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REVIEW ARTICLE

Liver Protective Effects of Extra Virgin Olive Oil: Interaction between Its Chemical Composition and the Cell-signaling Pathways Involved in Protection

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Abstract: ***Background and Objective:*** The liver is an organ susceptible to a multitude of injuries that causes liver damage, like steatosis, non-alcoholic steatohepatitis, cirrhosis, hepatocellular carcinoma, and ischemia-reperfusion injury. Extra virgin olive oil (EVOO), presents several protective effects on the liver, reducing hepatic steatosis, hepatocyte ballooning, fibrogenesis, preventing lipid peroxidation, among other effects. Due to its high levels of monounsaturated fatty acids, mainly oleic acid and phenolic compounds, such as hydroxytyrosol and oleuropein, EVOO is able to participate in the activation of different signaling pathways in the hepatocytes involved in the prevention of inflammation, oxidative stress, endoplasmic reticulum stress, mitochondrial dysfunction, and insulin resistance, allowing the prevention or resolution of liver damage. The aim of this work is to offer an update of the molecular effects of EVOO in the liver and its protective properties to prevent the establishment of liver damage through the regulation of different cell-signaling pathways.

Methods: Searches that considered the effects of EVOO in *in vivo* and *in vitro* models, whith emphasis in the molecular mechanism of liver tissue damage and prevention and/or treatment of steatosis, steatohepatitis, cirrhosis, hepatocellular carcinoma, and ischemia-reperfusion injury.

Conclusion: The most relevant molecular effects of EVOO involved in the prevention or resolution of liver damage are: *(i)* Activation of the nuclear transcription factor erythroid-derived 2-like 2 (Nfr2), inducing the cellular antioxidant response; *(ii)* Inactivation of the nuclear transcription factor-κB (NF-κB), preventing the cellular inflammatory response; and *(iii)* Inhibition of the PERK pathway, preventing endoplasmic reticulum stress, autophagy, and lipogenic response.

Keywords: Extra virgin olive oil, liver damage, protective molecular mechanism, hydroxytyrosol, cell-signaling pathways.

1. INTRODUCTION

The liver is considered as one of the largest organs in the human body, playing vital roles in metabolic, digestive, immunological, reservoir, and homeostatic functions, the functional unit being the hepatocyte representing about 80% of the liver cells in man [1]. Metabolically, the liver performs most of the intermediary pathways, biotransformation of xenobiotics, and plasma protein biosynthesis, with excretion and secretion of several types of biomolecules including Kupffer-cell dependent cytokines and hepatokines produced by the hepatocytes [2, 3]. The high energy requirements for the maintenance of these hepatic functions and cell proliferation are mainly provided by fatty acid (FA) β-oxidation, which make the liver susceptible to O_2 availability such as hypoxia [2], pathogens of viral origin, and toxic substances, leading to acute or chronic liver damage [4, 5]. In the case of chronic alcohol consumption, which promotes lipid peroxidation, necrosis, and eventually liver damage [6], a decreased expression and activity of peroxisome proliferator-activated receptor alpha (PPARα), involved in FA oxidation, and increases in those of sterol regulatory element binding protein-2 (SREBP-2) and SREBP-1c is observed, transcriptional factors related to the regulation of cholesterogenic and lipogenic enzymes, respectively, thus leading to fat accumulation in the hepatocytes [6]. Also, toxicants such as benzopyrene, carbon tetrachloride (CCl_4), and cadmium that induce hepatic lipid peroxidation with reduction in the levels and activity of superoxide dismutase (SOD) and catalase (CAT) significantly enhance the oxidative stress (OS) status of the liver [7-9] (Table **1**). In addition, metabolic conditions such as obesity and insulin resistance (IR) that trigger the onset of steatosis, inflammation, and mitochondrial dysfunction also develop an enhancement in the OS status of the liver, allowing tissue damage [10-12]. All of these aggres-

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sions may lead to the development of multiple diseases, such as acute and chronic hepatitis, alcoholic fatty liver disease, and non-alcoholic fatty liver disease (NAFLD), among others.

Olive oil is a vegetable product obtained from the mesocarp of the fruit of the olive tree by a process of cold pressing [13]. The product of the first pressing of the olive is the extra virgin olive oil (EVOO), which is considered the gold standard of edible oils. The EVOO composition is rich in monounsaturated fatty acids (MUFAs, 55-83%), mainly oleic acid (OA, C18: 1 n-9), followed by 4-20% of polyunsaturated fatty acids (PUFAs), and 8-14% of saturated fatty acids (SFAs) [13].

The most abundant triglyceride in olive oil is that containing three oleic acid (OA) molecules [14], the digestion of which begins in the stomach by the action of gastric lipase. Once in the intestine, the action of various enzymes, especially pancreatic lipase, degrades the triglycerides to monoglycerides, mixed micelles are formed and absorbed through the membrane of enterocytes, where they are re-esterified into triglycerides to form chylomicrons for transport. About 15% of the absorbed OA is directly transported by the portal vein to the liver and 85% passes through the lymph [14].

