PROTECTIVE EFFECT OF EVENING PRIMROSE OIL AND EXTRA VIRGIN OLIVE OIL AGAINST AUTOIMMUNE HEPATIC INFLAMMATION

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ABSTRACT

The evening primrose plant (Oenotherabiennis L.) is commonly known as tree primrose and sun drop. Evening primrose oil (EPO) has been used to treat a variety of ailments. It is classified as a "dietary supplement" under the Dietary Supplement Health and Education Act of 1994. These wide uses related to its high content of omega-6 (n-6) polyunsaturated fatty acids specially γ-linolenic acid (GLA) also beneficial effects of Extra Virgin Olive oil (EVOO) are not only related to its high content of oleic acid, but also to the antioxidant potential of its polyphenols. Also Extra Virgin Olive Oil (EVOO) is the primary source of fat in the Mediterranean diet. The current study performed to evaluate the effect of EPO and EVOO on acute hepatic inflammation results from CFA administration. Also we summarize recent findings emphasizing the role of main inflammatory markers and oxidative stress in acute hepatic inflammation case. Hepatic inflammation was induced by a single dose of CFA (100 µl), injected subcutaneously. These rats received EPO and EVOO through gastric intubation daily for 21 days after CFA injection. The current results revealed that EPO and EVOO treatment ameliorated significantly the elevated levels of the hepatic cytokines which elevated as a result to CFA injection. Moreover, EPO and EVOO treatment ameliorated the non-enzymatic antioxidant, liver malondialdehyde and glutathione (GSH) concentration and the enzymatic antioxidant, liver catalase, super oxide dismutase and liver GSH-peroxidase activities.

KEYWORDS: CFA; Primrose oil; Olive oil; Liver Injury; Inflammation; Oxidative Stress.

1- INTRODUCTION:

The incomplete Freund adjuvant (IFA) and complete Freund adjuvant (CFA), which is heat-killed Mycobacterium tuberculosis bacilli (Mtbc) dissolved in IFA. (Billiau and Matthys, 2001). This killed mycobacteria contain various pathogen-associated molecular patterns including toll-like receptor 2,4 and 9 agonists (Akira and Takeuchi, 2006). These adjuvants have been used extensively to establish experimental animal models of autoimmune diseases, e.g. experimental autoimmune encephalitis (EAE), neuritis (EAN), uveitis (EAU), thyroiditis.
(EAT) and orchitis, and to produce antibodies (Billiau and Matthys, 2001). Although liver is the primary target organ in host–microbe interaction and a major response organ in systemic inflammation as it is highly responsive to microbial toxins. Studies on the effects of Freund adjuvants on the liver have been largely neglected. The liver is regarded as a privileged site of immune tolerance. The disruption of immune tolerance in the liver can lead to immune-mediated liver diseases (Kumiko et al., 2015). Autoimmune hepatitis (AIH) is increasingly recognized as a liver-specific autoimmune disease that results, at least in part, from the perturbation of immune tolerance in the liver (Czaja and Manns, 2010). The disease is characterized histologically by chronic infiltration of inflammatory cells in the liver and the destruction of hepatocytes. AIH is characterized clinically as a chronic liver disorder and sometimes as an acute onset and severe liver disorder. AIH can lead to liver cirrhosis and hepatocellular carcinoma, which are often indications for liver transplantation. However, the pathogenesis of this disease remains poorly understood (Kumiko et al., 2015).

Progressive systemic TNF-α, IL-1β, IL-6 and NO increase the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), and decrease the activities of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) in tissues. These consequent events further aggravate protein and lipid oxidation, and injury tissues. Thus, oxidative damage of the vital organs, particularly the liver, is considered as secondary complications of arthritis (Sundaram et al., 2014). It is observed that the release of hepatic transaminases such as glutamic oxaloacetic transaminase (GOT) and glutamate-pyruvate transaminase (GPT) into the blood was found in AIA-mediated liver damage (Comaret et al., 2013 and Shusong et al., 2015).

