive correlation between platelet aggregation % and IL-6 and COX-2 respectively. This paid our attention to the link between platelet aggregation % and these cholesterol as well as inflammation determining factors. So, EPO may ameliorate the condition of the ischemic myocardium with HFD mainly via interruption of this link; modulation of hyperaggregability. Thus, modulation of platelet aggregation % using EPO may potentiate the effect of other medications commercially used in MI subjects with hypercholesterolemia.

Overall, we can say that our results agrees in conclusion with aggregate data from randomized trials, case-control, cohort studies, and long-term animal feeding experiments indicate that the consumption of omega-6; reduces the risk of CVD [59, 62], protects against CVD and lowers incidence of myocardial infarction or CHD death [14].

Conclusion

Finally, we concluded that EPO has a potent anti-inflammatory, anti-aggregatory and hypocholesterolemic effect. These effects can ameliorate MI even in a state of hyperaggregability

produced by concomitant HFD. These therapeutic effects of EPO in general are attributed to direct hypocholesterolemic effect and the indirect effect on the synthesis of eicosanoids (prostaglandins, cytokines, cytokine mediators). Further studies are still needed to justify the proper EPO dosage and timing of administration as well as its interaction with other drugs used in hypercholesterolemic MI subjects.

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References

[1] Suminori K; The Fukuoka Heart Study Group. Medication for Hypercholesterolemia nd the Risk of Nonfatal Acute Myocardial Infarction A Case-Control Study in Japan. Circ J 2002; 66: 463-468.

[2] Surya I, Mommersteeg M, Gorter G, Erkelens DW, Akkerman JWN. Abnormal platelet functions in a patient with abetalipoproteinemia. Thromb Haemost 1991; 65: 306-11.

[3] Block LH, Knorr M, Vogt E. Low density lipoprotein cause general cellular activation with increased phosphatidylinositol turnover and lipoprotein catabolism. Proc Natl Acad Sci U S A 1998; 85: 885-9.

[4] Knorr M, Locker R, Vogt E. Rapid activation of human platelets by low concentration of LDL via phosphatidylinositol cycle. Eur J Biochem 1998; 172: 753-9.

[5] Relou IA, Hackeng CM, Akkerman JW, Malle E. Low-density lipoprotein and its effect on human blood platelets. Cell Mol Life Sci 2003; 60: 961-70.

[6] Hackeng CM, Huigsloot M, Pladet MW, Nieuwenhuis HK, van Rijn HJ, Akkerman JW. Low-density lipoprotein enhances platelet secretion via integrin-alphaIIbbeta3-mediated signaling. Arterioscler Thromb Vasc Biol 1999; 19: 239-47.

[7] Czernichow S, Thomas D, Brukert E. N-6 fatty acids and cardiovascular health- Dietary intake recommendations. Med Sci 2011; 27: 614-618.

[8] Simopoulos AP. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp Biol Med 2008; 233: 674-688.

[9] Wang C, Harris WS, Chung M, Lichtenstein AH, Balk EM, Kupelnick B, Jordan HS, Lau J. n-3 Fatty acids from fish or fish-oil supplements, but not alpha-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: a systematic review. Am J Clin Nutr 2006; 84: 5-17.

[10] Harris WS, Mozaffarian D, Rimm E, Kris-Etherton P, Rudel LL, Appel LJ, Engler MM, Engler MB, Sacks F. Omega-6 fatty acids and risk for cardiovascular disease: a science advisory from the American Heart Association Nutrition Subcommittee of the Council on Nutrition, Physical Activity, and Metabolism; Council on Cardiovascular Nursing; and Council on Epidemiology and Prevention. Circulation 2009; 119: 902-907.

[11] Pan M, Song YL, Xu JM, Gan HZ. Melatonin ameliorates nonalcoholic fatty liver induced by high-fat diet in rats. J Pineal Res 2006; 41: 79-84.

[12] Piccione G, Grasso F, Fazio F. The effect of physical exercise on the daily rhythm of platelet aggregation and body temperature in horses. Vet J 2008; 176: 216-220.

