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Review

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Bioactive Properties of the Main Triterpenes Found in Olives, Virgin Olive Oil, and Leaves of *Olea europaea*

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ABSTRACT: Oleanolic acid, maslinic acid, uvaol, and erythrodiol are the main triterpenes present in olives, olive tree leaves, and virgin olive oil. Their concentration in virgin olive oil depends on the quality of the olive oil and the variety of the olive tree. These triterpenes are described to present different properties, such as antitumoral activity, cardioprotective activity, anti-inflammatory activity, and antioxidant protection. Olive oil triterpenes are a natural source of antioxidants that could be useful compounds for the prevention of multiple diseases related to cell oxidative damage. However, special attention has to be paid to the concentrations used, because higher concentration may lead to cytotoxic or biphasic effects. This work explores all of the bioactive properties so far described for the main triterpenes present in virgin olive oil.

KEYWORDS: *Olea europaea, virgin olive oil, antioxidant activity, oleanolic acid, maslinic acid, uvaol, erythrodiol, cancer, cardiovascular, inflammation, oxidative stress*

INTRODUCTION

Nowadays there is an increasing interest in healthy eating habits and physical care to improve our health and quality of life. In fact, government and educational agencies are trying to re-educate the eating habits of the population.

Mediterranean habits are known to be among the healthiest to improve age-dependent vascular activity,[1] and they have proved to be beneficial for several diseases such as the metabolic syndrome or coronary heart disease.[2,3]

Mediterranean habits include exercising regularly and following the so-called Mediterranean diet. This diet consists of bread, cereal, rice, pasta, fruits, and vegetables mainly and olive oil as the principal source of fat.[4] Virgin olive oil has been described to possess bioactive properties such as cardioprotective effects, commonly associated with high levels of monounsaturated fatty acids (MUFA),[5] but these effects would not necessarily be promoted by MUFA alone. Antioxidant and antiatherogenic activities,[6] antiproliferative and pro-apoptotic capacities on human cancer cell lines,[7,8] protection against oxidative DNA damage,[9] and anti-inflammatory properties[10] have been described mostly in its minor compounds. Virgin olive oil is composed by triacylglycerides and 1−2% of minor components (about 230 different compounds). It can be divided into two fractions, the unsaponifiable fraction, extracted with solvents after the saponification of the oil, and the saponifiable fraction. In the unsaponifiable fraction of virgin olive oil there are triterpenic alcohols and other pentacyclic triterpenes, which together form the main triterpenes of virgin olive oil.

However, very little is known about the activity of this group of compounds, known as triterpenes, present in the leaves and skin of olives and in virgin olive oil, too.[11] In this paper, we summarize the effects of the major triterpenes present in virgin olive oil described so far, and the key factors of their action regarding their role in the oxidation mechanism of the cell.

OLIVE OIL TRITERPENES

The main triterpenes of virgin olive oil are oleanolic acid, maslinic acid, uvaol, and erythrodiol.[11] These two hydroxyl pentacyclic triterpene acids (oleanolic and maslinic acid) and these two dialcohols (uvaol and erythrodiol) are differentiated according to the function present at the C-17 position. Maslinic acid has two vicinal hydroxyl groups at the C-2 and C-3 positions, besides the carboxyl radical. Uvaol and erythrodiol possess two hydroxyl groups in remote positions and are different with regard to the methyl group location (Figure 1). These triterpenes are found in olive skin and the leaves of olive trees (*Olea europaea*). The Picual variety showed the highest content of triterpenes in olives. The various types of commercial black and green olives ranged from 460 to 1470 mg/kg fruit. Natural black olives, not treated with NaOH (which debitters black and green olives for commercial treatments), showed concentration >2000 mg/kg in the olive flesh.[107] The leaf contains important amounts of oleanolic acid (3.0−3.5% DW), followed by maslinic acid and minor levels of erythrodiol and uvaol. The content of triterpenoids changes during leaf ontogeny.[108] Otherwise, in virgin olive oil, the concentration oscillated between 8.90 and 112.36 mg/kg.[11] Allouche et al. concluded that the high

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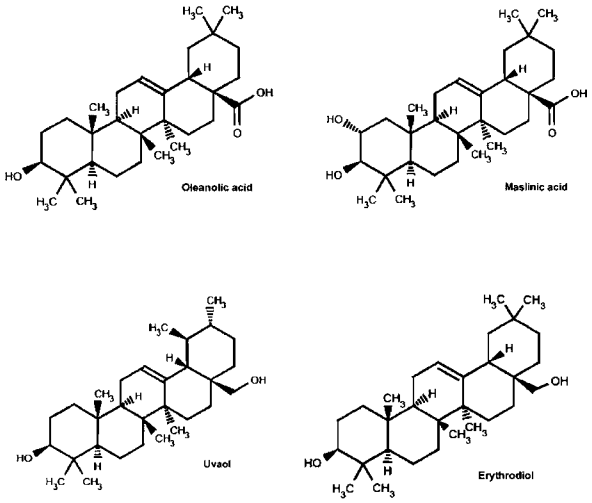

Figure 1. Chemical structures of oleanolic and maslinic acids and uvaol and erythrodiol dialcohols of olive oil.

variability observed in virgin olive oil triterpenic composition was due to genetic factors. High triterpenic content was obtained from 4 of the 40 varieties (Lechin de Granada, Dolce Agogia, Cornicabra, and Salonenque).[11] Other authors point out that the concentration of hydroxyl pentacyclic triterpene acids depends on the quality of olive oil.[12] Finally, it is recognized that triterpene concentration varies depending on the type of cultivation and the handling of olive oil. In fact, triterpenes are present in higher concentrations in olive pomace oil than in virgin olive oils.[12]

Very few papers describe the bioavailability of pentacyclic triterpenes from virgin olive oil intake, but some reveal interesting data from bioavailability in humans and rats.

Oleanolic Acid. After oral administration of 50 mg/kg to rats, a maximum concentration of 0.29 ± 0.26 μM was observed at 21 ± 17 min; oleanolic acid was minimally absorbed, with an absolute oral bioavailibity of 0.7%.[103] In humans, the plasmatic concentrations determined in healthy male volunteers after a single oral administration of 40 mg was 26.5 ± 15 nM at 5.2 ± 2.9 h.[104] Rada et al.[106] show that oleanolic acid and human serum proteins have molecular interactions between them, and these serum proteins are known for the important role in the binding of basic and neutral drugs. These authors demonstrated the formation of complexes between human serum proteins and OA.

Maslinic Acid. Maslinic acid after a single oral administration (50 mg/kg) to rats is absorbed in the intestine and reaches the blood, where it is found 10 min after the oral administration and can still be detected in plasma after 60 min.[105]

Uvaol and Erythrodiol. For uvaol and erythrodiol, we have not found in the literature consulted any reference concerning bioavailability.

Another important issue is the concentration used by the different authors in all of the different studies. Sánchez-González et al.[109] described an interesting property of maslinic acid, namely, its safety. In this paper, authors examined the administration of 50 mg/kg of maslinic acid for 28 days and a single oral administration of 1000 mg/kg to mice. Their results show that this compound does not produce any adverse effects on the variables tested in mice (morbidity, mortality, toxicity, body weight...), suggesting its use as a nutraceutical. We have to pay attention to the concentrations used with each triterpene because Lu et al. described another aspect of oleanolic acid, its hepatotoxic effect in mice in a dose-dependent manner. Oleanolic acid causes body weight loss, inflammation and hepatocellular apoptosis, necrosis, and feathery degeneration (indicative of cholestasis)[14] in mice. Depending on the concentration used and exposure time, the effect could be contrarily adverse or even have a biphasic effect; for example, Allouche et al.[6] observed an antithrombotic effect after 10 min of incubation period and a prothrombotic effect when the incubation period was prolonged to 20 min, which was attributed to alteration of maslinic acid after an extended exposure time with prothrombinase complex. Marquez-Martin et al. showed that the behavior of oleanolic acid and uvaol was suggestive of a biphasic response in terms of TNF-α production. There was an increase at low concentrations (10 μM) and a decrease at higher ones (100 μM).[15] Therefore, it seems that depending on the concentration and time used, these triterpenes have different effects on body response. Consequently, more studies are needed about the bioavailability and metabolism of these triterpenes with virgin olive oil and olive ingestion to be able to assess the potential effect that they could have with diet intake. Future studies should be adapted to the bioavailability concentration found for each triterpene; in this way the results obtained could be more reliable.