Olive oil is an integral ingredient in the Mediterranean diet. There is growing evidence that it may have great health benefits including the reduction in coronary heart disease risk, the prevention of some cancers and the modification of immune and inflammatory responses. Olive oil, a widely applied omega-9 enriched dietary lipid, has attracted much interest in its effects against liver injuries (Tanaka et al., 2009 and Hualin et al., 2014).

Evening primrose oil has a high concentration of polyunsaturated fatty acids, and has been used for more than 30 years as a dietary supplement. Simultaneously there were many reports published about the antitumor and immunotrophic activity of evening primrose oil (Guanget et al., 2013).
Therefore the aim of this study was to evaluate the protective effect of Evening primrose oil and extra virgin olive oil against hepatic inflammation in rheumatic rats.

2- Materials and methods:

2.1. Experimental animals:

White male albino rats (*Rattus norvegicus*) weighing between 100 g and 120 g were used as experimental animals in the present investigation. They were obtained from the animal house of National Research Institute, El-Giza, Egypt. They were kept under observation for about 15 days before the onset of the experiment to exclude any inter current infection. The chosen animals were housed in metal (stainless steel) separate bottom cages at normal atmospheric temperature (25±5°C) as well as under good ventilation and received water and standard balanced diet. All the procedures were performed in accordance with the Institutional Animal Ethics Committee in Beni-Suef University recommendations.

2.2. Chemicals and oils:

Complete Freund adjuvant (CFA), evening primrose oil and extra virgin olive oil were purchased from Sigma Chemical Company, St. Louis, MO, USA. They were stored at 2- 4ºC and protected from sun light. The dose selection for each compound was based on previously published studies.

2.3. Doses and Treatment:

A single dose of CFA used in this study was (100 µl), injected subcutaneously (Livia et al., 2011). Evening primrose oil (EPO) and Extra virgin olive oil (EVOO) were administered to male albino rats by gastric intubation at dose 5 mg / kg. b.w. / day (Silva et al., 2014 and Nakbi et al., 2010 respectively) the treatment began on the day of CFA injection and continued daily up to the 21th day after arthritis induction.

2.4. Experimental design:

The number of rats used in the present study is 24. They were allocated into 4 groups designed as follow:

**Group 1:** This group was regarded as control group and given distilled water by gastric intubation for 21 days

**Group 2:** It was given single dose of CFA (100 µl) injected subcutaneously in male rats.

**Group 3:** The rats of this group were administered single dose of CFA (100 µl) at beginning of the experiment and were also treated with evening primrose oil (EPO) (5 mg /kg b. w. /day), by gastric intubation for 21 days.
Group 4:

The rats of this group were administered single dose of CFA (100 µl) at beginning of the experiment and were also treated with extra virgin olive oil (EVOO) (5 mg /kg b. w. /day), by gastric intubation for 21 days. At the end of 21 days, six animals of normal, CFA-administered control rats and CFA-administered rats treated with EPO and EVOO were sacrificed under mild diethyl ether anesthesia. Liver from each animal was rapidly excised after dissection. 0.5g was homogenized in 5ml 0.9% sterilized NaCl (10% w/v) using Teflon homogenizer (Glas-Col, Terre Haute, USA).

2.5. Biochemical analyses:

Hepatic Tumor necrosis factor-α (TNF-α) was measured according to the method of Seckinger et al., (1998), Lantez et al., (1990) and Engelmann et al., (1990). Interleukins 6 and 1β were determined by the method of Dinarello, (1990) and Herzyk et al., (1992) by using specific ELISA kits Quantikine Rat Total Adeponecin Immunoassay (USA). Liver lipid peroxidation was determined by measuring thiobarbituric acid reactive substances (TBARS) according to the method of Preuss et al., (1998). Catalase activity determined according to Cohen et al., (1970) which follows the first-order kinetics as given by the equation: K = log (S0 / S3) X 2.3 / t. The SOD activity was measured in liver homogenate according to the method described by Marklund and Marklund,(1984). Liver glutathione (GSH) was determined according to the method of Beulter et al., (1963) and finally Glutathione peroxidase determined according Matkovics et al., (1998).