1-2% of the weight of EVOO is represented by other minor components, including *(i)* A non-saponifiable (apolar) fraction, represented by squalene, triterpenes, sterols, tocopherols and pigments, which can be extracted with solvents; and *(ii)* A polar fraction, where phenolic compounds are prominent, and to which many beneficial effects on human health have been attributed [15] (Fig. **1**). The content of these components varies according to the type of crop, climate, maturity of the olive at the time of harvest, and the industrial process used to produce EVOO [15]. Among phenolic compounds of EVOO, it is possible to find simple phenols (hydroxytyrosol (HT) and tyrosol) or secoiridoids (derivatives of oleuropein and ligstroside) [16]. After consumption, the secoiridoids are hydrolyzed in the stomach in a time-dependent manner, which increases the amount of free HT and tyrosol. The main site for the absorption of these polyphenols is the small intestine through passive diffusion [16], which mainly accumulates in plasma, urine, and liver in a dose-dependent manner [17].

There is important evidence that indicates that some of the components of EVOO, such as OA and polyphenols like HT, show protective effects on the liver in various experimental models, especially in cellular cultures and animal protocols [18, 19] (Table **1**). These components significantly diminish hepatic fibrogenesis [6, 20], liver steatosis [19, 21], and hepatocyte ballooning, preventing lipid peroxidation in rat liver [22] and various other metabolic parameters such as IR induced by high fat diets in animal models. These effects are associated with the modulation of various enzymatic pathways that promote FA oxidation, reduce inflammation and OS, and prevent hepatic tissue damage [18, 23]. In view of these evidences, the aim of this review was to present an update of the molecular effects of EVOO in the liver and its protective properties to prevent the establishment of liver damage (Fig. **1**).

2. METHODOLOGY

The review included various searches that considered the effects of EVOO in *in vivo* and *in vitro* models, using the PubMed database from the National Library of Medicine-National Institutes of Health and the Sciencedirect database from Elsevier. The emphasis of this review was placed in the participation of EVOO and its components on the molecular mechanism of tissue damage in the liver especially on the prevention and/or treatment of steatosis, steatohepatitis, cirrhosis, hepatocellular carcinoma, and ischemia-reperfusion injury.

3. EVOO PROTECTION IN LIVER DAMAGE

3.1. EVOO and Hepatic Steatosis

Hepatic steatosis is a condition with few associated complications, characterized by accumulation of triacylglycerols (TGs) in lipid vacuoles within hepatocytes [24]. There are two forms of steatosis, namely, microvesicular and macrovesicular, the latter being the most common form of TG accumulation in the liver that is characterized by the presence of one or two large fat globules displacing the nucleus to the edge of the hepatocyte [24, 25]. Different molecular events can result in the accumulation of lipids in hepatocytes, including (*i)* Increased lipid uptake in the liver due to increased lipolysis in white adipose tissue; *(ii)* Enhanced hepatic lipogenesis; *(iii)* Deficient synthesis and/or secretion of very-low density lipoproteins (VLDLs), sequestering TG and cholesterol in the liver; *(iv)* Reduction of mitochondrial β-oxidation; and *(v)* Deficiency of peripheral lipid storage due to pathologies such as IR, leading to TG accumulation in the liver [10].

EVOO, rich in MUFAs, has been used for preventive and treatment purposes in liver steatosis, both alone [26, 27] (Table **1**) or in conjunction with other compounds, such as n-3 PUFAs especially EPA (C20:5 n-3) and DHA (C22:6 n-3) [28]. However, there is a dual effect when we referrer to OA, considering that different studies in cellular and animal models suggest that OA is able to induce or prevent steatosis through the attenuation of inflammation and oxidative stress, therefore more studies are needed to elucidate this discrepancy [29]. Also, an animal study determined that EVOO supplementation did not affect the levels of OA in phospholipids from hepatocytes, hence the multiple effects seen regarding the improvement of the antioxidant status in the liver could be attributed to the phenolic fraction of olive oil [30]. EVOO, which is in particular rich in polyphenols, decreases FFA-induced steatosis in HepG2 cells, through reduction in the number and size of fat globules and TG accumulation [31], and is capable of mitigated fat accumulation in mice [32]. A study in high-fat diet (HFD) fed rats with hepatic steatosis showed that HT supplementation (10 mg/kg/day) restored PPARα and carnitine palmitoyltransferase 1 (CPT1) levels, with promotion of β-oxidation. In addition, HT, one of the most effective antioxidants of natural origin and present in EVOO, was able to decrease the production of proinflammatory cytokines such as TNF-α, which resulted in a histological decrease of steatosis and inflammation [18].