BIOACTIVE PROPERTIES OF OLIVE OIL TRITERPENES

In the past years, there have been a growing number of studies focusing on the activity of the virgin olive oil triterpenes. Antitumoral, anti-inflammatory, antioxidant, hepatoprotective, cardioprotective, and antimicrobial activities have been recently described.[6,7,16−19] Here we analyze the different bioactivities of these compounds against different diseases and conditions and future possible applications.

Cardiovascular Disease. Some of the risk factors of cardiovascular diseases are age, sex, and genetic makeup. These are not modifiable, but there are other risk factors that could be altered. Among the modifiable risk factors the following, among others, should be included: levels of high-density lipoprotein (HDL) cholesterol, levels of low-density lipoprotein (LDL) cholesterol, obesity, tobacco, levels of circulating oxidized LDL, hypertension, endothelial dysfunction, and oxidative stress, among others. Nowadays, a high number of myocardial heart attacks could be prevented by these modifiable factors, which are influenced by the diet.[20] The diet followed is responsible, to a greater or lesser extent, for atherosclerosis. Atherosclerosis is an oxidative, inflammatory, and thrombotic disease characterized by the deposition of lipid and other bloodborne material within the arterial wall of almost all vascular territories, which is the prelude to atheroma emergence.[29]

The connection between high levels of LDL oxidation and the increase in cardiovascular disease risk[21−23] and an early event in atherosclerosis has already been described.[24] Several studies interconnect certain foodstuffs and the oxidation process of LDL,[25] so that it seems that diet and cardiovascular disease are strongly linked. Indeed, at present, diet is considered an important determinant in the prevention of cardiovascular diseases.[26] In this way, triterpenes may play a key role in decreasing this LDL oxidation and, hence, in decreasing cardiovascular disease incidence. Oxidation of LDL may play a critical role in the early stages of the disease, whereas thrombosis acts at the latest stages, it being one of the fatal clinic consequences of this pathology.[30] Apart from preventing LDL oxidation, these compounds have been described as antiathero-

Table 1. Bioactive Properties of the Main Triterpenes Found in Olives, Olive Tree Leaves, and Virgin Olive Oil in Cardiovascular Disease

Cardiovascular Disease	Triterpene	Action	Doses	Assay	Reference
	Oleanolic acid	Protection against LDL oxidation	10 - 20 μM	*In vitro*	27, 28
		Antiatherogenic	100 mg/kg/day	*In vivo* (apoE knouckout mice) 8 weeks of treatment	31
		Antihyperlipidemic and antihypertensive	60 mg/kg/day	*In vivo* (DSS rats) 6 weeks of treatment	37
		Hypoglycemic effect	60 mg/kg/day	*In vivo* (DSS rats) 6 weeks of treatment	37
		Antioxidant and nitric oxide releasing action	60 mg/kg/day	*In vivo* (Wistar rats) 5 weeks of treatment	39
		Vasorelaxation in aortic rings	orujo oil intake	*In vivo* (rats)1 dosage	40
		Endothelium-dependent release of NO	3 - 30 μM	*In vivo* (Wistar rats) 12-16 weeks old rats	41
	Maslinic acid	Inhibition of LDL oxidation	12.5 – 400 μM 10 - 20 μM	*In vitro*	6,28
		Cardioprotective	15 mg/kg	*In vivo* (Wistar rats) 7 days of treatment	33
	Uvaol	Protection against LDL oxidation	10 - 20 μM	*In vitro*	28
		Antiatherogenic	12.5 – 400 μM	*In vitro*	6
		Cardiac hypertrophy reduction and left ventricle remodelling	50 mg/kg/day	*In vivo* (mice) 2 weeks of treatment	42
	Erythrodiol	Antiatherogenic	12.5 – 400 μM	*In vitro*	6
		Vaxorelaxation in aortic rings	orujo oil intake	*In vivo* (rats) 1 dosage	40
		Cardiac hypertrophy reduction and left ventricle remodelling	50 mg/kg/day	*In vivo* (mice) 2 weeks of treatment	42

genic, because of the role that the triterpenic diols, uvaol and erythrodiol, play in preventing LDL-supporting thrombin generation in vitro.[6] Table 1 shows triterpene actions in the different stages of the development of cardiovascular disease.

Oleanolic Acid. Oleanolic acid was described as playing a protection role against LDL oxidation like other triterpenes with the effect of chiosmastic gum (CMG), the most effective protecting human LDL oxidation yet known.[27,28] Thus, oleanolic acid exerts potent antiatherogenic effects independent of plasma lipid levels in apolipoprotein E knockout mice.[31] Previous studies of isolated oleanolic acid describe its action in preventing hypertension and hyperlipidaemia in Dahl salt-sensitive (DSS) rats with genetic hypertension. In this study, oleanolic acid is described as preventing the development of severe hypertension through its potent diuretic−natriuretic−saluretic activity, its direct cardiac effect, and its antihyperlipidemic, antioxidant, and hypoglycemic effects on DSS rats.[37] Other authors show its possible action in inhibiting the progress of fibrosis and in decreasing the portal pressure in CCl_4-induced portal hypertensive rats, which could be related to the increase of eNOS expression and enhancement of nitric oxide (NO) level in the liver.[38] This prevention of hypertension has also been attributed to the antioxidant and nitric oxide releasing action of oleanolic acid.[39] Indeed, Rodríguez-Rodríguez et al. showed how oleanolic acid together with erythrodiol was able to promote vasorelaxation in aortic rings with endothelium precontracted in rats.[40] This effect seemed to be mainly mediated by endothelial production of NO. Later, this effect was studied,[41] and oleanolic acid was shown to activate endothelium-dependent release of NO and to decrease smooth muscle cell calcium followed by relaxation. This oleanolic acid-evoked endothelium-derived NO release was independent of endothelial cell calcium and involved phosphoinositide-3-kinase-dependent phosphorylation of Akt-Ser(473) followed by phosphorylation of eNOS-Ser(1177).

Oleanolic acid is also involved in atherosclerosis protection also, with antihyperlipidemic effects in Wistar rats, decreasing hepatic expression levels of lipogenic genes, and several cytochrome P450 genes.[32]

Maslinic Acid. Maslinic acid strongly inhibits in vitro LDL oxidation.[6] However, maslinic acid showed both pro- and antithrombotic effects depending on the concentration used.[6] Thus, special attention has to be paid to the concentration of these compounds employed, because depending on that, the effects could change, which has been already described above. Another cardioprotective activity described for maslinic acid was its effect on isoproterenol-induced myocardial infarcted albino Wistar rats; maslinic acid reduced the effects of isoproterenol on body weight, heart weight, lipids, lipoproteins, lipid peroxidation, cardiac marker enzymes, and paraoxonase,[33] so it that seems maslinic acid has cardioprotective effects, influencing more than one pathway.

Consequently, maslinic acid may act both at the beginning and at the latest stage of atherosclerosis. Indeed, it has been described that this compound has been shown to be involved in atherosclerosis protection, with potential antioxidant and hypoglycemic effects by reducing insulin resistance in a mouse model of genetic type-2 diabetes.[32] However, more studies are needed to evaluate the precise mechanism of action of these compounds in atherosclerosis prevention.

