Chapter 1

Olive Oil: An Angel of Protection Against Cancer

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First Published \textbf{July 24, 2017}
Abstract

Cancer is one of the leading causes of death worldwide. Despite concerted efforts to improve the outcomes of various cancer therapies, the prognosis of cancer still remains very poor. Diet has been shown to play a vital role both in cancer development as well as in cancer prevention. The ultimate effect of diet on cancer progression and/or cancer prevention is determined by what we eat or the way we eat. Cancer, no doubt, is a preventable disease. Modern research has shown that healthy diet and dietary pattern could prevent about 30-35% incidents of cancer. Olive tree has a very long history and has been looked upon as a sign of hope, peace and sacredness since antiquity, as evidenced by references in ancient religious scriptures including Bible and Quran. Modern research has shown that Extra Virgin Olive Oil (EVOO), the principal source of dietary fat in Mediterranean basin, is quite different in composition from other dietary fats and plays important role in cancer chemoprevention. The cancer chemopreventive effect of EVOO has been attributed to its unique composition represented by mano-unsaturated
fatty acids (MUFA), phenolic compounds, squalene and melatonin. This book chapter will focus on the role of EVOO in cancer chemoprevention with special emphasis on the components of EVOO accounting for these effects and their underlying molecular mechanisms.

Introduction

Cancer is one of the most horrible diseases of the present era and represents the 2\textsuperscript{nd} leading cause of death worldwide with approximately 14 million new cases and 8.2 million cancer-related deaths in 2012 [1]. According to World Health Organization estimate, 17.5 million cancer deaths are projected to occur in the world, by 2050 [1]. After a careful evaluation of extensive studies conducted to determine the factors that contribute to the onset of cancer, it has been suggested that genetic factors account for only about 5\% of cancers while remaining 95\% cancers are linked with life style, diet and other environmental factors. Diet is one of the most important factors and accounts for about 30-35\% of cancer risk [2]. Among various risk factors, dietary factors are modifiable factors and cancer could be effectively prevented by modifying dietary patterns. Dietary fats are one of the main components of human diet. This book chapter will critically shed light on chemopreventive and anticancer effects of extra virgin olive oil (EVOO), the principal component of Mediterranean dietary fat.
The olive tree (*Olea europaea*) is an evergreen tree of the Oleaceae family which is native to the dry, subtropical Mediterranean region and various parts of Africa. It is also cultivated in Australia, California and South Africa. It is well adapted for survival in extreme temperatures and periods of drought. It grows up to a height of 50 feet with a spread of about 30 feet. The normal life expectancy of olive tree is about 500 years [3]. The consumption of wild olive has been believed to date back as far as 8000 BC while domestic cultivation of olive trees can be traced back as far as 1500-3000 BC. The olive fruit has been looked upon as a sign of hope, peace and sacredness by different cultures since time immemorial [4]. In the Quran, The Holy Book of Muslims, olive has been mentioned at least 7 times and has been called as “Blessed Tree”.

**Olive Oil Production and its Types**

Olive oil is produced from flesh of sun-ripened olive by simple application of pressure. In the first step, olive are crushed to form a pomace. The oil from pomace is extracted by mechanical or physical processes such as use of hydraulic press plates or application of continuous horizontal centrifuge. The oil obtained from the first press is of highest quality and is called as Extra Virgin Olive Oil (EVOO). It should be taken into account that not all the EVOO are of same quality. The quality of olive oil depends on various factors such as quality of olive fruit, geographical distribution and environmental factors. The olive oil
obtained from the 2\textsuperscript{nd} press of residual pomace is called as Virgin Olive Oil (VOO) and is of lesser quality \cite{3,4}. If the residual pomace or husk is processed with organic solvent, the oil produced as a result of this process is of lowest quality and is termed as refined husk olive oil or refined olive oil. Refining decreases the phenolic contents of oil and thus decreasing the antioxidant potential of oil \cite{3}.