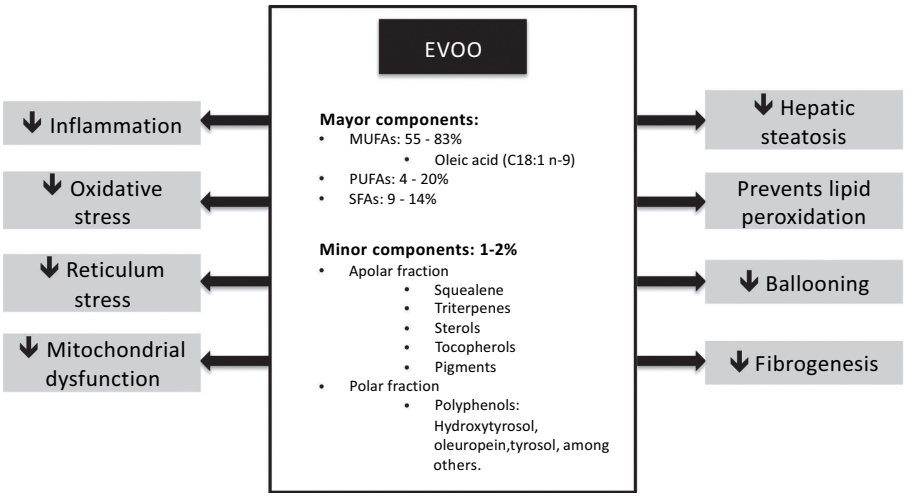

Fig. (1). EVOO components and actions. Bioactive compounds and beneficial effects of EVOO.

3.2. EVOO and Non-Alcoholic Steatohepatitis

Non-alcoholic steatohepatitis is a condition characterized by the presence of predominantly macrovesicular steatosis and lobular and portal inflammation with hepatocyte ballooning, with or without fibrosis [33, 34]. EVOO supplementation plays a important role diminishing the inflammation and OS, preventing the progression of liver damage to steatohepatitis [30]; EVOO rich in polyphenols has been able to increased the antioxidant parameters in the liver and reduces the pro-oxidant ones [30]. HT has important reducing effects on inflammation and OS, thus preventing the progression of liver damage to steatohepatitis. Within the anti-inflammatory mechanisms of HT, characterized in a human cell line of THP-1 monocytes, inhibition of nitric oxide synthase and cyclooxygenase-2 (COX-2) expression was identified, concomitantly with decreased TNF-α transcription and suppression of lipopolysaccharide-dependent nitric oxide formation [35]. In addition, HT is able to eliminate reactive oxygen species (ROS) and activate the endogenous defense system mediated by Nrf2 [21] (Table **1**), thus decreasing OS that allows the progression to steatohepatitis, with improvement of the general clinical prospect.

3.3. EVOO and Cirrhosis

Hepatic cirrhosis is a chronic disease considered irreversible that alters the structure and function of the liver. Histopathologically, it is defined as a triad of cellular necrosis, fibrosis, and regeneration nodules, hepatic dysfunction being the most important clinical manifestation [36]. Cirrhosis is the end stage of various chronic liver diseases and a terminal phase of liver damage [37], in which fibrosis is characterized by increased production of the extracellular matrix by the stellate cells and myofibroblasts [38]. An important number of studies have evaluated the effects of EVOO on fibrosis induced by CCl_4 administration. Wang *et al*. showed that rats intoxicated with CCl_4 and fed with EVOO had less derangements in the hepatic architecture with lower formation of fibrous tissue [7]. EVOO was also able to induce decreased lipid peroxidation and expression of smooth muscle actin alpha (α-SMA), a protein involved in cell structure, thus decreasing fibrosis [7], as also found in the CCl_4 model [20].

3.4. EVOO and Hepatocellular Carcinoma

Inflammation and tissue damage generated by liver cirrhosis can lead to dysplastic lesions in hepatocytes involving DNA damage, which may eventually trigger liver cancer [39]. Hepatocellular carcinoma is the most common primary liver cancer in adults [40] and hepatic cirrhosis is the most important predisposing factor for the development of hepatocellular carcinoma [41].

Several studies have tested the effect of polyphenols of EVOO on different types of cancer [42]. Referred to hepatocellular carcinoma, HT was shown to inhibit the enzyme xanthine oxidase with reduction in superoxide anion production, thus protecting against DNA damage [19] (Table **1**). A study conducted in cell lines of human hepatocellular carcinoma showed that HT is able to induce apoptosis and inhibit the proliferation of cancer cells by suppressing the activation of nuclear factor-κB (NF-κB), a transcription factor involved in proinflammatory signaling, with the consequent decrease in the transcription of genes regulated by NF-κB [19]. This is accomplished by a HT-induced suppression of AKT activation, which otherwise accelerates the degradation of the inhibitor of κB (IκB) and phosphorylates NF-κB-p65 subunit promoting the translocation of NF-κB to the nucleus and its transcriptional activity [19, 43]. In agreement with these views, 10-80 μM of the HT precursor oleuropein present in EVOO and added to human hepatoma cell lines showed a dose-dependent enhancement in cellular apoptosis, with inhibition of cell growth and colony formation, as results of the inactivation of the PI3K/AKT pathway [44] (Table **1**).