Table 2. Bioactive Properties of the Main Triterpenes Found in Olives, Olive Tree Leaves, and Virgin Olive Oil in Cancer

Cancer	Triterpene	Action	Doses	Assay	Reference
	Oleanolic acid	Invasion and migration decrease, ROS decrease, NO decrease, VEGF expression decrease	2 - 4 μM	*In vitro* (human liver cancer cells)	43
		Antitumoral activity	10 - 100 μM	*In vitro* (skin, hepatocellular, colon, lung, breast, pancreatic cancer cell lines and myelogenous leukemia)	7, 43-48
		Apoptosis induction by mitochondrial pathway	12,5 – 200 μM	*In vitro* (hepatocellular carcinoma and human pancreatic cancer cell line)	44, 45, 53, 54
		Cell cycle arrest	0 - 50 μg/ml	*In vitro* (hepatocellular carcinoma and human pancreatic cancer cell line)	44, 54
		Inhibit proliferation and colony formation. Apoptosis by mTOR signaling	12.5 – 100 μM	*In vitro* (osteosarcoma cells)	59
		Apoptosis by p53, Bax, Bcl-2 and caspase-3	2, 4 or 8 μM	*In vitro* (melanoma, colon and liver cancer cells)	55-58
	Maslinic acid	Invasion and migration decrease, ROS decrease, NO decrease, VEGF expression decrease	2 - 4 μM	*In vitro* (human liver cancer cells)	43
		Antitumoral	0 -100 μM	*In vitro* (skin, hepatocellular, colon, lung, breast, pancreatic cancer cell lines and myelogenous leukemia)	7, 43-48
		Chemopreventive	3.75 – 30 μM 100 mg/kg/day	*In vitro* (colorectal cancer)& *In vivo* (6 weeks of treatment)	46, 60
		Suppression of COX-2 expression, NFκβ and AP-1 inhibition	Unknown	*In vitro* (Raji cells)	61
		Antimetastatic activity	0-25 μM	*In vitro* (DU145 human prostate cancer cell line)	62
		Apoptosis induction through caspase 3	0 -100 μM	*In vitro* (different cancer cell lines)	63-67
		Suppression of NFκβ	0 – 50 μM	*In vitro* (pancreatic cancer cell line)	68
	Uvaol	Pro-apoptotic potential through JNK activation	0 -100 μM	*In vitro* (breast cancer cell, astrocytoma cells)	7,74,76
		Pro-apoptotic associated to ROS	0 -100 μM	*In vitro* (human breast cancer cells,	7, 76
		Antitumoral	0 -100 μM	*In vitro* (murine and human cancer cell lines)	7, 73-76
		Inhibition of proliferation	0 – 300 μM	*In vitro* (gastric cancer cell line)	70
	Erythrodiol	Pro- apoptotic potential	0 - 100 μM	*In vitro* (breast cancer cells, colon cancer cells astrocytoma cells)	7, 72,74, 76
		Antitumoral	0 -100 μM	*In vitro* (murine and human cancer cell lines)	7, 73-76
		Antiproliferative	0 – 150 μM	*in vitro (colon cancer cells)*	72

Uvaol and Erythrodiol. The action of erythrodiol and uvaol in reducing cardiac hypertrophy and left ventricle remodeling induced by angiotensin II in mice, through diminishing fibrosis and myocite area, has been recently described. They seem to

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modulate growth and survival of cardiac myofibroblasts, and both of them inhibit the angiotensin II-induced proliferation in a PPAR-γ-dependent manner, whereas at high doses they activate pathways of programmed cell death that are dependent on JNK and PPAR-γ.[42]

There are several studies on olive pomace oil, which has high triterpenic content, and on its improvement of the endothelial function,[34−36] so it seems reasonable to conclude that these compounds could have an active role in cardiovascular prevention.

In view of the actions described above, virgin olive oil triterpenes could have an interesting therapeutic potential as cardiovascular drugs, and furthermore they may fulfill a role in preventing, through diet, different kinds of cardiovascular disorders. Although more evidence will be necessary to identify the mechanism involved and their interactions, it will be necessary to determine the most effective dose and exposure time for treatments.

Cancer. A diversity of studies highlight different aspects of the function that triterpenes seem to play in cancer. So far, it is clear that triterpenes affect tumorigenesis and key factors for its development, such as angiogenesis.[43] Apart from this, various studies note the antitumor activities of triterpenes in different cancers such as hepatocellular carcinoma, skin cancer, colon cancer, lung cancer, breast cancer, myelogenous leukemia, and pancreatic cancer.[7,13,44−49]

In this line, the antiangiogenic effects of oleanolic and maslinic acids in human liver cancer cell lines have been studied. In a dose-dependent manner they reduced cell invasion and migration, decreasing reactive oxygen species (ROS) and NO levels and decreasing expression of vascular endothelial growth factor (VEGF).[43] In Table 2 the kinds of action exerted by each triterpene in the different types of cancer studied are specified.

Several studies have focused on the antitumoral activity of these triterpenes in the synthesis of new molecules derived from them and assessed their roles as anticancer drugs.[50−52]

Oleanolic Acid. The mechanism of action of oleanolic acid has been studied in different types of cancer cells. On hepatocellular carcinoma, oleanolic acid exhibited inhibitory effects through induction of apoptosis and cell cycle arrest.[44,53] Apoptosis was induced through the mitochondrial pathway, and this could be due to ROS generated by mitochondrial fatty acid oxidation. Wei et al. also described the arrest of cell cycle and induction of apoptosis in human pancreatic cancer cell line (Panc-28) by ROS-mediated mitochondrial depolarization and lysosomal membrane permeabilization.[54] Apoptosis was also induced in several cancer cell lines, including multidrug resistance cancer cells, non-small-cell lung cancer cell lines, lung adenocarcinoma, B16F10 melanoma cells, breast cancer, and colon cancer by oleanolic acid. This compound activates caspase-3, decreases the expression of Bcl-2 antiapoptotic gene, and increases the expression of pro-apoptotic protein Bax. Along with this, oleanolic acid is capable of decreasing angiogenic VEGF and decreasing the development of melanoma-induced lung metastasis of the B16F10 melanoma model in vivo.[7,47,48,55−58] In osteosarcoma cells, oleanolic acid inhibits proliferation and colony formation, induces G1 arrest, and promotes apoptosis, through mTOR signaling, a central regulator of cell growth, proliferation, survival, and metabolism.[59]

Maslinic Acid. Recent studies report the chemopreventive potential of maslinic acid in colorectal cancer in vitro[46] and in vivo.[60] This compound has not been as thoroughly studied as oleanolic acid in cancer, but there is increasing interest in the preventive action that it seems to possess. Hsum et al. studied the chemopreventive action that maslinic acid showed in Raji cells. It suppressed COX-2 expression and inhibited NF-κB and AP-1 binding activities.[61] Targeting pro-inflammatory pathways by dietary phytochemicals as a strategy for cancer prevention is one of the current issues studied, but at a later stage, inflammation and triterpene action will also be discussed.

One aspect of cancer development is the metastatic potential of the tumor. Many authors have recently studied the antimetastatic activity of maslinic acid in DU145 human prostate cancer cells and its mediation via hypoxia-inducible factor-1α signaling (HIF-1α).[62] In these cancer cells, maslinic acid acts by inhibiting uPAR, E-cadherin, VEGF, and matrix metalloproteases (MMPs) expression and dramatically reduces the levels of HIF-1α. Consequently, maslinic acid inhibits the migration, invasion, and adhesion of DU145 prostate cancer cells. As oleanolic, this acid induces apoptosis in specific cancer cell lines.[63−67] In some of them, maslinic acid promotes apoptosis by a mechanism similar to the one of oleanolic acid: a JNK-p53-dependent mechanism, the mitochondrial apoptotic pathway, the increase of expression of Bid and Bax, repression of Bcl-2, release of cytochrome *c*, and increase in caspase-9, -3, and -7 expression. Another potential antitumor activity of maslinic acid is its enhancement of the antitumor activity of TNF-α by suppressing NF-κB action and downstream gene expression, apart from activating the caspase-dependent apoptotic pathway.[68]

Uvaol and Erythrodiol. The two dialcohols of olive oil have been targeted for research in recent years. It was in 1976 when uvaol was first described to possess tumor inhibitory effects, along with ursolic acid and betulinic acid.[69] Until 1994, there were not any additional studies on the effects of any of these compounds. Then, Es-Saady et al. described uvaol, ursolic acid, and oleanolic acid inhibition in leukemic cell line proliferation.[70]

Erythrodiol effects on skin tumor formation in mice were described in 1988,[71] and until 2008, no author had described its cytotoxic effect.[72] Since then, several works have described uvaol and erythrodiol antitumoral effects in murine and human cancer cell lines.[7,73−76] The most remarkable effect of both is their pro-apoptotic potential, which they exert in two different ways: associated with ROS and by c-Jun N-terminal kinase JNK activation.[7,74,76] Again, it seems clear that ROS are crucial in the mechanism of action of these four compounds.

Inflammation, Oxidative Stress, and Oxidative Damage to DNA. Inflammation is related to several diseases, for example, as a prelude for cancer development and interrelating different kinds of cells for the development of a response to a trauma or strange antigen.

Virgin olive oil triterpenes have been recently studied for the modulation that they exert in the inflammatory response.