2.6. Statistical analysis of the results

The Statistical Package for the Social Sciences (IBM SPSS for WINDOWS7, version 20; SPSS Inc, Chicago) was used for the statistical analysis. Comparative analysis was conducted by using the general linear models procedure (IBM SPSS). p>0.05 were considered statistically non significant, while p<0.05 were considered statistically significant.

3- Results:

Data summarized in Table 1 and figures 1, 2 and 3 show the effect of CFA administration and treatment with EPO and EVOOO on hepatic cytokines, TNF-α,IL-6 and IL-1β markers. Our results revealed that the administration of CFA produced marked impairment demonstrated by significant increase in these hepatic inflammatory factors as compared to normal rats, while Oral administration of primrose oil and olive oil significantly decreased these elevated levels of Hepatic TNF-α,IL-6 and IL-1β when compared with the CFA-administered rats recording noticeable amelioration as compared to normal ones.
Table 2 and figures 5-8 show the effect of the tested evening primrose oil (EPO) and extra virgin olive oil (EVOO) on the liver oxidative stress markers and antioxidant defense system of CFA-administered rats. Hepatic lipid peroxidation (LPO) product was significantly increased as a result of CFA administration while the treatment of CFA-administered rats with EPO and EVOO for 21 days significantly decreased these elevated values and becomes near to those of normal values. On the other hand, rats injected with CFA exhibited a noticeable decrease in values of catalase (CAT) activity, superoxide dismutase (SOD) activity, total glutathione content (GSH) and glutathione peroxidase (GPX) level as compared to normal rats. While, the treatment with EPO and EVOO after CFA administration produced a significant increase of these oxidative stress values as compared to the corresponding CFA administered group pointing to a marked normalization as compared to normal group.

Table 1: Effect of evening primrose oil and extra virgin olive oil on hepatic cytokines, TNF-α, IL-6 and IL-1β activities in CFA-administered rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameter</th>
<th>Tumor Necrosis Factor-α (TNF-α (pg/ml))</th>
<th>Interleukin-6 (IL-6 (pg/ml))</th>
<th>Interleukin-1β (IL-1β (pg/ml))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td>31.29±1.01 a</td>
<td>33.47±1.48 a</td>
<td>28.74±1.29 a</td>
</tr>
<tr>
<td>CFA</td>
<td></td>
<td>122.12±1.43 d</td>
<td>124.45±1.24 c</td>
<td>114.29±1.35 d</td>
</tr>
<tr>
<td><strong>Evening Primrose Oil (EPO)</strong></td>
<td></td>
<td>52.92±1.93 c</td>
<td>58.55±1.44 b</td>
<td>75.78±0.90 c</td>
</tr>
<tr>
<td><strong>Extra virgin olive oil (EVOO)</strong></td>
<td></td>
<td>60.06±1.22 b</td>
<td>49.64±2.06 b</td>
<td>63.23±1.57 b</td>
</tr>
<tr>
<td><strong>LSD value at 0.05</strong></td>
<td></td>
<td>2.42</td>
<td>2.24</td>
<td>1.84</td>
</tr>
</tbody>
</table>