3.5. EVOO and Ischemia/Reperfusion (I/R) Injury

I/R is a condition characterized by a transient or permanent decrease of the blood flow to an organ for a given time period, followed by the restoration of blood flow [45]. During ischemia of the liver, the lack of oxygen in the tissue and the conversion of the hepatocellular metabolism to anaerobic

pathways [46] induce a proinflammatory state that leaves the tissue vulnerable to reperfusion [47]. Rapid re-establishment of blood flow is necessary to restore the cellular functions lost during ischemia, but reperfusion is also capable of initiating a cascade response of OS causing deep injury in hepatocytes [46]. Hepatic injury by I/R is characterized by OS, inflammation, and cellular apoptosis [48] (Table **1**).

There are few studies evaluating the effect of EVOO or some of its components on hepatic I/R damage. In this respect, Pan *et al*. [48] evaluated the effect of HT on an *in vivo* mouse model of surgery-induced I/R. HT (10 mg/kg) administered 4 hours before ischemia achieved *(i)* Reduced serum aspartate transaminase (AST) and alanine transaminase (ALT) levels compared to controls; *(ii)* Diminished cellular apoptosis and accumulation of ROS and malondialdehyde (MDA) as indicators of oxidative damage; *(iii)* Attenuation of the increase in the inflammatory mediators TNF-α, IL-6, and macrophage inflammatory protein 2 (MIP-2) [48]. Furthermore, using an *in vitro* model of anoxia/reoxygenation in an anaerobic chamber, HT (100 μM) decreased the number of apoptotic cells and the levels of ROS [48], whereas in I/R injury subsequent to liver transplantation or surgical resection, HT increased the activity of SOD 1 and SOD 2, enzymes that undertake superoxide radical elimination thus protecting the hepatocyte from oxidative damage [48].

4. EVOO AND THE MOLECULAR MECHANISMS OF HEPATIC PROTECTION

The effect of EVOO on hepatocytes from healthy rats, compared to refined olive oil lacking polyphenols, but with the same fatty acid profile, highlighted the synergistic effect of polyphenols on the inhibition of FA release and cholesterol synthesis [26]. Moreover, oleuropein and HT in particular (25 μM) inhibited the synthesis of FA and cholesterol [47] (Table **1**), which is related to the decrease in the enzymatic activity of lipogenic acetyl-CoA carboxylase (ACC), thus promoting FA β-oxidation [49, 50]. An experimental model of NAFLD induced by a HFD showed that EVOO reduces glycaemia and insulin resistance due to greater AKT phosphorylation and GLUT2 expression in hepatocytes, by improving glucose uptake and the approach of the insulin receptor to the membrane [51, 52]. Additionally, EVOO *(i)* improves lipid metabolism by increasing AMPK phosphorylation; *(ii)* decreases inflammation by lowering TNF-α and IL-1 levels; *(iii)* diminishes the expression of COX-2 and lipid peroxidation, thus favoring the integrity of the hepatocyte; and *(iv)* induces PPARα upregulation and SREBP-1c downregulation, with enhancement of FA β-oxidation and inhibition of hepatic lipogenesis that decrease the accumulation of TG [32, 51, 53, 54] (Table **1**).

HT is known to mediate lipid metabolism with reduction of the size of the lipid globules and the accumulation of TG in the hepatocytes [18]. Molecularly, HT binds to the cytoplasmic domain of PPARα favoring PPARα heterodimerization with 9-cis-RXR [55-57] and the translocation of the PPARα-RXR dimer to the nucleus, binding to the PPAR response zone in the promoter region of the target genes regulated by this transcription factor [58-60]. Among these genes is that coding for CPT-1α, which is involved in the transport of FAs to the interior of the mitochondria through the carnitine-dependent system that promotes β-oxidation. On the other hand, HT also increases the translation of ACOX-1, a key peroxisomal enzyme indispensable for the oxidation of long hydrocarbon chain FAs [47, 53, 59, 60]. Besides, HT triggers ACC phosphorylation through activation of the AMPK pathway, leading to ACC inactivation, which decreases malonyl CoA levels, reduces hepatic CPT-1α inhibition, favors β-oxidation, and diminishes FA synthesis to attenuate steatosis [50]. By suppressing NF-kB, HT reduces inflammation and OS [19], which with the concomitant activation and greater translocation of Nrf2 to the nucleus, HT upregulates the endogenous antioxidant defense system that reduces lipid peroxidation and hepatocyte ballooning, thus avoiding the progression of liver steatosis to steatohepatitis or cirrhosis [18, 19, 21, 23]. Likewise, polyphenols from EVOO such as oleuropein (10-80 μM) inhibit cell growth and increase cell apoptosis in human hepatoma cell lines by inducing *(i)* The cleavage of caspases, which are essential initiators or effectors of cell apoptosis; *(ii)* Upregulation of the expression of BAX mRNA and protein and downregulation of Bcl-2, mitochondrial proteins that are involved in apoptosis; and *(iii)* Reduction in the levels of phosphorylated active AKT, favoring apoptosis by suppression of the PI3K/AKT pathway [44] (Fig. **2**).