Oleanolic acid has been described as an anti-inflammatory molecule in vivo[77−79] and in vitro.[80,81] This compound promotes an anti-inflammatory status inhibiting the activation of nuclear factor-κB (NF-κB) and the production of tumor necrosis factor-α (TNF-α) in human umbilical vein endothelial cells (HUVECs).[82] The suppressive effect of triterpenes in the activation of NF-κB seems to be extensive to the four triterpenes in different types of cells.[61,80,81,83] It has been described that the efficient activation of NF-κB-dependent genes by TNF-α requires a cell to be in an oxidized redox state, suggesting that stimuli such as TNF may exert only a limited response if the cell is not in an appropriate redox equilibrium;[84] thus, the link between ROS generation and activation of the NF-κB pathway seems to be recognizable.[85] Most studies focus on the role that triterpenes

could play against certain diseases, their apoptotic role against tumor cells, or the protective action in vascular alteration, but a principal feature of these compounds is their antioxidant effect (Figure 2).

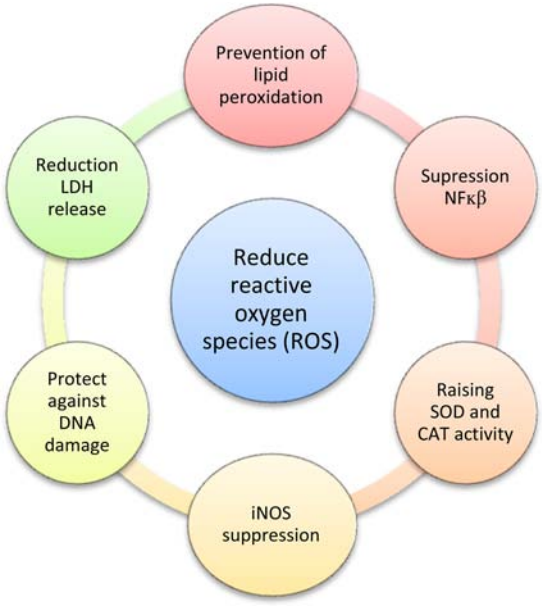

Figure 2. Oxidative mechanisms affected by triterpenes.

The chemical antioxidant role of the triterpenic fraction of virgin olive oil is well documented, although the free radical scavenging activity is almost absent in oleanolic acid, uvaol, and erythrodiol. Maslinic acid also exhibited a weak antiradical activity up to 800 μM and 2.50 mol ratio, but up to 5.00 mol a high DPPH scavenging activity was observed.[7] It acts as an efficient peroxyl radical scavenger by the ORAC assay.[6]

Balanehru et al. described the protection offered by oleanolic acid, isolated from *Eugenia jumbolana*, against hepatic microsome lipid peroxidation in rats.[86] Maslinic acid was described to prevent hepatocyte membrane from lipid peroxidation in rats, induced by the hydroxyl radical (OH*).[87] According to this, some authors tried to study this prevention of lipid peroxidation in hepatic microsomes of rats that were fed, for 3 weeks, high-oleic-acid oils (of sunflower oil, olive oil, and olive pomace oil) containing different concentrations of the antioxidants α-tocopherol, erythrodiol, and oleanolic acid. They concluded that oleanolic acid and erythrodiol protect against, at least partly, microsomal lipid peroxidation in rats fed olive pomace oil.[88]

Oxidative stress and inflammation are closely related, not only because of the NF-κB pathway but also on account of other signals such as ROS and reactive nitrogen species (RNS) produced by macrophages and other mediated immune cells. With this signal, macrophages activate other immune cells that, with them, will try to mediate inflammation and revert to the initial health status. In this way, any compound that acts directly or indirectly in oxidative stress will act in inflammation and, thereby, in the prelude of several diseases.

According to this, oleanolic acid has been one of the triterpenes most studied in inflammation and oxidative stress. This compound is an effective inhibitor of cyclo-oxygenase (COX) and of 5-lipoxygenase (5-LOX),[89] both present in the arachidonic acid synthesis pathway. The anti-inflammatory effects of suppressing COX-2 action, like the reduction of several pro-inflammatory cytokines such as IL-6, IL-1β, and TNF-α, are well-known.

Other studies in PC12 cells show the influence of oleanolic acid in reducing subsequent H_2O_2- or MMP^+-induced cell death and lactate dehydrogenase (LDH) release, which leads to alleviated oxidative stress in PC12 cells H_2O_2- or MMP^+-induced injury. It spares GSH, raising the activity of SOD and catalase and reducing the release of IL-6 and TNF-α.[93,94] Another antioxidant effect of oleanolic acid was studied by Tsai et al.[95] The study was undertaken in mouse brain, where, dose-dependently, oleanolic acid diminished ROS and proteins related with oxidative stress, showing neuroprotective effects in vivo.

COX-2 and inducible nitric oxide synthethase (iNOS) expression are suppressed at protein and mRNA levels by maslinic acid, and likewise in the translocation of NF-κB to the nucleus (and IκBα phosphorylation), in a concentration-dependent manner in cultured cortical astrocytes.[90] These last actions (reduction of IL-6, IL-1β, and TNF-α) are produced by maslinic acid in mouse macrophages[91] and by the four triterpenic compounds of olive oil in human peripheral blood mononuclear cells.[15] These authors observed that maslinic acid significantly inhibits the enhanced production of NO induced by lipopolysaccharide (LPS), measured by the nitrite production with an IC_{50} value of 25.4 μM. This seems to be in correlation with an action in the iNOS gene expression rather than a direct inhibitory effect on the enzyme activity. ROS were reduced in a dose-dependent manner (IC_{50} = 43.6 μM) showing a preventive effect in oxidative stress in murine macrophages. The inhibition of NO production by oleanolic and maslinic acid was described by Yang et al. in murine RAW 264.7 cells.[92] In breast cancer cells ROS production was decreased by uvaol, oleanolic acid, and maslinic acid.[7]

Interestingly, triterpenes are capable of protecting against H_2O_2-induced DNA damage in several leukemic[96] and human breast cancer cell lines.[7] There are not many studies about antioxidant effects of triterpenes in DNA damage, but attending to the effects observed in different types of cells on oxidative stress, and with these previous studies in leukemic and breast cancer cell lines, probably these triterpenic acids and dialcohols play an important role in the oxidative stress mechanism of the cell, even at nucleus level, protecting against oxidative damage to DNA. Because of that, these olive oil triterpenes could be a good option for preventing different diseases related with oxidative stress, such as cardiovascular diseases,[97] cancer,[98] or even Parkinson's disease[99] and Alzheimer's disease.[100,101]

Another potentially interesting role of triterpenes is their predictable antioxidant capacity in aging. Aging is associated with the accumulation of inactive or less active forms of numerous enzymes. The possibility that these age-related changes are due, at least in part, to oxidative modification is indicated by Berlett et al.[102] There is no scientific evidence of the action of these compounds in the oxidative modification of a protein, but they are modulators of the proteic activity in the cell and could protect against the loss of their activity or oxidative modification; additional studies are required to ensure this.

Oleanolic acid, maslinic acid, uvaol, and erythrodiol are the main triterpenes found in virgin olive oil, but they are not present in other edible oils. They appear in olive leaves and olive skin, and their concentrations depend on the variety selected and the culture handling. These triterpenes possess antioxidant properties per se, and in different cellular types, they affect some central proteins of oxidative stress and inflammation (NF-κB and COX-2); it is still unknown what actual pathways they affect and how.

Although the origins of the diseases described above are varied, oxidative stress is a common condition in them. Compounds that protect against oxidative stress may be useful to prevent these diseases. As we have already showed, triterpenes exert a protective role against oxidant environment, regulating it or, even more, diminishing it. Therefore, the main triterpenes of virgin olive oil could have a critical role in preventing a group of several diseases related with oxidative stress, such as cancer or cardiovascular disease.

More bioavailability studies about these triterpenes are needed to obtain reliable information about the range in which they are present in the cellular metabolism.

Taking into account all available scientific evidence, the beneficial effects of the major triterpenes present in virgin olive oil could prevent certain diseases. For all of these reasons, more studies on the mechanism of action of these triterpenes in oxidative stress are required; indeed, these studies could probe the potential role of triterpenes in preventing the appearance of different diseases.

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REFERENCES

(1) Klonizakis, M.; Alkhatib, A.; Middleton, G.; Smith, M. F. Mediterranean diet- and exercise-induced improvement in age-dependent vascular activity. *Clin. Sci.* **2013**, *124*, 579−587.