*Values significantly different to control at (p≤0.05). *Data are expressed as mean ± SE. *Values which share the same superscript symbol are not significantly different. *F-Probability: P < 0.05
Table 2: Effect of evening primrose oil and extra virgin olive oil on hepatic oxidative stress in CFA-administered rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameter</th>
<th>Lipid peroxidation (n mole/mg)</th>
<th>Catalase (u/g.tissue)</th>
<th>Super oxide dismutase (n mole/mg)</th>
<th>Total glutathione content (n mole/mg)</th>
<th>Glutathione Peroxidase (U/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td>1.49±0.08</td>
<td>121.08±1.07</td>
<td>2.70±0.11</td>
<td>54.64±0.5</td>
<td>43.69±1.81</td>
</tr>
<tr>
<td>CFA</td>
<td></td>
<td>17.44±0.93</td>
<td>64.25±1.18</td>
<td>0.28±0.01</td>
<td>22.81±0.97</td>
<td>8.82±0.64</td>
</tr>
<tr>
<td>Evening Primrose Oil (EPO)</td>
<td></td>
<td>5.64±0.26</td>
<td>108.69±1.24</td>
<td>1.35±0.1</td>
<td>41.49±0.98</td>
<td>30.39±0.98</td>
</tr>
<tr>
<td>Extra virgin olive oil (EVOO)</td>
<td></td>
<td>3.56±0.2</td>
<td>97.71±1.1</td>
<td>1.62±0.13</td>
<td>44.39±1.09</td>
<td>28.75±0.48</td>
</tr>
<tr>
<td>LSD value at 0.05</td>
<td></td>
<td>0.7</td>
<td>1.62</td>
<td>0.14</td>
<td>1.29</td>
<td>1.56</td>
</tr>
</tbody>
</table>

*Values significantly different to control at (p≤0.05).  
*Data are expressed as mean ± SE.  
*Values which share the same superscript symbol are not significantly different.  
*F-Probability: P < 0.05

Fig. 1: The effect of primrose oil and olive oil administration on hepatic Tumor Necrosis Factor-α (TNF–α) in liver inflamed rats

Fig. 2: The effect of primrose oil and olive oil administration on hepatic interleukin-6 in liver inflamed rats
Fig. 3: The effect of primrose oil and olive oil administration on hepatic interleukine-1β in liver inflammed rats

Fig. 4: The effect of primrose oil and olive oil administration on hepatic lipid peroxidation in liver inflammed rats

Fig. 5: The effect of primrose oil and olive oil administration on hepatic Catalase Activity in liver inflammed rats
Fig. 6: The effect of primrose oil and olive oil administration on hepatic superoxide dimutase Activity in liver inflamed rats.

Fig. 7: The effect of primrose oil and olive oil administration on hepatic total glutathione content in liver inflamed rats.

Fig. 8: The effect of primrose oil and olive oil administration on hepatic glutathione peroxidase activity in liver inflamed rats.
Discussion

The liver has a unique endothelium that consists of fenestrated endothelial cells lining hepatic sinusoids, allowing hepatocytes to contact immune cells directly. The sinusoids contain endothelial cells, Kupffer cells (resident macrophages), stellate cells (important in remodeling and fibrosis) and intrahepatic lymphocytes. The liver plays a critical role in first-line host defense against incoming foreign antigens absorbed through the gut into the portal venous system. It must maintain a balance between tolerance to incoming antigens and generation of an immune response. Tolerance is essential to avoid food allergy and explains graft survival of liver transplants across major histocompatibility complex (MHC) antigen differences. Loss of tolerance or a hyper immune response may lead to autoimmunity (Marion, 2002).

Freund adjuvants are used extensively to establish experimental animal models of autoimmune diseases and to produce antibodies. However, studies on their mechanisms of action have been largely neglected, particularly their effects on liver, the primary target organ for host–microbe interaction (Sai-Kiang Lim, 2003).