5. METABOLIC PATHWAYS MODULATED BY EVOO IN THE LIVER

5.1. Activation of Transcription Factor Nrf2

The activation of Nrf2 induces a cellular antioxidant response through the gene expression of antioxidant enzymes and enzymes involved in cell detoxification, which are capable of protecting the cell from OS and the related damage it produces, both factors being relevant in the progression of liver damage. Among the antioxidant enzymes involved are *(i)* Glutathione peroxidase (GPx) and glutathione S-transferase (GST) involved in the removal of peroxides that induce oxidative damage; *(ii)* Glutathione reductase (GR), which reduces oxidized glutathione to reduced glutathione, a strong non-enzymatic antioxidant molecule [61-63]. HT, known for its antioxidant activity, is able to increase the levels of phosphorylated AKT and ERK in hepatocytes, increasing Nrf2 phosphorylation at serine or tyrosine residues, which achieves the dissociation of the Nrf2/Keap1 complex that maintains Nrf2 in the cytosol.

This increases the translocation of Nrf2 to the nucleus where it binds to the antioxidant responsive element (ARE) to increase the expression and activity of GPx, GST, and GR, resulting in a decrease of the OS status and in the associated damage [21, 64]. However, no increase in the activity of Nrf2-dependent phase II enzymes was observed in a randomized, double-blind study in healthy humans subjected to 5 and 25 mg HT/day for a week, which could be due to the doses or the short time of the treatment [65]. Therefore, more studies in humans are needed to verify the possible protective action of HT (Table **1**; Fig. **1**).

5.2. Inactivation of Transcription Factor NF-κB

NF-kB is a transcription factor that plays a key regulatory role in the expression of genes coding for cytokines and for

Table 1. Summary of the effects of EVOO and its components on liver protection in different study models.

	References	Model	Intervention	Results/Findings
a) Steatosis	Pirozzi *et al*. 2016 [18]	Animal	Spraugue-Dawley male rats were divided into three groups: Control group, HFD group and HFD with HT (10 mg/kg/day)	- ↓ microvesicular steatosis and ballooning - Avoid liver inflammation - ↓ glycemia, insulin and HOMA index - Restoration of PPARα, CPT1α and FGF21 mRNA levels - ↑ ACC phosphorylation - Prevention of increased TNF-α and IL-6 mRNA - ↓ COX-2 expression - ↓ Lipid peroxidation and ROS production
	Priore *et al*. 2015 [26]	Cellular	Hepatocytes of healthy Wistar rats were incubated for four hours with olive oil high in polyphenols (HPOO) or olive oil poor in polyphenols.	- HPOO ↓ FA and cholesterol synthesis - HPOO ↓ ACC activity
	Rincon-Cervera *et al*. 2016 [30]	Animal	C57BL/6J male mice randomly assigned to one of eight groups with EVOO supplementation with ascending levels of polyphenols: CD; CD + EVOO I; CD + EVOO II; CD + EVOO III; HFD; HFD + EVOO I; HFD + EVOO II; HFD + EVOO III.	- EVOO supplementation was able to restored DHA levels, and n6/n3 ratio levels in the liver that was altered by HFD. - EVOO ↑ the antioxidant parameters in the liver (glutathione, glutathione equivalents and glutathione/glutathione disulfide ratio). - EVOO III ↓ pro-oxidant parameters (F2-isoprotones and TBARS). - EVOO III restored to CD values the activity of Δ5 and Δ6 desaturase in the liver. - EVOO ↓ SREBP 1c expression and DNA-binding activity. - EVOO ↑ SOD, CAT, GPx and GR activity to control values. - EVOO regulated ACC, FAS and CPT-1 levels altered by HFD.
	Valenzuela *et al*. 2017 [32]	Animal	C57BL/6J male mice randomly assigned to one of four experimental groups: CD (10% Kcal as fat); CD supplemented with HT (5 mg/day); HFD (60% Kcal as fat); HFD supplemented with HT.	- HT improved serum parameters like triacylglycerol, total cholesterol and LDL-cholesterol and markers of insulin resistance (Fasting glucose, insulin and HOMA index) in HFD mice. - HT mitigated the fat accumulation in hepatocytes of HFD mice, and attenuated the score of steatosis in the same group. - HT recovered to control values the activity of Δ-5 and Δ-6 desaturases in the HFD group, also recover control values of the mRNA expression of both desaturases that was augmented whit HFD. - HT reduced both the expression and DNA binding activity of SREBP 1-c.
	Priore *et al*. 2014 [49]	Cellular	Hepatocytes of healthy Wistar rats were incubated for two hours with HT, tyrosol or oleuropein (Ole).	- ↓ 50% in lipid synthesis (HT y Ole) - ↓ 30% in cholesterol synthesis - ↓ 40% of ACC activity (HT y Ole) - ↓ Diglyceride acyltransferase (DGAT) and Hydroxymethylglutaryl-CoA Reductase (HMGCR) activity - 40% activation of ACC phosphorylation (HT y Ole) - 60% activation of AMPK phosphorylation (HT y Ole)

Table (1) contd….