\textbf{Composition of Olive Oil}

Olive oil is composed of two main fractions, saponifiable or major fraction and non-saponifiable or minor fraction. The saponifiable fraction represents more than 98\% of oil by weight and mainly consists of mono- and poly-unsaturated fatty acids. Fatty acid composition of olive oil is quite different from that of other seed oils. Olive oil contains high amounts of mono-unsaturated fatty acids (MUFAs) especially oleic acid, the concentration of which ranges from 56\%-84\% of total fatty acids \cite{5} while other vegetable oils contain considerably low level of oleic acid such as corn (30\%), palm (43\%), peanut (49\%), soybean (25\%) and sunflower seed (33\%) \cite{6}. On the other hand, the concentration of poly-unsaturated Linoleic acid which is abundantly present in other seed oils, ranges from 3\%-21\% in olive oil \cite{5,6}. Very small quantities of saturated fatty acids such as stearic, palmitic acids (8\%-14\%) and omega-3 fatty acids such as \(\alpha\)-linolenic acids are also found in olive oil \cite{5,7}. The non-saponifiable fraction of olive oil
constitutes less than 2% of the total weight and consists of over 230 chemical compounds including phenols, sterols, hydrocarbons, pigments, volatile compounds, aliphatic compounds and antioxidants. The hydrocarbon profile of olive oil mainly consists of squalene, phenanthrene, perilene, chrysene, and pyrene. Squalene, the most abundant hydrocarbon component of olive fruit is present in much higher quantity in olive oil (0.7%) compared to other vegetable oils such as corn oil, sunflower oil and peanut oil ranging from 0.002%-0.03%. [6]. Phenolic compounds of olive oil are of special interest. To date, at least 30 phenolic compounds has been identified in olive oil. The phenolic compounds of olive oil can be categorized into four main groups; which include simple phenols such as phenolic acid and phenolic alcohols, flavonoids, lignans and secoiridoids. The antioxidant and radical scavenging ability of EVOO is mainly attributed to the phenolic contents of oil. The total phenolic contents in various EVOOs are highly variable ranging from 50 to 940 mg/kg. The concentration of phenols in EVOO depends on various factors such as the area of origin, the cultivar, climate, the degree of ripeness of the olives and the procedures followed for oil extraction [7]. Tyrosol and hydroxytyrosol are the most abundant simple phenols in EVOO which make about 30% of total phenol contents in oil [5]. Like the total phenol contents, the concentration of tyrosol and hydroxytyrosol in different brands of EVOO is also variable.
Chemopreventive and Anticancer Activities of EVOO

Diet accounts for approximately 30-35% of cancer risk [2]. Dietary fat is one of the major components of human diet. The quantity as well as the quality of dietary fat has been associated with cancer risk either positively or negatively [8]. The observational analysis showed that the incidence of various cancers in Mediterranean population is relatively lower compared to the other European and North American countries [9]. This observation led to the formulation of hypothesis that lower cancer risk in Mediterranean population might be associated with Mediterranean dietary pattern. Indeed, this hypothesis was supported by a large number of epidemiological studies [10]. EVOO, the principal dietary fat in Mediterranean countries has been shown to exhibit multiple beneficial effects on human health including anti-oxidant, anti-inflammatory, anti-aging, neuroprotective and cardioprotective effects [11]. To test this hypothesis, in the early 20th century, many in vivo studies have been conducted to explore anticancer effects of EVOO. These studies mainly focused on the role of olive oil in preventing transplanted cancers in mice [12]. Starting from late 20th century to date, a large number of epidemiological studies including case control and cohort studies have been conducted to investigate the possible chemoprotective and anticancer effect of EVOO in Mediterranean diet [13]. Collectively, the data indicate
that the consumption of EVOO has been associated with reduced risk of several cancers especially those of upper digestive and respiratory cancers. Contrary to Mediterranean diet, more consumption of the Western diet has been associated with 60% increase of endometrial cancer as determined by a study conducted on women in San Francisco [14]. Overall, the incidence and death rate of some major cancers including breast, ovary, colon, stomach, endometrium and prostate in Mediterranean countries are lower than in Scandinavian countries, United Kingdom and the United State of America [3,9]. Current estimates indicate that if the population of highly developed Western countries shifts from Western diet to a traditional Mediterranean diet, about 25% of colorectal cancers, 15% of breast cancers and 10% of prostate, pancreas and endometrial cancer could be effectively prevented [9]. Very recently, a systematic review and meta-analysis of observational studies in which 1.78 million subjects were included, was conducted to find out the association between Mediterranean diet and various human cancers. The data suggest that adherence to the highest degree of Mediterranean diet is associated with significant lower incidence of various cancers such as colorectal (17%), breast cancer (7%), gastric cancer (27%), prostate cancer (4%), liver cancer (42%) and head and neck cancer (60%) [15].
What Makes EVOO a Chemopreventive and Anticancer Dietary Fat

A large body of literature evidence indicates the protective role of EVOO against various human cancers. In order to understand the chemopreventive potential of EVOO, a plethora of research studies have been conducted till now. Collective data from multitudinous studies demonstrate that chemopreventive and anticancer activity of EVOO is attributed to its unique composition which remarkably differs from other vegetable oils such as corn oil, sunflower oil, soybean oil and peanut oil. The EVOO is a unique mixture of various biologically active molecules, the concentrations of which are quite different from other seed oils. These includes MUFAs especially oleic acid, which makes about 80% of the total fatty acids; phenolic compounds which are characteristic features of EVOO; squalene, the concentration of which is 350-24 times higher in EVOO than other vegetable oils [6] and melatonin which is twofold higher in EVOO than in refined olive oil and sunflower oil [16]. The chemopreventive as well as anticancer effects of these unique components are briefly discussed here.