[13] Machado RM, Nakandakare ER, Quintao EC, Cazita PM, Koike MK, Nunes VS, Ferreira FD, Afonso MS, Bombo RP, Machado-Lima A, Soriano FG, Catanozi S, Lottenberg AM. Omega-6 polyunsaturated fatty acids prevent atherosclerosis development in LDLr-KO mice, in spite of displaying a pro-inflammatory profile similar to trans fatty acids. Am J Clin Nutr 2013; 97: 66-71.

[14] Czernichow S, Thomas D, Bruckert E. n-6 Fatty acids and cardiovascular health: a review of the evidence for dietary intake recommendations. Br J Nutr 2010; 104: 788-96.

[15] Choo J, Ueshima H, Curb JD, Shin C, Evans RW, El-Saed A, Kadowaki T, Okamura T, Nakata K, Otake T, Miura K, Abbott RD, Sutton-Tyrrell K, Edmundowicz D, Kuller LH, Sekikawa A. Serum n-6 fatty acids and lipoprotein subclasses in middle-aged men: the population-based cross-sectional ERA-JUMP study. Am J Clin Nutr 2010; 91: 1195-203. doi: 10.3945/ajcn.2009.28500.

[16] Chandrasekharan NV, Dai H, Roos KLT, Evanson NK, Tomsik J, Elton TS, Simmons DL. COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. Proc Natl Acad Sci U S A 2002; 99: 13926-31.

[17] Calder PC. n-3 Fatty acids and cardiovascular disease: evidence explained and mechanisms explored. Clin Sci 2004; 107: 1-11.

[18] Koga T, Azma T, Yuge O. Prostaglandin E1 at clinically relevant concentrations inhibits ag-