(2) Mitjavila, M. T.; Fandos, M.; Salas-Salvadó, J.; Covas, M.; Borrego, S.; Estruch, R.; Lamuela-Raventós, R.; Corella, D.; Martínez-Gonzalez, M. T.; Sánchez, J. M.; Bulló, M.; Fitó, M.; Tormos, C.; Cerdá, C.; Casillas, R.; Moreno, J. J.; Iradi, A.; Zaragoza, C.; Chaves, J.; Sáez, G. T. The Mediterranean diet improves the systemic lipid and DNA oxidative damage in metabolic syndrome individuals. A randomized, controlled, trial. *Clin. Nutr.* **2013**, *32*, 172−178.

(3) De Lorgeril, M.; Renaud, S.; Mamelle, N.; Salen, P.; Martin, J.-.; Monjaud, I.; Guidollet, J.; Touboul, P.; Delaye, J. Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. *Lancet* **1994**, *343*, 1454−1459.

(4) Willett, W. C.; Sacks, F.; Trichopoulou, A.; Drescher, G.; Ferro-Luzzi, A.; Helsing, E.; Trichopoulos, D. Mediterranean diet pyramid: a cultural model for healthy eating. *Am. J. Clin. Nutr.* **1995**, *61*, 1402S−1406S.

(5) Covas, M. I. Olive oil and the cardiovascular system. *Pharmacol. Res.* **2007**, *55*, 175−186.

(6) Allouche, Y.; Beltran, G.; Gaforio, J. J.; Uceda, M.; Mesa, M. D. Antioxidant and antiatherogenic activities of pentacyclic triterpenic diols and acids. *Food Chem. Toxicol.* **2010**, *48*, 2885−2890.

(7) Allouche, Y.; Warleta, F.; Campos, M.; Sanchez-Quesada, C.; Uceda, M.; Beltran, G.; Gaforio, J. J. Antioxidant, antiproliferative, and pro-apoptotic capacities of pentacyclic triterpenes found in the skin of olives on MCF-7 human breast cancer cells and their effects on DNA damage. *J. Agric. Food Chem.* **2011**, *59*, 121−130.

(8) Warleta, F.; Quesada, C. S.; Campos, M.; Allouche, Y.; Beltran, G.; Gaforio, J. J. Hydroxytyrosol protects against oxidative DNA damage in human breast cells. *Nutrients* **2011**, *3*, 839−857.

(9) Warleta, F.; Campos, M.; Allouche, Y.; Sánchez-Quesada, C.; Ruiz-Mora, J.; Beltrán, G.; Gaforio, J. J. Squalene protects against oxidative DNA damage in MCF10A human mammary epithelial cells but not in MCF7 and MDA-MB-231 human breast cancer cells. *Food Chem. Toxicol.* **2010**, *48*, 1092−1100.

(10) Huang, L.; Guan, T.; Qian, Y.; Huang, M.; Tang, X.; Li, Y.; Sun, H. Anti-inflammatory effects of maslinic acid, a natural triterpene, in cultured cortical astrocytes via suppression of nuclear factor-kappa B. *Eur. J. Pharmacol.* **2011**, *672*, 169−174.

(11) Allouche, Y.; Jimenez, A.; Uceda, M.; Aguilera, M. P.; Gaforio, J. J.; Beltran, G. Triterpenic content and chemometric analysis of virgin olive oils from forty olive cultivars. *J. Agric. Food Chem.* **2009**, *57*, 3604−3610.

(12) Perez-Camino, M. C.; Cert, A. Quantitative determination of hydroxy pentacyclic triterpene acids in vegetable oils. *J. Agric. Food Chem.* **1999**, *47*, 1558−1562.

(13) Juan, M. E.; Wenzel, U.; Ruiz-Gutierrez, V.; Daniel, H.; Planas, J. M. Olive fruit extracts inhibit proliferation and induce apoptosis in HT-29 human colon cancer cells. *J. Nutr.* **2006**, *136*, 2553−2557.

(14) Lu, Y. F.; Wan, X. L.; Xu, Y.; Liu, J. Repeated oral administration of oleanolic acid produces cholestatic liver injury in mice. *Molecules* **2013**, *18*, 3060−3071.

(15) Marquez-Martin, A.; De La Puerta, R.; Fernandez-Arche, A.; Ruiz-Gutierrez, V.; Yaqoob, P. Modulation of cytokine secretion by pentacyclic triterpenes from olive pomace oil in human mononuclear cells. *Cytokine* **2007**, *36*, 211−217.

(16) Moreno, J. J. Effect of olive oil minor components on oxidative stress and arachidonic acid mobilization and metabolism by macrophages RAW 264.7. *Free Radical Biol. Med.* **2003**, *35*, 1073−1081.

(17) Brunelleschi, S.; Bardelli, C.; Amoruso, A.; Gunella, G.; Ieri, F.; Romani, A.; Malorni, W.; Franconi, F. Minor polar compounds extra-virgin olive oil extract (MPC-OOE) inhibits NF-kappa B translocation in human monocyte/macrophages. *Pharmacol. Res.* **2007**, *56*, 542−549.

(18) Giner-Larza, E. M.; Manez, S.; Recio, M. C.; Giner, R. M.; Prieto, J. M.; Cerda-Nicolas, M.; Rios, J. L. Oleanonic acid, a 3-oxotriterpene from *Pistacia*, inhibits leukotriene synthesis and has anti-inflammatory activity. *Eur. J. Pharmacol.* **2001**, *428*, 137−143.

(19) Martin, R.; Ibeas, E.; Carvalho-Tavares, J.; Hernandez, M.; Ruiz-Gutierrez, V.; Nieto, M. L. Natural triterpenic diols promote apoptosis in astrocytoma cells through ROS-mediated mitochondrial depolarization and JNK activation. *PLoS One* **2009**, *4*, e5975.

(20) De Caterina, R.; Zampolli, A.; Del Turco, S.; Madonna, R.; Massaro, M. Nutritional mechanisms that influence cardiovascular disease. *Am. J. Clin. Nutr.* **2006**, *83*, 421S−426S.

(21) Holvoet, P.; Harris, T. B.; Tracy, R. P.; Verhamme, P.; Newman, A. B.; Rubin, S. M.; Simonsick, E. M.; Colbert, L. H.; Kritchevsky, S. B. Association of high coronary heart disease risk status with circulating oxidized LDL in the well-functioning elderly: findings from the Health, Aging, and Body Composition study. *Arterioscler. Thromb. Vasc. Biol.* **2003**, *23*, 1444−1448.

(22) Meisinger, C.; Baumert, J.; Khuseyinova, N.; Loewel, H.; Koenig, W. Plasma oxidized low-density lipoprotein, a strong predictor for acute coronary heart disease events in apparently healthy, middle-aged men from the general population. *Circulation* **2005**, *112*, 651−657.

(23) Mertens, A.; Verhamme, P.; Bielicki, J. K.; Phillips, M. C.; Quarck, R.; Verreth, W.; Stengel, D.; Ninio, E.; Navab, M.; Mackness, B.; Mackness, M.; Holvoet, P. Increased low-density lipoprotein oxidation and impaired high-density lipoprotein antioxidant defense are associated with increased macrophage homing and atherosclerosis in dyslipidemic obese mice: LCAT gene transfer decreases atherosclerosis. *Circulation* **2003**, *107*, 1640−1646.

(24) Matsuura, E.; Hughes, G. R.; Khamashta, M. A. Oxidation of LDL and its clinical implication. *Autoimmun. Rev.* **2008**, *7*, 558−566.

(25) Lapointe, A.; Couillard, C.; Lemieux, S. Effects of dietary factors on oxidation of low-density lipoprotein particles. *J. Nutr. Biochem.* **2006**, *17*, 645−658.

(26) Ferdowsian, H. R.; Barnard, N. D. Effects of plant-based diets on plasma lipids. *Am. J. Cardiol.* **2009**, *104*, 947−956.