	Reference	Model	Intervention	Results/Findings
	Lama *et al*. 2017 [51]	Animal	Male Sprague-Dawley rats were divided into four groups based on the different types of diet: standard diet (STD); HFD; HFD containing Olive oil without polyphenols (WPOO), and HFD containing Polyphenol-rich virgin olive oil (HPCOO).	- HPCOO ↓ALT and TG that were increased by HFD. - HPCOO improved the unbalance between pro-inflammatory (TNF-alpha and IL-1) and anti-inflammatory mediators (IL-10). - HPCOO ↓ TNF-alpha and COX-2 mRNA levels, ROS production, and MDA levels.
	Valenzuela, *et al*. 2017 [53]	Animal	C57BL/6J male mice randomly assigned to one of four experimental groups: CD (10% Kcal as fat); CD supplemented with HT (5 mg/day); HFD (60% Kcal as fat); HFD supplemented whit HT.	- HT was able to restore the activity of GST and GGT that were decrease by HFD. - HT improved the effects of HFD on the activity of ACC and FAS (both increased by HFD) and CPT-1 (decrease by HFD). - HT recovered the mRNA expression of PPARα, ACOX-1 and CPT-1 that were reduced by HFD, and partially recover the normal values of gene expression of NF-κB (increase by HFD). - HT achieved a partial recovery of the DNA-binding activity of PPARα (decreased by HFD) and NF-κB (increased by HFD), and total recover the values of Nrf2 (decreased by HFD).
	Jurado-Ruiz *et al*. 2017 [54]	Animal	C57BL/6J male mice randomly assigned to standard diet or HFD for 12 weeks, this last group was then divided into: HFD-L (where the main source of fat is lard); HFD-EVOO (main source of fat is EVOO); HFD-OL (main source of fats is from EVOO rich in polyphenols); and R group (low fat diet) for 24 weeks.	- EVOO supplementation groups had more OA in the phospholipids in the liver. - HFD-OL ↑ cd36 fatty acids transporter expression. - HFD-EVOO and HFD-OL ↑ expression of Pnpla3 (which activated triglyceride hydrolase). - HFD-OL group show a trend to augmented the expression of PPARα.
b) Non-alcoholic steatohepatitis	Devi *et al*. 2008 [8]	Cellular	Male Wistar Albino rats microsomes were incubated according to one of three groups: Vehicle control, Benzopyrene treatment (25 uM) and olive oil cotreatment (10 µL of olive oil).	Olive oil exposure attenuated the decreased of antioxidant enzymes and the increased of lipid peroxidation products (effect of benzopyrene).
	Martín *et al*. 2010 [21]	Cellular	Human cell line HepG2 were cultured with HT dissolved in it at 0.5, 1, 5 and 10 µM. To evaluated the protective effect of HT, after HT treatment t-BOOH was added to the plates.	- ↓ Cell death induced by t-BOOH - ↑ GPx, GST and GR activity - ↑ GPx, GST and GR mRNA expression levels and protein expression - ↑ Protein levels of Nrf2 in the nucleus and ↓ in the cytosol, therefore ↑ the translocation of Nrf2 - ↑ AKT and ERK phosphorylated levels → which leads to translocation of Nrf2 to the nucleus
	Baraldi *et al*. 2015 [27]	Animal	C57BL/6J male mice were randomly divided into four groups: Control (corn il; 60% of linoleic acid (LA)); Conjugated linoleic fatty acid (CLA) group; EVOO (78% of oleic acid) and CLA + EVOO group for 60 days.	- EVOO prevented IR - EVOO improved lipid profile - EVOO prevented the increase in ACC1 and UCP2 expression - Lower production of hydrogen peroxide
	Crespo *et al*. 2015 [65]	Human	21 healthy volunteers between 20 and 40 years old. Double-blind, randomized, placebo-controlled study. They tested two HT doses (5 and 25 mg/day)	- There was no significant effect on anthropometric variables. - There were significant time of study and treatment whit HT interaction for isoprostanes (marker of oxidation) and high-sensitive C-reactive protein (marker of inflammation). - There were only significant time effect on GST01 and GSTP1 levels (phase II enzymes).

Table (1) contd…..