Fatty Acid Profile of EVOO and Cancer Chemoprevention

To date, a large number of research studies have been conducted to find out the association of EVOO unique
components with cancer. Firstly, EVOO differs remarkably in its fatty acids profile from other vegetable oils. Chemopreventive and/or anticancer effects of MUFAs in EVOO remain controversial [10]. However, most of the studies reported protective effect of MUFAs of olive oil against human cancers [3,17]. In one such study, Yaqoob et al., have shown that olive oil and fish oil have a similar suppressive effect on lymph node lymphocyte proliferation while this effect was significantly higher compared to other oils such as safflower oil or coconut oil [18]. Further studies showed that sunflower oil with high oleic acid content exerted similar growth suppressive effects on lymphocyte proliferation as that of olive oil, indicating the anti-proliferative effect of oleic acid [19]. Unlike olive oil, fish oil contains high contents of omega-3 polyunsaturated fatty acids (n−3 PUFAs) eicosapentaenic acid (EPA) and docosahexaenoic acid (DHA). Several in vivo animal model studies have shown that feeding animals with oil having high content of n-6 PUFAs such as Linoleic acid enhanced tumor growth, progression and metastasis where as fish oil which contains high contents of n-3 PUFAs (EPA & DHA) has tumor inhibitory effects [20,21]. It is well established now that n-6 PUFAs are converted by COX-2 enzyme into prostaglandin E2 (PGE2), a proinflammatory cytokine that not only facilitate cancer initiation but also promote tumor progression while n-3 PUFAs are converted by COX-2 enzyme into prostaglandin E3 that does not exhibit tumorigenic properties [2].
Excessive production of PGE2 has been implicated in various human cancers. In addition to anticancer effect mediated through inhibition of PGE2, n-3 PUFAs (EPA, DHA) have been shown to inhibit proliferation, induce apoptosis, suppress inflammation, regulate transcription factors such as NF-κB and diminish formation of estrogen [22]. It should be noted that both fish oil and EVOOO have been shown to exhibit similar chemopreventive activity, however; the composition of both oils is quite different. The anti-tumor effects of fish oil could be partly explained by the possible mechanisms described above, however; the anticancer mechanism of EVOO fatty acid profile remains largely unclear. One possibility is that the low level of linoleic acid in EVOO is associated with cancer prevention. Another possibility is, increasing oleic acid and α-linolenic acid would decrease the relative proportion of other fatty acids consumed. A decrease in linoleic acid could result in conversion of α-linolenic acid into n-3 PUFA (EPA). This might explain the similar cancer chemopreventive effects of EVOO and fish oil. Yet another possibility is that EVOO with high contents of MUFAs is less likely to induce oxidative stress which is associated with initiation and progression of cancer.

In addition to chemoprevention, there are now evidence that oleic acid which makes about 80% of total fatty acid profile of olive oil, can inhibit growth and induce cell death in cancer cells by suppressing HER2/erbB-2, a member of the type 1 receptor tyrosine kinases (RTKs)
expression which is one of the most commonly analyzed genes in human cancers. Exogenous supplementation of oleic acid at a concentration of 10 μM for 48 h has been shown to decrease about 50% the expression of HER2-coded p185\textsuperscript{Her2/neu} protein in BT-474 and SK-Br3 breast cancer cell lines. The effect of oleic acid at this concentration was comparable to the effect of HER2 monoclonal antibody trastuzumab at its optimal concentration in BT-474 and SK-Br3 breast cancer cells [23]. Oleic acid treatment has also been shown to repress the activity of HER2 gene promoter in HER2 over-expressing SK-Br3 breast, SK-OV3 ovarian and NCI-N87 gastric cancer cell lines. Further data showed that in combine treatment, oleic acid synergistically enhanced trastuzumab-mediated down-regulation of HER2 and augmented anticancer efficacy of trastuzumab by increasing DNA fragmentation, caspases-3 activation and PARP cleavage which are the characteristic features of apoptotic cell death. Moreover, in endothelial cells, oleic acid has been shown to inhibit angiogenesis by inhibiting VEGF-induced tyrosine phosphorylation of VEGFR2 [24]. Further study demonstrated that oleic acid significantly inhibited TNF-α-induced COX-2 gene and protein expression as well as PGE2 secretion in U87 glioblastoma cells [25]. COX-2/PGE2 signaling pathway plays important role in cancer progression by promoting migration and angiogenesis of cancer cells. The molecular mechanism by which oleic acid has been reported to inhibit angiogenesis and HER2 expression is shown in Figure 1.
Figure 1: Molecular mechanism of oleic acid-induced angiogenesis inhibition and HER2/erbB-2 suppression. Oleic acid inhibits angiogenesis by inhibiting phosphorylation of VEGFR2 and expression and secretion of COX-2 and PGE2, respectively. HER2 gene expression is negatively regulated by Polyomavirus Enhancer Activator 3 (PEA3) transcriptional factor. The PEA3 binding motif on HER2 gene promoter itself acts as a positive regulator for HER2 gene expression. If this binding site is occupied by PEA3, the activity of HER2 promoter is repressed. HER2 over-expressing cancer cells express very low to undetectable trans-repressor PEA3 level which allow The PEA3 binding motif on HER2 gene promoter to continue the transcription of HER2 gene. Oleic acid down-regulate Fatty Acid Synthase (FASN) which results in accumulation of FASN substrate Malonyl-CoA in the cells. Malonyl-CoA through an unknown mechanism induces the expression of transcriptional factor PEA3 which ultimately inhibits HER2 gene transcription by binding with the PEA3 binding motif on HER2 gene promoter.
**Phenolic Components of EVOO and Cancer Chemoprevention**