Our results obtained in the present work can be discussed in two main aspects: first, the development of autoimmune hepatic inflammatory response and metabolic changes in the liver of animals induced by complete Freund adjuvant (CFA); second, the effects of EPO and EVOO against these inflammatory processes in the liver of induced rats. According to the first aspect, it was possible to observe that the injection of CFA at the dose of 100 µg of Mycobacterium tuberculosis induce autoimmune hepatic inflammation. These results run parallel to those of Sai-Kiang Lim.,(2003) whose reported that Freund adjuvant induced a 5–10-fold increase in toll-like receptor (TLR) 2 mRNA but not TLR4 mRNA in livers of mice. Since CFA is essentially made of killed Mycobacterium tuberculosis bacilli (Mtb) dissolved in Incomplete Freund adjuvant (IFA), it is the solvent in CFA that induced an increase in TLR2 expression. As TLR2 is the receptor activated by killed Mtb, this solvent-mediated increase in TLR2 expression will result in enhanced recognition of killed Mtb by hepatocytes during CFA administration. We propose that the potency of Freund adjuvant in eliciting an immune response lies in their ability to induce expression of the appropriate TLR, TLR2, for the active ingredient, killed Mtb, in CFA. Hence, adjuvant administration has been known to stimulate innate immunity, induce expression of cytokines, enhance phagocytosis and trigger autoimmune-like diseases (Billiau and Matthys, 2001). Consequently, the inflammatory indicators as TNF-α, IL-1β, IL-6 were increased significantly in the liver of CFA-induced
rats, and then oral administration with EPO and EVOO reduced their accumulation in a dose-dependent manner, these results are in accordance with Shusong et al., (2015).

The liver resembles a central organ of cytokine activity due to the fact that it hosts hepatocytes, which are highly susceptible to the activity of cytokines in a variety of physiological and pathophysiological processes. Moreover, the non-parenchymal cells of the liver, in particular Kupffer cells (KCs), the resident tissue macrophages of the liver, are able to synthesize a variety of cytokines that may act systemically on any other organ of the body, or in a paracrine manner on hepatocytes and other non-parenchymal liver cells. (Giuliano and Thomas, 2001). In the last few years, it has been suggested that in most cases hepatocellular injury is due not to the damaging agent itself but to the inflammatory cells that have been attracted by the stressed hepatocytes. In contrast to sustained hepatocellular damage, acute hepatitis is a temporary event that finishes with normal liver histology and function (restitutio ad integrum). In fact, hepatotoxins (drugs, infectious agents) may induce a stress situation in hepatocytes with subsequent release of chemokines followed by accumulation of inflammatory cells and subsequent hepatocellular damage (Fig. 9) (Diehl, 2000). Hepatotropic infectious agents such as viruses or activated T-cells may act in a similar manner (Tiegs, 1997) and induce accumulation of inflammatory cells that kill hepatocytes (Arii and Imamura, 2000).

![Fig 9. Acute liver injury. (Diehl, 2000)](image-url)
Hepatocellular stress may activate resident liver macrophages. Moreover, KCs can be activated directly, e.g. by binding of endotoxins via the CD14 receptor. Proinflammatory cytokines such as IL-1α, IL-1β and TNF-α are released from KCs, which induces cell adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) on sinusoidal endothelial cells involved in the recruitment of inflammatory cells, such as blood monocytes (Neubauer et al., 1998). Moreover, pro-inflammatory cytokines may be released from mesenchymal liver cells. In fact, it is debated whether mononuclear phagocytes of the liver are the main source of pro-inflammatory cytokines. Further induction of chemokines amplifies the inflammatory cascade (Marra et al., 1998). Deteriorated hepatocytes may then be removed by mononuclear phagocytes. The classical hypothesis that toxic liver injury is permitted by hepatocellular death and subsequent attraction of inflammatory cells, the latter removing dead hepatocytes, has come into debate, since newer data have shown that ICAM-1, which is crucial for immigration of inflammatory cells into the liver tissue, may be expressed from sinusoidal cells before the appearance of necrotic hepatocytes in the rat model of carbon tetrachloride-induced acute liver injury (Neubauer et al., 1998). TNF-α released early after carbon tetrachloride intoxication participates in the down-regulation of platelet endothelial cell adhesion molecule 1, which may represent an important event in the sinusoidal transmigration of inflammatory cells (Neubauer et al., 2000). According to all these findings we can say Cytokines plays a critical role in many aspects of immune system development, immune response regulation, and T cell-mediated tissue injury specially TNFα that has both proinflammatory and immunoregulatory properties. Also it considered as a critical growth factor for thymocytes and plays an important role in the peripheral immune system in antigen-presenting cell function and in regulating apoptosis of potentially autoreactive T cells. It may also foster tissue regeneration in the liver (Diehl, 2000).