	Reference	Model	Intervention	Results/Findings
	Giordano *et al*. 2014 [72]	Cellular	HepG2 cells were treated with 1 µM and 5 µM of HT and 100 µM lipoic acid and glutathione-ethyl ester (GSH) for 24 hours.	- HT ↓ Reticulum stress markers levels (mRNA levels of CHOP and BiP proteins involved in the UPR response).
c) Cirrhosis	Wang *et al*. 2014 [7]	Animal	Fischer male rats were arbitrarily divided into four groups: Corn oil normal group, olive oil normal group, corn oil CCl_4 group and olive oil CCl_4 group. CCl_4 administration was subcutaneous (0.1 mL/100 g body weight, 1:1 mixed with soybean oil)	- Olive oil ↓ CCl_4-induced lipid peroxidation, α-SMA expression and CCl_4-induced fibrosis
d) Carcinoma	Zhao *et al*. 2014 [19]	Cellular	Hepatocellular carcinoma human cell line HepG2, Hep3B and SK-HEP-1 Were incubated with HT (at a rising dose of 0 – 400 µM) for 48 – 72 hours.	- ↓ Cell proliferation - ↑ Cell apoptosis: ↓ expression of pro-caspase-3 and ↑ expression of cleaved PARP - ↓ Phosphorylated AKT concentration (dose dependent) - Suppression of NF-κB DNA binding activity, expression of NF-kB and it regulated genes (Bcl-xL, c-myc, COX-2, Cyclin-D1 and VEGF) (dose dependent)
	Yan *et al*. 2015 [44]	Cellular	HepG2 and Huh7 human hepatocellular carcinoma cell lines were treated with different concentrations of oleuropein (0, 20, 40, 60, 80 or 100 µM) for 24 hours.	- Oleuropein inhibited HepG2 cell growth in a dose-dependent manner and a reduction in the number of colonies formed. - Oleuropein induced cell apoptosis through suppression of the PI3K/AKT signal pathway.
e) I/R	Pan *et al*. 2013 [48]	Animal/Cellular	Mice had I/R injury induce through the clamp of the left and median lobule of the liver. Mice were administrated with 10 mg/kg of HT 12 hours before ischemia. Anoxia/reoxygenation damage (A/R) was induced in mouse hepatocytes. Cells were incubated with 25, 50 and 100 µM of HT 4 hours before anoxia.	- ↓ AST y ALT - ↓ Necrosis focus - ↓ ROS and hepatic oxidative damage - ↓ TNFα, IL-6 and MIP-2 - ↑ SOD and CAT activity - Inhibition of the decrease in cell viability - ↓ ROS - Inhibition of the decrease in the expression of SOD1, SOD2 and CAT

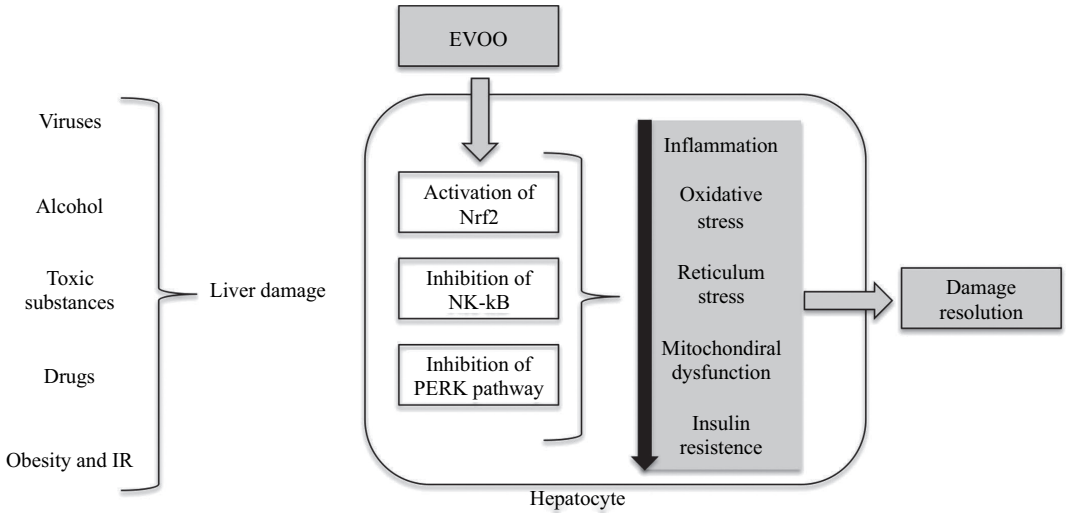

Fig. (2). Molecular effects of EVOO in hepatocytes. Molecular pathway's involved in liver protective effects of EVOO.

proteins involved in inflammation, cell proliferation, apoptosis, angiogenesis, and metastasis among others [66, 67]. NF-kB can be activated by the stimulation of the AKT pathway, *i.e*., when AKT is phosphorylated. In this scenario, EVOO is able to decrease the levels of phosphorylated AKT leading to NF-kB inactivation, thus preventing the cellular inflammatory response [19, 64]. A study conducted in hepatocellular carcinoma cells revealed that HT decreases AKT phosphorylation in a dose-dependently manner, which results in diminished NF-κB DNA binding and in the transcription of genes controlled by this transcription factor, with concomitant induction of apoptosis in the cells [19] (Fig. **1**).