Cancer chemopreventive ability of EVOO is mainly attributed to its phenolic contents. Unlike lipophilic phenols such as tocopherols and tocotrienols which are also present in other vegetable oils, EVOO contains a large amount of hydrophilic phenols (phenolic acid, phenolic alcohols, flavonoids, lignans and secoiridoids) which make it unique from other vegetable oils [26]. Despite multiple mechanisms reported in the literature, cancer chemoprevention by EVOO phenols through their antioxidant potential has been considered the most acceptable model. Reactive oxygen species (ROS) are oxygen containing reactive chemical entities which are continuously produced inside the cells during normal metabolic processes of the cells. In normal cells, there exists a sophisticated balance between ROS production and antioxidant system’s ability to neutralize ROS. However, an imbalance between ROS production and cells’ antioxidant ability to readily detoxify ROS results in over-production of ROS. Overproduction of ROS leads to oxidative damage including lipid peroxidation, protein oxidation and DNA damage [27]. DNA damage is potentially mutagenic. Although our body is blessed with effective defense mechanisms against ROS, the capacity of these mechanisms decreases with the passage of age [28]. Therefore, body should be constantly provided with antioxidants through dietary supplements to reduce the burden of ROS and to protect body from deleterious effects of ROS. The role of ROS in
tumor development as well as in tumor progression is well established now [29]. In this regard, EVOO phenols with potential antioxidant ability are considered worthy in cancer chemoprevention. Indeed, significant part of cancer prevention by EVOO phenolic compounds is attributed to their antioxidant potential. EVOO phenols have been shown to exert antioxidant effects through multiple mechanisms including metal ions chelation such as iron (Fe) and copper (Cu) as well as free radical scavenging [30]. The antioxidant ability of EVOO phenols has been extensively studied [31-35]. The antioxidant ability of phenolic compounds has been found to be associated with the number and position of hydroxyl group (OH) on phenol ring. Moreover, EVOO phenolic compounds have been shown to effectively protect ROS-induced DNA damage as witnessed from various in vitro and in vivo studies [36-39]. The results indicate that EVOO phenolic compounds mainly inhibit cancer initiation by scavenging free radicals and protecting DNA from ROS-induced mutation.

**EVOO’s Polyphenols Inhibit Formation of Carcinogens During Pan Frying and Grilling Meat**

In the last few decades, several epidemiological studies conducted in geographically distinct regions, have shown a strong association between diet and cancer risk especially those of gastric, colorectal, bladder, kidney and endo-
metrial tumors [2]. Improved technology in food industry, no doubt, has achieved the goal of preserving food for long time however; these processed and preserved foods are not associated with healthy effects. More consumption of red meat has been associated with increased risk of colorectal cancer [2]. However, it should be noted that carcinogenic effects of consuming red meat are mainly associated with chemical substances such as n-nitroso compounds, heterocyclic aromatic amines (HAs) and polycyclic aromatic hydrocarbons (PAHs) which are produced through meat processing, preservation and cooking at high temperature such as pan fry or grilling directly over an open flam [40]. Heterocyclic aromatic amines are produced by the reaction of amino acids, sugars and creatine when muscle meat, including beef, pork, fish, or poultry, is cooked using high temperature methods, such as pan frying or grilling directly over an open flame. Modern research has shown that application of EVOO or phenolic compound significantly inhibits formation of HAs during meat or fish roasting. Moreover, Monti et al., determined the formation of HAs by heating an aqueous solution of creatine, glucose and glycine in the presence of EVOO and found that EVOO significantly inhibited HAs formation in the given system compared to control. Inhibition of HAs have also been verified by phenolic compounds extracted from EVOO [41]. The findings suggest that EVOO plays a protective role during cooking/roasting of meat and fish by preventing the formation of potential carcinogenic HAs. PAHs are released from combustion related-activi-
ties such as tobacco smoke, coal tar burning, automobile exhausts, and wood burning. When meat is grilled or barbecued directly on an open flame, PAHs contaminate foodstuffs [42]. Benzo[a]Pyrene (BaP), a highly carcinogenic PAH has been reported to induce cancer in various organs including lung, liver, gastric and mammary glands through multiple mechanisms [43,44] and has now been categorized as Human Group 1 carcinogen by the International Agency for Research on Cancer [43]. Very recently, Banks et al., investigated the preventive effect of olive oil on BaP-induced colon cancer in adult Apc\textsuperscript{Min} mice. The data demonstrated that olive oil effectively inhibited BaP-induced cancer in mice by promoting rapid detoxification of BaP by decreasing its organic metabolites, preventing BaP-induced DNA damage and altering the expressions of drug metabolizing enzymes both in the liver and colon tissues [45]. These findings suggest that olive oil hold the promise to protect the body from the effects of potent carcinogens produced during cooking foodstuffs.