gregation of platelets under synergic interaction with endothelial cells. Acta Anaesthesiol Scand 2002; 46: 987-993.
[19] De La Cruz JP, Martín-Romero M, Carmona JA, Villalobos MA, Sánchez de la Cuesta F. Effect of evening primrose oil on platelet aggregation in rabbits fed an atherogenic diet. Thromb Res 1997; 87: 141-149.
[20] Banerjee SK, Sood S, Dinda AK. Chronic oral administration of raw garlic protects against isoproterenol-induced myocardial necrosis in rat. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 2003; 136: 377-386.
[21] Vogler GA. Anesthesia and analgesia. In: Suckow MA, Weisbroth SH, Franklin CL (Eds.). The Laboratory Rat. New York, USA: Elsevier Academic Press 2006; pp: 627-695.
[22] Afshin M, Ben Ayed I, Punithakumar K, Law MW, Islam A, Goela A, Ross I, Peters T, Li S. Assessment of regional myocardial function via statistical features in MR images. Med Image Comput Comput Assist Interv 2011; 14: 107-14.
[23] Buhl SN, Jackson KY. Optimal conditions and comparison of lactate dehydrogenase catalysis of the lactate-to-pyruvate and pyruvate-to-lactate reactions in human serum at 25, 30, and 37 degrees C. Clin Chem 1978; 24: 828-831.
[24] Szasz G. Proceedings of the Second International Symposium on Clinical Enzymology. 1975; Chicago, IL, USA.
[25] Wurzburg U, Hennrich N, Lang H. Determination of creatine kinase-MB in serum using inhibiting antibodies. Klin Wochenschr 1976; 54: 357-60.
[26] Benjamin IJ, Jalil JE, Tan LB, Cho K, Weber KT, Clark WA. Isoproterenol-induced myocardial fibrosis in relation to myocyte necrosis. Circ Res 1989; 65: 657-670.
[27] Bayles B, Usatine R. Evening Primrose Oil. Am Fam Physician 2009; 80: 1405-1408.
[28] Senapati S, Banerjee S, Gangopadhyay DN. Evening primrose oil is effective in atopic dermatitis: a randomized placebo-controlled trial. Indian J Dermatol Venereol Leprol 2008; 74: 447-452.
[29] Fan YY, Chapkin RS. Importance of dietary gamma-linolenic acid in human health and nutrition. J Nutr 1998; 128: 1411-4.
[30] Takai S, Jin D, Kawashima H, Kimura M, Shiraishi-Tateishi A, Tanaka T, Kakutani S, Tanaka K, Kiso Y, Miyazaki M. Anti-atherosclerotic effects of dihomo-gamma-linolenic acid in ApoE-deficient mice. J Atheroscler Thromb 2009; 16: 480-9.
[31] Kela AK, Reddy LP, Thrombe DP. ECG findings in normal rats and after administration of isoproterenol. Ind J Physiol Pharmacol 1980; 24: 84-90.
[32] Garjani A, Andalib S, Biabani S, Soraya H, Doustar Y, Garjani A, Maleki-Dizaji N. Combined atorvastatin and coenzyme Q10 improve the left ventricular function in isoproterenol-induced heart failure in rat. Eur J Pharmacol 2011; 666: 135-141.
[33] Srivastava S, Chandrasekar B, Gu Y, Luo J, Hamid T, Hill BG, Prabhu SD. Downregulation of CuZn-superoxide dismutase contributes to beta-adrenergic receptor-mediated oxidative stress in the heart. Cardiovasc Res 2007; 74: 445-455.
[34] Mohamed TS, lokanath N, Prasanthi A, madhavi M, Mallika G, Vishnu MN. Aqueous extract of Saussurea lappa root ameliorate oxidative myocardial injury induced by isoproterenol in rats. J Adv Pharm Technol Res 2013; 4: 94-100.
[35] Johnson M, Swan D, Surette M, Stegner J, Chilton T, Fontech A, Chilton F. Dietary supplementation with γ-linolenic acid alters fatty acid content and eicosanoid production in healthy humans. J Nutr 1997; 127: 1435-1444.
[36] Zurier R, Rossetti R, Jacobson E, DeMarco D, Liu N, Temming J, White B, Laposata M. Gamma-linolenic acid treatment of rheumatoid arthritis. A randomized, placebo-controlled trial. Arthritis Rheum 1996; 39: 1808-1817.
[37] Hussein N, Ah-Sing E, Wilkinson P, Leach C, Griffin B, Millward D. Long-chain conversion of [13C] linoleic acid and alpha-linolenic acid in response to marked changes in their dietary intake in men. J Lipid Res 2005; 46: 269-280.
[38] Fan Y, Chapkin R. Mouse peritoneal macrophage prostaglandin E1 synthesis is altered by dietary γ-linolenic acid. J Nutr 1992; 122: 1600-1606.
[39] Thippeswamy BS, Thakker SP, Tubachi S, Kalyani GA, Netra MK, Patil U, Desai S, Gavimath CC and Veerapur VP. Cardioprotective Effect of Cucumis trigonus Roxb on Isoproterenol-Induced Myocardial Infarction in Rat. Am J Pharmacol and Toxicol 2009; 4: 29-37.
[40] Kawashima H, Tateishi N, Shiraishi A, Teraoka N, Tanaka T, Tanaka A, Matsuda H, Kiso Y. Oral administration of dihomo-gamma-linolenic acid prevents development of atopic dermatitis in NC/Nga mice. Lipids 2008; 43: 37-43.
[41] Post JA, Verkleij AJ, Langer GA. Organization and function of sarcolemmal phospholipids in control and ischemic/reperfused cardiomyocytes. J Mol Cell Cardiol 1995; 27: 749-60.
[42] Senanayake N, Shahidi F. Incorporation of docosahexaenoic acid (DHA) into evening primrose oil (Oenothera biennis L.) oil via lipase-catalyzed transesterification. Food Chemistry 2004; 85: 489-496.
[43] Hassig A, Liang WX, Stampfli K. Bronchial asthma: information on phytotherapy with essen-