Our results exhibited a marked amelioration in hepatic cytokines (TNF-α, IL-6 and IL-1β) as a result of EPO and EVOO administration; referring to their highly potent immune-modulatory and anti-inflammatory effects. These results are in agreement with those of (Yan et al., 2013; Sekhon-Looduet al., 2014 and Claudio et al., 2015). Beneficial effects for EPO have been reported in several diseases as eczema, asthma, rheumatoid arthritis, breast problem, fibromyalgia syndrome, premenstrual, menopausal syndrome, diabetic neuropathy, mastalgia, atopic dermatitis, several autoimmune conditions and gastrointestinal symptoms(Rodgers et al., 2009; Ola and Omran, 2012). EPO is a rich source of the ω-6
essential fatty acid, linoleic acid, and γ-linolenic acid (GLA), precursors of the series-1 prostaglandins (*FIG. 10*).

**N-6 FATTY ACIDS**

Linolenic Acid (LA)
Vegetable oils

γ-Linolenic Acid (GLA)
human milk
evening primrose oil

DIHOMOGAMMA-LINOLENIC ACID (DHLA)

ARACHIDONIC ACID ➔ PG 2 Series, LT 4 Series

**LONG CHAIN FATTY ACIDS**

*FIG. 10* long chain fatty acids in evening primrose oil. (Jennifer, 2003).

Essential fatty acids are incorporated into cell membranes where they play a vital role in the structure of cell membranes, influencing membrane flexibility, fluidity, and the behavior of membrane-bound proteins. Essential fatty acids serve as a source of eicosanoids. Consumption of GLA (18:3 ω-6) favors an increase in the dihomogammalinolenic acid (DGLA) content of cell membranes without a corresponding increase the arachidonic acid concentration (Fan and Chapkin, 1998). Ingestion of EPO elevates concentrations of DGLA (20:3 ω-6), enhancing production of eicosanoids of the prostaglandin 1 series (PG1). In addition, DGLA, which itself cannot be converted to leukotrienes, can form a 15-hydroxyl derivative that blocks the transformation of arachidonic acid to leukotrienes. Increased DGLA may act as a competitive inhibitor of the proinflammatory eicosanoids, prostaglandin 2 and leukotriene 4 series, which promotes TNF-α, IL-6 and IL-1β production (Cuiying et al., 2016) are produced from arachidonic acid (20:4 ω-6) (see Figure 10). Membranes rich in DGLA favor formation of the prostaglandin 1 series of eicosanoids and reduce leukotriene synthesis. Membranes rich in DGLA favor a less inflammatory state. On stimulation, DGLA can be converted by inflammatory cells into compounds that possess both anti-inflammatory and anti-proliferative properties. Chronic inflammation is reduced by suppression of T lymphocytes. EPO is also used to correct an ω-6 fatty acid deficiency (Jennifer, 2003).
The olive fruit and virgin olive oil are essential components of the Mediterranean diet, a nutritional regimen gaining ever-increasing recognition for its beneficial effects on human health (Urpi-Sardà et al., 2012 and Escrich et al., 2011). There is some evidence that the effects of olive oil on immune function in animal studies are due to high content of oleic acid, but there is also growing evidence that the high content of phenolic compounds in virgin olive oil have demonstrated anti-inflammatory, antioxidant and antineoplastic activities (Procopio et al., 2009 and Yaqoob, 2013). Among these, hydroxytyrosol (HT), which is abundant in the aqueous fraction of olive pulp, is a simple phenolic compound with marked antioxidant activity (Jemai et al., 2009 and Tutino et al., 2012).