**EVOO’s Polyphenols Inhibit Formation of Acrylamide in Potato Chips**

Potato chips are predominant part of the snack food worldwide with various brand names. Modern research has shown that during frying, acrylamide, a classified carcinogen is formed in potato chips. The intensity of color in fried potato chips is positively associated with acrylamide
contents. The exact molecular mechanism of acrylamide formation during frying process is not clear however, it appears to result from Maillard reaction involving amino acids especially asparagine and reducing sugars [46,47]. Very recently, Napolitano et al., evaluated the effect of EVOOs differing in phenolic contents, on acrylamide formation during potato chips frying for various time points. They found that EVOO with higher phenolic contents decreased the formation of acrylamide in potato chips compared to control. Moreover, they found that EVOO enriched with ortho-diphenolic compounds (such as hydroxytyrosol, cafeic acid, oleuropein) could effectively inhibit acrylamide formation in chips cooked in mild to moderate frying conditions [48].

**EVOO Inhibits Formation of Hydroxy-alkenal at High Temperature**

Hydroxy-alkenals are potentially toxic and carcinogenic compounds formed from thermal decomposition of PUFAs during heating at high temperature [49]. Sacchi et al., heated EVOO, sunflower oil and soybean oil in a thermostatic bath fryer at 180°C for 6 h and determined the level of hydroxy-alkenals at the end. They found that trans-2-alkenals were equally formed in all tested oils while the production of alka-2,4-dienals was low in EVOO compared to other seed oils. Unlike sunflower oil and soybean oil, 4-hydroxy-2-alkenals, were not detected in EVOO after 6 h of heating. In contrast to vegetable oils,
EVOO is enriched with MUFAs, higher concentration of PUFAs in sunflower oil and soybean oil and quite low level of PUFAs in EVOO explains these finding [49].

EVOO Improves Nutritional Value of Cooked Food

In addition to inhibiting the formation of toxic chemical, foodstuffs cooked in EVOO, offer an additional intake of antioxidants, thus improving the nutritional value of cooked food. To date, various studies have been conducted to determine the uptake of antioxidants and other healthy components in foodstuffs cooked or fried in EVOO. In one such study, French fries were fried in EVOO and crust of French fries was subjected to LC-MS for detection of EVOO’s phenolic compounds. Significant amount of phenolic compounds were found in crust of French fries [50]. In another study, Kalogeropoulos et al., pan fried finfish in EVOO. Fresh and fried fish and oil samples were subjected to GC/MS and HPLC for the analyses of polyphenols, hydroxy pentacyclic triterpene acids (HPTAs) and α-tocopherol. Nine polyphenols were detected in frying oil, out of which 6 were also found in fried fish. The terpenic acids oleanolic, maslinic and ursolic were also found both in frying oils and fried fish, however; no polyphenol and/or HPTA were detected in raw fish samples [51]. The same authors provided further evidence of uptake of polyphenols, HPTAs and α-tocopherol in vegetables (Potatoes, green peppers, zucchinis and eggplants) shallow fried in
EVOO [52]. The effect of heating on α-tocopherol (Vitamin E) contents in the presence of varying concentrations of EVOO phenols has also been investigated by Pellegrini et al., [53]. They prepared four oil samples from refined olive oil with fixed amount of α-tocopherol and increasing amount of polyphenols extracted from EVOO. The samples were heated for 30-120 min at a temperature of 160-190°C and measured the level of α-tocopherol in all samples. The data demonstrate that polyphenols from EVOO act as an efficient stabilizers for α-tocopherol during olive oil heating. Collective data from these studies suggest that foodstuffs cooked in EVOO provide an additional intake of antioxidants and terpenic acids.

**EVOO’s Polyphenols Improve the Quality of Canned Fish**

Canning is a method of preserving food, in which food contents are processed and sealed in an airtight container. Tuna fish is canned in edible oils, in brine or in sauces. Tuna fish is processed and canned. The sealed cans are then heated for 2-4 hours to kill bacteria. This thermal treatment could induce lipid oxidation. Medina et al., conducted several studies [54,55] in which they canned Tuna muscles in EVOO and determined its preservative effect on n-3 PUFAs present in fresh fish before tuna canning. The level of n-3 PUFAs was significantly higher in tuna canned in EVOO compared to those canned and sterilized in soybean or refined olive oils as well as in brine.
Similarly, the level of oxidation found in lipids extracted from canned tuna muscles was also lower in EVOO. This protective effect of EVOO during/after the thermal treatment of cans appears to come from natural antioxidants of EVOO which are not present in other filling oils and brine [56,57].