tial fatty acids. Med Hypotheses 2000; 54: 72-74.
[44] Balasinska B. Hypocholesterolemic effect of dietary evening primrose oil (Oenothera paradoxa) cake extract in rats. Food Chemistry 1998; 63: 453-459.
[45] Riaz A, Khan RA, Ahmed SP. Assessment of anticoagulant effect of evening primrose oil. Pak J Pharm Sci 2009; 22: 355-359.
[46] Dhikav V, Anand KS, Sudha R. Omega-3 Polyunsaturated Fatty Acid and Cardiovascular Disorders. JIACM 2004; 5: 182-5.
[47] Liang XC, Guo SS. Effect of jiang-zhi zhong-yaopian on total cholesterol, triglycerides, TXB2,6-keto-PGF1 alpha in hyperlipemic ptients. Zhong Xi Yi Jie He Za Zhi 1999; 11: 20-24.
[48] Wijendran V, Hayes KC. Dietary n-6 and n-3 fatty acids balance and cardiovascular health. Annu Rev Nutr 2004; 24: 597-615.
[49] Harris WS, Poston WC, Haddock CK. Tissue n-3 and n-6 fatty acids and risk for coronary heart disease events. Atherosclerosis 2007; 193: 1-10.
[50] Das UN. Hypothesis: Essential fatty acids and their metabolites could function as endogenous HMG-CoA reductase and ACE enzyme inhibitors, anti-arrhythmic, anti-hypertensive, anti-atherosclerotic, anti-inflammatory, cytoprotective, and cardioprotective molecules. Lipids Health Dis 2008; 7: 37. doi: 10.1186/1476-511X-7-37.
[51] Istvan ES, Deisenhofer J. Structural mechanism for statin inhibition of HMGCoA reductase. Science 2001; 292: 1160-1164.
[52] Strano A, Davi G, Averna M. Platelet sensitivity to prostacyclin and thromboxane production in hyperlipidernic patients. Thromb Haernost 1982; 48: 18-20.
[53] Renaud S, Morazain R, Mc Grego L, Baudie F. Dietary fats and platelet functions in relation to atherosclerosis and coronary heart disease. Haemostasis 1979; 8: 234-251.
[54] Mikhailidis P, Jeremeny Y. Platelet function: the role of essential fatty acids and eicosanoids. Prostaglandin Leukoc Essent Fat Acids 1989; 35: 187-188.
[55] Pischon T, Hankinson SE, Hotamisligil GS, Rifai N, Willett WC, Rimm EB. Habitual dietary intake of n-3 and n-6 fatty acids in relation to inflammatory markers among US men and women. Circulation 2003; 108: 155-160.
[56] Ferrucci L, Cherubini A, Bandinelli S, Bartali B, Corsi A, Lauretani F, Martin A, Andres-Lacueva C, Senin U, Guralnik J. Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers. J Clin Endocrinol Metab 2006; 91: 439-446.
[57] Das UN. Essential fatty acids as possible mediators of the actions of statins. Prostaglandins Leukot Essent Fatty Acids 2001; 65: 37-40.
[58] Mitchell JA, Warner TD. COX isoforms in the cardiovascular system: understanding the activities of non-steroidal anti-inflammatory drugs. Nat Rev Drug Discov 2006; 5: 75-86.
[59] Fiorucci S, Antonelli E. Cyclo-oxygenase isoenzymes. Structural basis for selective inhibition of cyclo-oxygenases by anti-inflammatory agents. Dig Liver Dis 2001; 33 Suppl 2: S2-7.
[60] Carnieto A Jr, Dourado PM, Luz PL, Chagas AC. Selective cyclooxygenase-2 inhibition protects against myocardial damage in experimental acute ischemia. Clinics (Sao Paulo) 2009; 64: 245-52.
[61] Barham JB, Edens MB, Fonteh AN, Johnson MM, Easter L, Chilton FH. Addition of eicosapentaenoic acid to gamma-linolenic acid-supplemented diets prevents serum arachidonic acid accumulation in humans. J Nutr 2000; 130: 1925-31.
[62] Kark JD, Kaufmann NA, Binka F, Goldberger N, Berry EM. Adipose tissue n-6 fatty acids and acute myocardial infarction in a population consuming a diet high in polyunsaturated fatty acids. Am J Clin Nutr 2003; 77: 796-802.