(27) Andrikopoulos, N. K.; Kaliora, A. C.; Assimopoulou, A. N.; Papapeorgiou, V. P. Biological activity of some naturally occurring

resins, gums and pigments against in vitro LDL oxidation. *Phytother. Res.* **2003**, *17*, 501−507.
(28) Andrikopoulos, N. K.; Kaliora, A. C.; Assimopoulou, A. N.; Papageorgiou, V. P. Inhibitory activity of minor polyphenolic and nonpolyphenolic constituents of olive oil against in vitro low-density lipoprotein oxidation. *J. Med. Food* **2002**, *5*, 1−7.
(29) Badimon, J. J.; Ibanez, B.; Cimmino, G. Genesis and dynamics of atherosclerotic lesions: implications for early detection. *Cerebrovasc. Dis.* **2009**, *27* (Suppl. 1), 38−47.
(30) Lusis, A. J. Atherosclerosis. *Nature* **2000**, *407*, 233−241.
(31) Buus, N. H.; Hansson, N. C.; Rodriguez-Rodriguez, R.; Stankevicius, E.; Andersen, M. R.; Simonsen, U. Antiatherogenic effects of oleanolic acid in apolipoprotein E knockout mice. *Eur. J. Pharmacol.* **2011**, *670*, 519−526.
(32) Lou-Bonafonte, J. M.; Arnal, C.; Navarro, M. A.; Osada, J. Efficacy of bioactive compounds from extra virgin olive oil to modulate atherosclerosis development. *Mol. Nutr. Food Res.* **2012**, *56*, 1043−1057.
(33) Hussain Shaik, A.; Rasool, S. N.; Abdul Kareem, M.; Krushna, G. S.; Akhtar, P. M.; Devi, K. L. Maslinic acid protects against isoproterenol-induced cardiotoxicity in albino Wistar rats. *J. Med. Food* **2012**, *15*, 741−746.
(34) Rodriguez-Rodriguez, R.; Herrera, M. D.; de Sotomayor, M. A.; Ruiz-Gutierrez, V. Effects of pomace olive oil-enriched diets on endothelial function of small mesenteric arteries from spontaneously hypertensive rats. *Br. J. Nutr.* **2009**, *102*, 1435−1444.
(35) Rodriguez-Rodriguez, R.; Herrera, M. D.; de Sotomayor, M. A.; Ruiz-Gutierrez, V. Pomace olive oil improves endothelial function in spontaneously hypertensive rats by increasing endothelial nitric oxide synthase expression. *Am. J. Hypertens.* **2007**, *20*, 728−734.
(36) Rodriguez-Rodriguez, R.; Perona, J. S.; Herrera, M. D.; Ruiz-Gutierrez, V. Triterpenic compounds from "orujo" olive oil elicit vasorelaxation in aorta from spontaneously hypertensive rats. *J. Agric. Food Chem.* **2006**, *54*, 2096−2102.
(37) Somova, L. O.; Nadar, A.; Rammanan, P.; Shode, F. O. Cardiovascular, antihyperlipidemic and antioxidant effects of oleanolic and ursolic acids in experimental hypertension. *Phytomedicine* **2003**, *10*, 115−121.
(38) Liu, C. H.; Huang, X. T.; Li, Y. Y.; Zheng, X.; Li, N.; Mi, S. Q.; Wang, N. S. The anti-portal hypertension effect of oleanolic acid in CCl_4-induced cirrhosis rats. *Zhong Yao Cai* **2012**, *35*, 930−935.
(39) Bachhav, S. S.; Patil, S. D.; Bhutada, M. S.; Surana, S. J. Oleanolic acid prevents glucocorticoid-induced hypertension in rats. *Phytother. Res.* **2011**, *25*, 1435−1439.
(40) Rodriguez-Rodriguez, R.; Herrera, M. D.; Perona, J. S.; Ruiz-Gutierrez, V. Potential vasorelaxant effects of oleanolic acid and erythrodiol, two triterpenoids contained in 'orujo' olive oil, on rat aorta. *Br. J. Nutr.* **2004**, *92*, 635−642.
(41) Rodriguez-Rodriguez, R.; Stankevicius, E.; Herrera, M. D.; Ostergaard, L.; Andersen, M. R.; Ruiz-Gutierrez, V.; Simonsen, U. Oleanolic acid induces relaxation and calcium-independent release of endothelium-derived nitric oxide. *Br. J. Pharmacol.* **2008**, *155*, 535−546.
(42) Martin, R.; Miana, M.; Jurado-Lopez, R.; Martinez-Martinez, E.; Gomez-Hurtado, N.; Delgado, C.; Bartolome, M. V.; San Roman, J. A.; Cordova, C.; Lahera, V.; Nieto, M. L.; Cachofeiro, V. DIOL triterpenes block profibrotic effects of angiotensin II and protect from cardiac hypertrophy. *PLoS One* **2012**, *7*, e41545.
(43) Lin, C. C.; Huang, C. Y.; Mong, M. C.; Chan, C. Y.; Yin, M. C. Antiangiogenic potential of three triterpenic acids in human liver cancer cells. *J. Agric. Food Chem.* **2011**, *59*, 755−762.
(44) Wang, X.; Bai, H.; Zhang, X.; Liu, J.; Cao, P.; Liao, N.; Zhang, W.; Wang, Z.; Hai, C. Inhibitory effect of oleanolic acid on hepatocellular carcinoma via ERK-p53-mediated cell cycle arrest and mitochondrial-dependent apoptosis. *Carcinogenesis* **2013**, *34*, 1323−1330.
(45) George, V. C.; Kumar, D. R.; Suresh, P. K.; Kumar, R. A. Apoptosis-induced cell death due to oleanolic acid in HaCaT keratinocyte cells − a proof-of-principle approach for chemopreventive drug development. *Asian Pac. J. Cancer Prev.* **2012**, *13*, 2015−2020.
(46) Rufino-Palomares, E. E.; Reyes-Zurita, F. J.; Garcia-Salguero, L.; Mokhtari, K.; Medina, P. P.; Lupianez, J. A.; Peragon, J. Maslinic acid, a triterpenic anti-tumoural agent, interferes with cytoskeleton protein expression in HT29 human colon-cancer cells. *J. Proteomics* **2013**, *83C*, 15−25.
(47) Lúcio, K. A.; da Graça Rocha, G.; Monção-Ribeiro, L. C.; Fernandes, J.; Takiya, C. M.; Gattass, C. R. Oleanolic acid initiates apoptosis in non-small cell lung cancer cell lines and reduces metastasis of a B16F10 melanoma model in vivo. *PLoS One* **2011**, *6*, e28596.
(48) Shan, J. Z.; Xuan, Y. Y.; Ruan, S. Q.; Sun, M. Proliferation-inhibiting and apoptosis-inducing effects of ursolic acid and oleanolic acid on multi-drug resistance cancer cells in vitro. *Chin. J. Integr. Med.* **2011**, *17*, 607−611.
(49) Li, C.; Yang, Z.; Zhai, C.; Qiu, W.; Li, D.; Yi, Z.; Wang, L.; Tang, J.; Qian, M.; Luo, J.; Liu, M. Maslinic acid potentiates the anti-tumor activity of tumor necrosis factor alpha by inhibiting NF-kappaB signaling pathway. *Mol. Cancer* **2010**, *9*, 73.
(50) Petronelli, A.; Pannitteri, G.; Testa, U. Triterpenoids as new promising anticancer drugs. *Anticancer Drugs* **2009**, *20*, 880−892.
(51) Sogno, I.; Vannini, N.; Lorusso, G.; Cammarota, R.; Noonan, D. M.; Generoso, L.; Sporn, M. B.; Albini, A. Anti-angiogenic activity of a novel class of chemopreventive compounds: oleanic acid terpenoids. *Recent Results Cancer Res.* **2009**, *181*, 209−212.
(52) Leal, A. S.; Wang, R.; Salvador, J. A.; Jing, Y. Synthesis of novel heterocyclic oleanolic acid derivatives with improved antiproliferative activity in solid tumor cells. *Org. Biomol. Chem.* **2013**, *11*, 1726−1738.
(53) Shyu, M. H.; Kao, T. C.; Yen, G. C. Oleanolic acid and ursolic acid induce apoptosis in HuH7 human hepatocellular carcinoma cells through a mitochondrial-dependent pathway and downregulation of XIAP. *J. Agric. Food Chem.* **2010**, *58*, 6110−6118.
(54) Wei, J.; Liu, M.; Liu, H.; Wang, H.; Wang, F.; Zhang, Y.; Han, L.; Lin, X. Oleanolic acid arrests cell cycle and induces apoptosis via ROS-mediated mitochondrial depolarization and lysosomal membrane permeabilization in human pancreatic cancer cells. *J. Appl. Toxicol.* **2013**, *33*, 756−765.
(55) Pratheeshkumar, P.; Kuttan, G. Oleanolic acid induces apoptosis by modulating p53, Bax, Bcl-2 and caspase-3 gene expression and regulates the activation of transcription factors and cytokine profile in B16F. *J. Environ. Pathol. Toxicol. Oncol.* **2011**, *30*, 21−31.
(56) Feng, L.; Au-Yeung, W.; Xu, Y. H.; Wang, S. S.; Zhu, Q.; Xiang, P. Oleanolic acid from *Prunella vulgaris* L. induces SPC-A-1 cell line apoptosis via regulation of Bax, Bad and Bcl-2 expression. *Asian Pac. J. Cancer Prev.* **2011**, *12*, 403−408.
(57) Juan, M. E.; Planas, J. M.; Ruiz-Gutierrez, V.; Daniel, H.; Wenzel, U. Antiproliferative and apoptosis-inducing effects of maslinic and oleanolic acids, two pentacyclic triterpenes from olives, on HT-29 colon cancer cells. *Br. J. Nutr.* **2008**, *100*, 36−43.
(58) Yan, S. L.; Huang, C. Y.; Wu, S. T.; Yin, M. C. Oleanolic acid and ursolic acid induce apoptosis in four human liver cancer cell lines. *Toxicol. In Vitro* **2010**, *24*, 842−848.
(59) Zhou, R.; Zhang, Z.; Zhao, L.; Jia, C.; Xu, S.; Mai, Q.; Lu, M.; Huang, M.; Wang, L.; Wang, X.; Jin, D.; Bai, X. Inhibition of mTOR signaling by oleanolic acid contributes to its anti-tumor activity in osteosarcoma cells. *J. Orthop. Res.* **2011**, *29*, 846−852.
(60) Sanchez-Tena, S.; Reyes-Zurita, F. J.; Diaz-Moralli, S.; Vinardell, M. P.; Reed, M.; Garcia-Garcia, F.; Dopazo, J.; Lupianez, J. A.; Gunther, U.; Cascante, M. Maslinic acid-enriched diet decreases intestinal tumorigenesis in Apc(Min/+) mice through transcriptomic and metabolomic reprogramming. *PLoS One* **2013**, *8*, e59392.
(61) Hsum, Y. W.; Yew, W. T.; Hong, P. L.; Soo, K. K.; Hoon, L. S.; Chieng, Y. C.; Mooi, L. Y. Cancer chemopreventive activity of maslinic acid: suppression of COX-2 expression and inhibition of NF-kappaB and AP-1 activation in Raji cells. *Planta Med.* **2011**, *77*, 152−157.
(62) Park, S. Y.; Nho, C. W.; Kwon, D. Y.; Kang, Y. H.; Lee, K. W.; Park, J. H. Maslinic acid inhibits the metastatic capacity of DU145 human prostate cancer cells: possible mediation via hypoxia-inducible factor-1alpha signalling. *Br. J. Nutr.* **2013**, *109*, 210−222.
(63) Parra, A.; Rivas, F.; Martin-Fonseca, S.; Garcia-Granados, A.; Martinez, A. Maslinic acid derivatives induce significant apoptosis in b16f10 murine melanoma cells. *Eur. J. Med. Chem.* **2011**, *46*, 5991−6001.