**EVOO’s Polyphenols Increase the Antioxidant Activity of Tomato Sauce**

Consumption of fresh tomato and tomato products have been associated with reduce risk of several types of cancers especially those of the prostate, lung, breast and digestive tract. The anticancer effect of tomato is associated with lycopenes (carotenoids) in high quantity [58]. In human diet, both fresh and transformed tomatoes (such as tomato sauce) are consumed worldwide. In Mediterranean region, a tomato sauce is typically prepared by heating at 70-80°C for about 6-10 h which ultimately results in loss of antioxidants [49]. A recent research report from Sacchi et al., showed that cooking tomato sauce in the presence of small amount of EVOO substantially increases antioxidant activity of tomato sauce during cooking. The effect appeared to come from the protective action of secoiridoids present in EVOO on tomato carotenoids [41]. Thus tomato sauce preparation with addition of small amount of EVOO could increase the chemopreventive effect of tomato sauce.
Chemopreventive and Anticancer Mechanism of some Important Bioactive Compounds of EVOO

In addition to chemopreventive and anticancer effects of EVOO, many studies have been conducted to investigate the major bioactive components of EVOO responsible for chemopreventive and anticancer effects of EVOO. To date, several compounds have been identified and characterized for their potential anticancer activities in various in vitro as well as in vivo animal model studies. In this section, we will discuss the chemoprotective and/or anticancer activity of some major bioactive molecules of EVOO, their cellular targets and underlying molecular mechanisms one by one.

Hydroxytyrosol (3,4-DHPEA)

Hydroxytyrosol is a simple phenol present in olive oil. Among phenolic components of olive oil, hydroxytyrosol is the most studied compound for its biological and pharmacological properties. It has been shown to possess antioxidant, anti-inflammatory, chemopreventive and anticancer activities [59]. Cancer chemopreventive effect of hydroxytyrosol appears to come from its antioxidant ability. ROS-induced DNA damage is implicated in cancer initiation. The protective effect of hydroxytyrosol against H₂O₂ induced DNA damage has been verified in
various human cells including chondrocytes [60], breast and prostate tumour cells [39,61], blood monocytes and neuroblastoma cell lines [62], Jurkat cells [63], human peripheral blood mononuclear cells (PBMC) [38, 64] and in a human promyelocytic leukaemia cells (HL60) [38].

In addition to cancer chemopreventive effects, hydroxytyrosol has been shown to exert anticancer activity by inhibiting proliferation and cancer progression through multiple mechanisms both in vitro cell studies and in vivo animal models. In vitro cell studies data show that hydroxytyrosol exerts anticancer activity by inhibiting growth and inducing apoptosis in various human cancer cell lines including HT-29, CaCo2 and WiDr colon cancer [59,65,66], M14 melanoma [67], HL-60 leukemia [65,68], and MCF-7 breast cancer [69]. The antiproliferative effect of hydroxytyrosol has also been tested by Rosignoli et al., [64] against MCF-7 and MDA-MB-231 (breast cancer), PC3 and LNCaP (prostate) and SW480 and HCT116 (colon) cancer cells.

Terzuoli et al., [59,70] evaluated the in vivo anticancer efficacy of hydroxytyrosol in HT-29 xenograft model in two independent experiments using the same model. Treatment of hydroxytyrosol at a dose of 10 mg/kg body weight for 2 weeks reduced the tumor growth by 50% compared to control group. Moreover, tumors from hydroxytyrosol treated mice exhibited clear reduction in vessel size with significantly lower expression of Ki-67 (proliferation marker), Epidermal growth factor receptor
(EGFR), hypoxia inducible factor-1α (HIF-1α), vascular endothelial growth factor (VEGF), microsomal prostaglandin-E synthase-1 (mPGES-1) and increase expression of cleaved caspases-3 (apoptosis marker). During whole experimental period no sign of toxicity has been detected in mice at given dose of hydroxytyrosol.

In another study, Li et al., [71] evaluated the anticancer effect of hydroxytyrosol in human cholangiocarcinoma cell lines TFK-1 and KMBC and gallbladder cell line GBS-SD using in vitro cell studies. The anticancer effects were further validated in TFK-1 xenografts in nude mice. Intraperitoneal injection of hydroxytyrosol at a dose of 500 mg/kg/day for 3 weeks significantly reduced the size and weight of tumor compared to the control groups. The expression of Ki-67 (Proliferation marker) was found to be significantly lower while the number of apoptotic cells was significantly higher in tumor tissues derived from hydroxytyrosol-treated mice. Western blot analysis of tumor homogenates showed that hydroxytyrosol treatment decreased the expressions of p-ERK, Pro-PARP, Bcl-2, cyclin B1 and, p-Cdc2 (Thr15) while increased the expressions of cleaved PARP, caspase-9, caspase-3, Bax and p-Cdc2 (Tyr161). These results were also verified in TFK-1, KMBC and GBS-SD cell lines using in vitro cell studies. It is important to note that no apparent change in liver, spleen and body weight was observed even at very high dose of hydroxytyrosol. Similarly, subcutaneous injection of hydroxytyrosol (100μg/day) for 5 days effectively in-
hibited the growth of C6 rat glioma xenografts. However, antitumor activity of hydroxytyrosol was completely diminished when animals were treated with a combination of hydroxytyrosol and oleuropein (powerful antioxidant), indicating that hydroxytyrosol-mediated antitumor activity is not derived from its antioxidant potential [72]. In addition to xenografts tumor models, the anticancer effect of hydroxytyrosol has also been evaluated in chemically (DMBA:7,12-dimethylbenz[a]anthracene)-induced mammary tumor model in female Sprague–Dawley rats [73]. Mammary tumor was developed by exposing animals to DMBA. Hydroxytyrosol treatment (0.5mg/kg body weight; 5 days/week, for 6 weeks) was started when tumor volume reached 2cm³. At the end, tumor volume was measured and tumor tissues were subjected for analysis of various genes expressions. Hydroxytyrosol significantly reduced breast tumor volume and Ki-67 expression in tumor tissues. Moreover, microarray analysis of cDNA from 595 identified sequences showed that 99 genes were up-regulated while 74 were down-regulated by hydroxytyrosol treatment. The microarray data was further validated by RT-PCR analysis which showed that hydroxytyrosol treatment significantly modulated the expressions of 13 genes, 6 genes were up-regulated and 7 genes were down-regulated. A dramatic increase (12 times) in the expression of Secreted frizzled-related protein 4 (SFRP4) gene was observed. SFRP4 gene is down-regulated in various cancers including breast cancer and its expression is
inversely associated with cancer growth and progression. SFRP4 has been shown to inhibit cancer cell proliferation by inhibiting wnt signaling pathway which is implicated in cancer progression [74].