Silva et al., reported that hydroxytyrosol has the most bioavailability in olive oil components (Silva et al., 2014). Olive oil phenolic compounds are mainly absorbed in the small intestine (Vissers et al., 2002) and therefore the increase of hydroxytyrosol bioavailability, in olive oil, might be related to the rate of gastric emptying and slow release of hydroxytyrosol from the oil matrix (Gonzalez et al., 2010). Moreover hydroxytyrosol is protected from degradation in the gastrointestinal tract, before absorption, due to the presence of other antioxidants, in olive oil, thus improving bioavailability (Tuck et al., 2001).

Claudio et al., observed that HT not only increases PPAR-α levels, but also restores the expression of its downstream regulated gene FGF2, a cytokine/hormone, in the liver (Claudio et al., 2015). It was found that PPAR-α is an important regulator of inflammatory process (Gervois and Mansouri, 2012). The anti-inflammatory effects of all classes of PPARs rely primarily on genomic mechanisms of transrepression of transcriptional factors, i.e. nuclear transcription factor-kB (NF-kB), which induce the reduction of pro-inflammatory cytokines and enzymes (Wahli and Michalik, 2012). Therefore, similarly to PPAR-γ, it is conceivable that PPAR-α activity could modulate metabolic disorders associated with inflammation either through its metabolic activity or its anti-inflammatory effects. Recently, the current knowledge concerning the main biological properties of HT was summarized, including its anti-inflammatory effects (Granados et al., 2010). Here, we demonstrated that HT significantly reduces the expression of inflammatory cytokines (TNF-α, IL-6 and IL-1β) in the liver,
confirming its anti-inflammatory activity, likely associated to its ability in controlling metabolic alterations through PPAR-α recover.

Antioxidants are substances that either directly or indirectly protects cells against adverse effects of xenobiotics, drugs, carcinogens and toxic radical reactions (Kamesh and Sumathi, 2012). Oxidative stress results from an imbalance between the cellular production of reactive oxygen species (ROS) and the antioxidant mechanisms that remove them (Posadas et al., 2009). Reactive oxygen species are different forms of activated oxygen inevitably produced in living organisms as by-products of regular metabolism or from external sources. These oxygen forms can easily react with most biological molecules including proteins, lipids, lipoproteins and nucleic acids, and cause progressive decline in cell function resulting in a variety of pathophysiological disorders (Botsoglou et al., 2010).