5.3. Inhibition of the PERK Pathway

OS-dependent carbonylation of amino acid residues in proteins is capable of altering protein folding, which upon overloading of unfolded/misfolded proteins constitutes the endoplasmic reticulum (ER) stress phenomenon. This process is sensed by ER of hepatocytes that triggers an adaptive response known as unfolded protein response (UPR), with the aim to decrease the load of unfolded proteins [68]. For this purpose, three transduction signals accounting for the presence of unfolded proteins in the lumen of the organelle are activated. *(i)* Activation of inositol requiring enzyme 1α (IRE-1α) by oligomerization and autophosphorylation, leading to the cut in two positions of the X-box binding protein 1 (XBP1) mRNA; the resulting exons are assembled to generate an mRNA that encodes for a transcription factor that promotes the expression of chaperones and other genes of the UPR [69]. *(ii)* Migration of activating transcription factor 6α (ATF6α) to the Golgi apparatus, where its transmembrane domain is cut off and the n-terminal fragment migrates to the nucleus, where it acts as a transcription factor to increase protein-folding capacity [70, 71]. *(iii)* Activation of double-stranded RNA-dependent protein kinase-like ER kinase (PERK), an enzyme that phosphorylates the alpha subunit of eIF2, leading to the inactivation of this translational initiation factor, thus decreasing protein synthesis that reduces ER stress [72-75] (Table **1**). When these cellular pathways are not sufficient to prevent cell damage and the accumulation of misfolded proteins, cellular autophagy is triggered, in addition to the upregulation of lipogenic transcription factors (SREBP-1c, PPAR-γ) and enzymes (ACC, diacylglycerol acyl transferase 2, stearoyl-CoA desaturase 1) [76].

CONCLUSION

The role of EVOO, especially through HT, is to activate the cellular antioxidant enzymatic machinery by increasing the activity and translocation to the nucleus of Nrf2. In this way, OS decreases or avoids ER stress, thus preventing autophagy, the continuous tissue damage generated by the accumulation of unfolded proteins, and the lipogenic response (Fig. **1**).

LIST OF ABBREVIATIONS

ACC	=	Acetyl-CoA carboxylase
ACOX-1	=	Peroxisomal acyl-coenzyme A oxidase 1
ALT	=	Alanine transaminase
A/R	=	Anoxia/reoxygenation
ARE	=	Antioxidant responsive element
AST	=	Aspartate transaminase
ATF6α	=	Activating transcription factor 6α
CAT	=	Catalase
CCl4	=	Carbon tetrachloride
CD	=	Control diet
CLA	=	Conjugated linoleic fatty acid
COX-2	=	Cyclooxygenase-2
CPT1	=	Carnitine palmitoyltransferasa 1
DGAT	=	Diglyceride acyltransferase
DHA	=	Docosahexaenoic fatty acid
EPA	=	Eicosapentaenoic fatty acid
ER	=	Endoplasmic reticulum
EVOO	=	Extra virgin olive oil
FA	=	Fatty acid
FFA	=	Free fatty acid
GPx	=	Glutathione peroxidase
GR	=	Glutathione reductase
GSG	=	Glutathione-ethyl ester
GST	=	Glutathione S-transferase
HDF	=	High fat diet
HMGCR	=	Hydroxymethylglutaryl-CoA reductase
HPCOO	=	Polyphenols-rich virgin olive oil
HPOO	=	High polyphenols olive oil
HT	=	Hydroxytyrosol
IR	=	Insulin resistence
I/R	=	Ischemia-reperfusion
IRE-1α	=	Inositol requiring enzyme 1α
LA	=	Linoleic acid
MDA	=	Malondialdehyde
MIP-2	=	Macrophage inflammatory protein 2
MUFA	=	Monounsaturated fatty acids
NAFLD	=	Non-alcoholic fatty liver disease
NF-kB	=	Nuclear factor kappa B
Nrf2	=	Nuclear transcription factor erythroid derived 2-like 2
OA	=	Oleic acid
OLE	=	Oleuropein
OS	=	Oxidative stress
PERK	=	Protein kinase-like ER kinase
PPARα	=	Peroxisome proliferator activated receptor alpha
PUFAs	=	Polyunsaturated fatty acids
ROS	=	Reactive oxygen species
SFAs	=	Saturated fatty acids
α-SMA	=	Smooth muscle actin alpha
SOD	=	Superoxide dismutase
SREBP	=	Sterol regulatory element binding protein
STD	=	Standard diet
TGs	=	Triglycerides
UPR	=	Unfolded protein response
VLDL	=	Very-low density lipoproteins
WPOO	=	Olive oil without polyphenols
XBP1	=	X-box binding protein 1

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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