(64) Reyes-Zurita, F. J.; Pachon-Pena, G.; Lizarraga, D.; Rufino-Palomares, E. E.; Cascante, M.; Lupianez, J. A. The natural triterpene maslinic acid induces apoptosis in HT29 colon cancer cells by a JNK-p53-dependent mechanism. *BMC Cancer* **2011**, *11*, 154.
(65) Wu, D. M.; Zhao, D.; Li, D. Z.; Xu, D. Y.; Chu, W. F.; Wang, X. F. Maslinic acid induces apoptosis in salivary gland adenoid cystic carcinoma cells by Ca^{2+}-evoked p38 signaling pathway. *Naunyn Schmiedebergs Arch. Pharmacol.* **2011**, *383*, 321−330.
(66) Reyes-Zurita, F. J.; Rufino-Palomares, E. E.; Lupianez, J. A.; Cascante, M. Maslinic acid, a natural triterpene from *Olea europaea* L., induces apoptosis in HT29 human colon-cancer cells via the mitochondrial apoptotic pathway. *Cancer Lett.* **2009**, *273*, 44−54.
(67) Juan, M. E.; Planas, J. M.; Ruiz-Gutierrez, V.; Daniel, H.; Wenzel, U. Antiproliferative and apoptosis-inducing effects of maslinic and oleanolic acids, two pentacyclic triterpenes from olives, on HT-29 colon cancer cells. *Br. J. Nutr.* **2008**, *100*, 36−43.
(68) Li, C.; Yang, Z.; Zhai, C.; Qiu, W.; Li, D.; Yi, Z.; Wang, L.; Tang, J.; Qian, M.; Luo, J.; Liu, M. Maslinic acid potentiates the anti-tumor activity of tumor necrosis factor alpha by inhibiting NF-kappaB signaling pathway. *Mol. Cancer* **2010**, *9*, 73.
(69) Trumbull, E. R.; Bianchi, E.; Eckert, D. J.; Wiedhopf, R. M.; Cole, J. R. Tumor inhibitory agents from *Vauquelinia corymbosa* (Rosaceae). *J. Pharm. Sci.* **1976**, *65*, 1407−1408.
(70) Es-Saady, D.; Najid, A.; Simon, A.; Denizot, Y.; Chulia, A. J.; Delage, C. Effects of ursolic acid and its analogues on soybean 15-lipoxygenase activity and the proliferation rate of a human gastric tumour cell line. *Mediators Inflamm.* **1994**, *3*, 181−184.
(71) Nishino, H.; Nishino, A.; Takayasu, J.; Hasegawa, T.; Iwashima, A.; Hirabayashi, K.; Iwata, S.; Shibata, S. Inhibition of the tumor-promoting action of 12-O-tetradecanoylphorbol-13-acetate by some oleanane-type triterpenoid compounds. *Cancer Res.* **1988**, *48*, 5210−5215.
(72) Juan, M. E.; Wenzel, U.; Daniel, H.; Planas, J. M. Erythrodiol, a natural triterpenoid from olives, has antiproliferative and apoptotic activity in HT-29 human adenocarcinoma cells. *Mol. Nutr. Food Res.* **2008**, *52*, 595−599.
(73) Min, B. S.; Kim, Y. H.; Lee, S. M.; Jung, H. J.; Lee, J. S.; Na, M. K.; Lee, C. O.; Lee, J. P.; Bae, K. Cytotoxic triterpenes from *Crataegus pinnatifida*. *Arch. Pharm. Res.* **2000**, *23*, 155−158.
(74) Martin, R.; Ibeas, E.; Carvalho-Tavares, J.; Hernandez, M.; Ruiz-Gutierrez, V.; Nieto, M. L. Natural triterpenic diols promote apoptosis in astrocytoma cells through ROS-mediated mitochondrial depolarization and JNK activation. *PLoS One* **2009**, *4*, e5975.
(75) Martins, A.; Vasas, A.; Schelz, Z.; Viveiros, M.; Molnar, J.; Hohmann, J.; Amaral, L. Constituents of *Carpobrotus edulis* inhibit P-glycoprotein of MDR1-transfected mouse lymphoma cells. *Anticancer Res.* **2010**, *30*, 829−835.
(76) Chen, H. L.; Lin, K. W.; Huang, A. M.; Tu, H. Y.; Wei, B. L.; Hour, T. C.; Yen, M. H.; Pu, Y. S.; Lin, C. N. Terpenoids induce cell cycle arrest and apoptosis from the stems of *Celastrus kusanoi* associated with reactive oxygen species. *J. Agric. Food Chem.* **2010**, *58*, 3808−3812.
(77) Singh, G. B.; Singh, S.; Bani, S.; Gupta, B. D.; Banerjee, S. K. Anti-inflammatory activity of oleanolic acid in rats and mice. *J. Pharm. Pharmacol.* **1992**, *44*, 456−458.
(78) Kapil, A.; Sharma, S. Effect of oleanolic acid on complement in adjuvant- and carrageenan-induced inflammation in rats. *J. Pharm. Pharmacol.* **1995**, *47*, 585−587.
(79) Manez, S.; Recio, M. C.; Giner, R. M.; Rios, J. L. Effect of selected triterpenoids on chronic dermal inflammation. *Eur. J. Pharmacol.* **1997**, *334*, 103−105.
(80) Suh, S. J.; Jin, U. H.; Kim, K. W.; Son, J. K.; Lee, S. H.; Son, K. H.; Chang, H. W.; Lee, Y. C.; Kim, C. H. Triterpenoid saponin, oleanolic acid 3-*O*-β-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside (OA) from *Aralia elata* inhibits LPS-induced nitric oxide production by down-regulated NF-κB in raw 264.7 cells. *Arch. Biochem. Biophys.* **2007**, *467*, 227−233.
(81) Marquez-Martin, A.; Puerta, R. D. L.; Fernandez-Arche, A.; Ruiz-Gutierrez, V.; Yaqoob, P. Modulation of cytokine secretion by pentacyclic triterpenes from olive pomace oil in human mononuclear cells. *Cytokine* **2006**, *36*, 211−217.
(82) Yang, E. J.; Lee, W.; Ku, S. K.; Song, K. S.; Bae, J. S. Anti-inflammatory activities of oleanolic acid on HMGB1 activated HUVECs. *Food Chem. Toxicol.* **2012**, *50*, 1288−1294.
(83) Lee, W.; Yang, E. J.; Ku, S. K.; Song, K. S.; Bae, J. S. Anti-inflammatory effects of oleanolic acid on LPS-induced inflammation in vitro and in vivo. *Inflammation* **2013**, *36*, 94−102.
(84) Buttke, T. M.; Sandstrom, P. A. Oxidative stress as a mediator of apoptosis. *Immunol. Today* **1994**, *15*, 7−10.
(85) Oita, R. C.; Ferdinando, D.; Wilson, S.; Bunce, C.; Mazzatti, D. J. Visfatin induces oxidative stress in differentiated C2C12 myotubes in an Akt- and MAPK-independent, NFkB-dependent manner. *Pfluegers Arch.* **2010**, *459*, 619−630.
(86) Balanehru, S.; Nagarajan, B. Protective effect of oleanolic acid and ursolic acid against lipid peroxidation. *Biochem. Int.* **1991**, *24*, 981−990.
(87) Montilla, M. P.; Agil, A.; Navarro, M. C.; Jimenez, M. I.; Garcia-Granados, A.; Parra, A.; Cabo, M. M. Antioxidant activity of maslinic acid, a triterpene derivative obtained from *Olea europaea*. *Planta Med.* **2003**, *69*, 472−474.
(88) Perona, J. S.; Arcemis, C.; Ruiz-Gutierrez, V.; Catala, A. Effect of dietary high-oleic-acid oils that are rich in antioxidants on microsomal lipid peroxidation in rats. *J. Agric. Food Chem.* **2005**, *53*, 730−735.
(89) Diaz, A. M.; Abad, M. J.; Fernandez, L.; Recuero, C.; Villaescusa, L.; Silvan, A. M.; Bermejo, P. In vitro anti-inflammatory activity of iridoids and triterpenoid compounds isolated from *Phillyrea latifolia* L. *Biol. Pharm. Bull.* **2000**, *23*, 1307−1313.
(90) Huang, L.; Guan, T.; Qian, Y.; Huang, M.; Tang, X.; Li, Y.; Sun, H. Anti-inflammatory effects of maslinic acid, a natural triterpene, in cultured cortical astrocytes via suppression of nuclear factor-kappa B. *Eur. J. Pharmacol.* **2011**, *672*, 169−174.
(91) Marquez Martin, A.; de la Puerta Vazquez, R.; Fernandez-Arche, A.; Ruiz-Gutierrez, V. Supressive effect of maslinic acid from pomace olive oil on oxidative stress and cytokine production in stimulated murine macrophages. *Free Radical Res.* **2006**, *40*, 295−302.
(92) Yang, Z. G.; Li, H. R.; Wang, L. Y.; Li, Y. H.; Lu, S. G.; Wen, X. F.; Wang, J.; Daikonya, A.; Kitanaka, S. Triterpenoids from *Hippophae rhamnoides* L. and their nitric oxide production-inhibitory and DPPH radical-scavenging activities. *Chem. Pharm. Bull. (Tokyo)* **2007**, *55*, 15−18.
(93) Tsai, S. J.; Yin, M. C. Antioxidative and anti-inflammatory protection of oleanolic acid and ursolic acid in PC12 cells. *J. Food Sci.* **2008**, *73*, H174−H178.
(94) Rong, Z. T.; Gong, X. J.; Sun, H. B.; Li, Y. M.; Ji, H. Protective effects of oleanolic acid on cerebral ischemic damage in vivo and H(2)O(2)-induced injury in vitro. *Pharm. Biol.* **2011**, *49*, 78−85.
(95) Tsai, S. J.; Yin, M. C. Anti-oxidative, anti-glycative and anti-apoptotic effects of oleanolic acid in brain of mice treated by D-galactose. *Eur. J. Pharmacol.* **2012**, *689*, 81−88.
(96) Ovesna, Z.; Kozics, K.; Slamenova, D. Protective effects of ursolic acid and oleanolic acid in leukemic cells. *Mutat. Res.* **2006**, *600*, 131−137.
(97) Hoeschen, R. J. Oxidative stress and cardiovascular disease. *Can. J. Cardiol.* **1997**, *13*, 1021−1025.
(98) Halliwell, B. Oxidative stress and cancer: have we moved forward? *Biochem. J.* **2007**, *401*, 1−11.
(99) Hwang, O. Role of oxidative stress in Parkinson's disease. *Exp. Neurobiol.* **2013**, *22*, 11−17.
(100) Markesbery, W. R. Oxidative stress hypothesis in Alzheimer's disease. *Free Radical Biol. Med.* **1997**, *23*, 134−147.
(101) Nunomura, A.; Perry, G.; Aliev, G.; Hirai, K.; Takeda, A.; Balraj, E. K.; Jones, P. K.; Ghanbari, H.; Wataya, T.; Shimohama, S.; Chiba, S.; Atwood, C. S.; Petersen, R. B.; Smith, M. A. Oxidative damage is the earliest event in Alzheimer disease. *J. Neuropathol. Exp. Neurol.* **2001**, *60*, 759−767.
(102) Berlett, B. S.; Stadtman, E. R. Protein oxidation in aging, disease, and oxidative stress. *J. Biol. Chem.* **1997**, *272*, 20313−20316.
(103) Jeong, D. W.; Wang, B. J.; Wei, C. M.; Yuan, G. Y.; Bu, F. L.; Guo, W. R.; Lee, S. M.; Han, C. K.; Lee, H. S. Dose-linear pharmacokinetics of