**Oleocanthal (p-HPEA-EDA)**

Oleocanthal is a phenolic component of olive oil. The peculiar pungent and irritant sensation of EVOO is mainly contributed by oleocanthal [75]. It has been reported to exhibit antioxidative, antibacterial, anti-inflammatory and antitumor activities [76]. The anticancer activities of oleocanthal has been verified in various human cancers including breast cancer [77], prostate cancer [78], liver cancer [76], colon cancer [78], multiple myeloma [80], melanoma [81] and human leukemia monocytes lymphoma [82] both *in vitro* and *in vivo*. Collective data from studies mentioned above revealed that oleocanthal anticancer activity is associated with its effects on multiple cellular targets including transcription factors such as STAT3 and Activator Protein-1 (AP-1); survival pathways such as AKT-mTOR pathway; Kinases such as extracellular regulated kinase (ERK)1/2, AMP activated protein kinase (AMPK); Non-receptor tyrosine kinases such as JAK1, JAK2 and Src; Phosphatases such as SHP-1; invasion and metastasis markers such as matrix metalloproteinases (MMP-2, MMP-9), VEGF, E-cadherin, N-cadherin, Vimentin, and Twist; anti-apoptotic proteins such
as Bcl-2, Bcl-xL, cyclin D1, and survivin and apoptosis regulators such as caspases activation and PARP cleavage.

To date, the *in vivo* anticancer activity of oleocanthal has been investigated in 3 independent studies. In first study, Akl et al., [77] established an orthotopic breast cancer model by injecting MDA-MB-231/GFP cells into the mammary gland fat pad of female athymic nude mice. Five days post-inoculation, mice were treated by intraperitoneal injection of oleocanthal (5mg/kg, 3x/week) for 33 days. At the end of experiment, tumor was excised, weighed and tumor tissues were subjected to immunohistochemistry and Western blot analysis for the expression of Ki-67, CD31, c-Met and PARP. The results showed that oleocanthal reduced the tumor weight by 60% compared to control group. Moreover, oleocanthal reduced the expression of Ki-67, CD31 and decreased the phosphorylation of c-Met in tumor tissues [77].

Very recently, Gu et al., [81] evaluated the anti-melanoma activity of oleocanthal both *in vitro* and *in vivo*. Oleocanthal inhibited proliferation, invasion and migration and induced apoptosis in A375 and G361 melanoma cells in a dose-dependent manner (0-60μM). Furthermore, oleocanthal suppressed tube formation in human umbilical vascular endothelial cells. These results were further supported by *in vivo* xenograft model. A375 cells were subcutaneously injected into the flank of nude mice and
treatment started when tumor volume reached 100mm$^3$. Oleocanthal was administered intraperitoneally at a dose of 10 mg/kg/day for a time period of 3 weeks. Oleocanthal significantly reduced tumor size with suppressive effect on the expression of Ki-67 and CD31. The anti-metastatic effect of oleocanthal has also been evaluated by injected A375 cells into nude mice through tail vein. Treatment was started after one week at a dose of 15mg/kg/week. Metastasis was measured by bioluminescence every two weeks. After 6 weeks, mice were sacrificed; lungs were excised for further analysis. Very weak illumination signals and fewer metastatic foci were detected in lungs of treated group compared to control group. Further *in vitro* mechanistic study demonstrated that oleocanthal inhibited STAT3 signaling pathway and down-regulated STAT3 down-stream target genes including Mcl-1, Bcl-xL, MMP-2, MMP-9, and VEGF.