Liver is the primary site of drug metabolism and has one of the highest antioxidant enzyme capacities in the body. These antioxidant enzymes limit the effects of oxidant molecules on tissues and are active in the defense against oxidative cell injury thanks to the fact that they are free radical scavengers (Nakbi et al., 2010). Hence, our study can suggest that the significant decrease of the antioxidant enzyme activities and the marked increment of MDA contents in the liver proved the failure of antioxidant defense system to overcome the influx of ROS generated by CFA administration which result in increased oxidative stress, protein damage and lipid damage. These results run parallel with Amanda et al., (2016). Moreover, the oral administration of EPO and EVOO recovered the activities of hepatic antioxidant enzymes (CAT, SOD and GPx), and reduced the product of lipid peroxidation (e.g. TBARS) and total glutathione content, which may have resulted from the stabilization of plasma membrane as well as the repair of the hepatic tissue damage induced by CFA which result in increased oxidative stress, protein damage and lipid damage. These results are in agreement with those of Shahidi and Ambigaipalan, (2015) and Kasdallah et al., (2008). Evening primrose contain three major low molecular-weight phenolic compounds, namely (+) catechin, (−) epicatechin, and gallic acid (Wettasinghe et al., 2002). Shahidi (2000) reported that the stronger antioxidant activity of evening primrose may be attributed to its tannin components. More recent studies have suggested that the antioxidant properties of evening primrose may arise from phenolic acids such as gallic, caffeic, p-hydroxybenzoic, vanillic, ferulic and salicylic acids, as well as proanthocyanidins and flavanols (Puri, 2004), catechin and epicatechin derivatives (Wettasinghe and Shahidi, 2002) or protocatechuic and gallic acids and esters (Peschel et al., 2007). Salicylic, p-hydroxybenzoic, 2-hydroxy-4-
methoxybenzoic, vanillic, m- and p-coumaric, gallic, ferulic and caffeic acids are found in smaller quantities in evening primrose seeds (Ribas-Agusti et al., 2011). Murat et al., 2011 reported that these phenolic constituents result in a tendency to bind to free radicals. Furthermore, essential fatty acids inhibitory effect on certain enzymes (i.e. cyclooxygenase and elastase), which trigger the generation of free radicals in the organism, by means of competitive binding to the enzymes, and its strengthening the glutathione-dependent antioxidant defense system, could be mentioned to explain the mode of action of evening primrose oil (Hamburger et al., 2002 and Puri, 2004). Kasdallah et al., showed that the oral supplementation of olive oil to rats administered ethanol chronically restored damage caused to the liver by inhibiting lipid peroxidation and improving enzymatic activities (Kasdallah et al., 2008). The mechanism proposed to explain the positive effects of olive oil may be attributed to its richness in MUFA, mainly oleic acid which has different effects on lipid profiles and peroxidation in hepatic mitochondria (Ochoa et al., 2001). Indeed, EVOO contains a considerable amount of phenolic compounds as oleuropein, hydroxytyrosol, tyrosol and caffeic acid (Fig. 12), which all have potent inhibition effects against ROS (Owen et al., 2000 and Feng et al., 2008). Hydroxytyrosol is highly effective against DNA damage by peroxynitrite in vitro. Caffeic acid phenethyl ester and its related compounds limit the functional alterations of the isolated mouse brain and liver mitochondria submitted to in vitro anoxia-reoxygenation (Feng et al., 2008).

Fig12. Chemical structures of some phenolics found in olive oil. (Shahidi and Ambigaipalan, 2015)

Mitochondrial impairment causes enhanced ROS production, which in turn self-sustains organelle damage. In particular, products of cellular lipid peroxidation (i.e. MDA) associated to inflammatory cytokines (i.e. TNF-α), contribute to mitochondrial dysfunction by interfering with mitochondrial respiratory chain and by forming superoxide anion
(Sanchez et al., 2000). Indirectly, TNF-α promotes mitochondrial dysfunction, increasing RNS as consequence of iNOS induction (Wu et al., 2009). For this reason, the antioxidant activity of hydroxytyrosol (HT) has been well examined. HT significantly reduces ROS production, MDA levels and protein nitrosylation. Our data are in agreement with (Claudio et al., 2015 and Zheng et al., 2015), where, HT improves mitochondrial function and reduce oxidative stress potentially through activation of the activated protein kinase (AMPK) pathway in the brain. In particular, AMPK induces the phosphorylation of acetyl CoA carboxylase (ACC), a modification that inactivates the enzyme, reducing the formation of malonyl-CoA. The decrease in malonyl-CoA synthesis, in turn, reduces liver carnitine palmitoyltransferase1 (CPT1) inhibition, thus prompting adequate shunting of fatty acids into the mitochondria and hence their oxidation (Aguilera et al., 2008). Here, we show that HT increases the phosphorylation of ACC and the transcription of liver CPT1, both remarkably reduced by EVOO. The restoration of CPT1 was consistent with the normalization of its transcriptional regulatory factor PPAR-α. Our results are in agreement with a previous study (Alberdi et al., 2013).

Conclusion:

EPO and EVOO administration exhibited a beneficial therapeutic effect on rats with hepatic inflammation due to the presence of omega-3 Fatty acids and polyphenols, which showed anti-inflammatory and antioxidant action.

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