oleanolic acid after intravenous and oral administration in rats. *Biopharm. Drug Dispos.* **2007**, *28*, 51−57.
(104) Song, M.; Hang, T. J.; Wang, Y.; Jiang, L.; Wu, X. L.; Zhang, Z.; Shen, J.; Zhang, Y. Determination of oleanolic acid in human plasma and study of its pharmacokinetics in Chinese healthy male volunteers by HPLC tandem mass spectrometry. *J. Pharm. Biomed. Anal.* **2006**, *40*, 190−196.
(105) Lozano-Mena, G.; Juan, M. E.; García-Granados, A.; Planas, J. M. Determination of maslinic acid, a pentacyclic triterpene from olives, in rat plasma by high-performance liquid chromatography. *J. Agric. Food Chem.* **2012**, *60*, 10220−10225.
(106) Rada, M.; Ruiz-Gutiérrez, V.; Guinda, Á. Determination of triterpenic acids in human serum by high-performance liquid chromatography: triterpenoid interaction with serum protein. *J. Agric. Food Chem.* **2011**, *59*, 2308−2313.
(107) Romero, C.; García, A.; Medina, E.; Ruiz-Mendez, M. V.; Castro, A. D.; Brenes, M. Triterpenic acids in table olives. *Food Chem.* **2010**, *118*, 670−674.
(108) Guinda, Á.; Rada, M.; Delgado, T.; Gutierrez-Adanez, P.; Castellano, J. M. Pentacyclic triterpenoids from olive fruit and leaf. *J. Agric. Food Chem.* **2010**, *58*, 9685−9691.
(109) Sánchez-Gonzalez, M.; Lozano-Mena, G.; Juan, M. E.; García-Granados, A.; Planas, J. M. Assessment of the safety of maslinic acid, a bioactive compound from *Olea europaea* L. *Mol. Nutr. Food Res.* **2013**, *57*, 339−346.