The anticancer activity of oleocanthal has also been determined in liver cancer both *in vitro* and *in vivo* studies. Oleocanthal suppressed growth, arrested cell cycle at G0/G1 phase and induced apoptosis in HepG2, HCCLM3 and Huh-7 liver cancer cells at a concentration range of 0-50 μM. Further mechanistic study showed that oleocanthal inhibited invasion and metastasis. These effects were found to be associated with inhibition of STAT3 activation, and its downstream target genes including Twist, MMP-2, cyclin D1, survivin, Bcl-2, N-cadherin, and vi-
mentin. These results were further verified in orthotopic liver cancer model. Oleocanthal at a dose of 5mg/kg/day and 10mg/kg/day significantly inhibited tumor growth and metastasis in animal mouse models. Similar expressions of proteins were detected in tumor tissues derived from oleocanthal-treated mice [76].

**Oleuropein (3,4-DHPEA-EA)**

Oleuropein is the most abundant phenolic secoiridoid glycoside present in olive leaves and unprocessed olive drups while in olive oil it is found in the form of oleuropein aglycon. It is one of the most extensively studied phenolic contents of EVOO for its chemopreventive and anticancer properties both *in vitro* and *in vivo*. A large number of *in vitro* studies demonstrated the anticancer effects of oleuropein against a wide range of human cancers including breast, colorectal, lung, bladder, prostate, kidney, skin and brain cancers [83]. Consistent with *in vitro* results, several *in vivo* animal model studies have shown that oleuropein is effective in preventing chemical- and radiation-induced cancer of skin, breast, tongue and soft tissue [84]. Hamdi et al., determined the anticancer effect of oleuropein in Swiss albino mice with soft tissue sarcoma. Treatment of Albino mice with 1% oleuropein in drinking water for 9-12 day induced complete tumor regression [85]. The anticancer effect of oleuropein was further evaluated in other carcinogenesis models in which tumors were induced either by UVB radiations or by azoxymethan (AOM).
In UVB-induced skin cancer mouse models, topical application as well as orally administered oleuropein effectively prevented skin cancer [86,87]. Orally administered oleuropein effectively prevented the radiation-induced expression of Ki-67, CD31, MMP-2, MMP-9, MMP-13, VEGF and Cox-2. The findings indicate that oleuropein is able to inhibit cancer initiation as well as cancer progression. ROS-induced DNA damage plays vital role in cancer initiation. The protective effect of oleuropein in UV irradiation- and ROS-induced DNA damage has been well documented. Indeed, oleuropein has effectively prevented UVB-induced and AOM-induced DNA damage in C57BL/6J and A/J mice, respectively [88,89]. The protective effect of oleuropein has also been verified in human blood monolayer cells exposed *ex vivo* to H$_2$O$_2$. Additional evidence supporting inhibitory effect of oleuropein in cancer initiation came from another study in which tumor was induced in rat tongue with 4-nitroquinoline 1-oxide (4-NQO) [90]. Very recently, Giner et al., [91] have demonstrated the chemopreventive effect of oleuropein in colitis-associated colorectal cancer in C57bl/6 mice. In this model, co-exposure of mice to AOM and dextran sodium sulfate (DSS) resulted in induction of intestinal inflammation as evidenced by increase level of IL-6, IFN-γ, TNF-α, IL-17A and COX-2 and colon tumor development in all mice. Oleuropein treatment efficiently suppressed inflammatory responses and tumor development in this model. Further data showed that oleuropein reduced the expres-
sion of Ki-67, increased apoptosis by up-regulating Bax, and inhibited NF-κB, wnt/β-catenin, AKT and STAT3 survival pathways. The molecular mechanism by which oleuropein prevent DNA damage and cancer initiation *in vivo* is not clear, however, it appears that oleuropein inhibits cancer initiation via its antioxidant activity as both AOM and 4-NQO initiate carcinogenesis by forming highly reactive intermediates that form adducts to DNA.

The inhibitory effect of oleuropein on cancer promotion/progression has also been investigated in MCF-7 xenograft mouse model. A significant inhibitory effect of oleuropein on xenograft tumor growth was detected in this model [77]. Collective data from various studies clearly indicate the suppressive effect of oleuropein on tumorigenesis and tumor progression.

**Squalene**

Squalene is a poly-unsaturated triterpene hydrocarbon present in high amount in shark liver oil and EVOO. Squalene is the major hydrocarbon in EVOO with its content about 7mg/g which is extremely higher compared to other vegetable oils including corn, peanut and sunflower oils. In US the average intake of squalene is about 30 mg/day where as in Mediterranean countries where EVOO is the main dietary fat, the average intake of squalene is about 200-400mg/day. Squalene is an intermediate metabolite in cholesterol synthesis and possesses multiple biological
activities including antioxidant and antitumor properties [92,93]. The absence of cancer in shark is correlated with the high level of squalene (>40%). Similarly, high level of squalene in EVOO is believed to be partially responsible for low cancer incidence in Mediterranean countries. The cancer chemoprotective effect of squalene has been tested in various animal models. Topical application of squalene completely inhibited Benzo(a)Pyrene (BaP)-induced skin cancer and suppressed tumor promoting effect of 12-O-tetradecanoylphorbol-13-acetate (TPA) in animal mouse models [94]. Other studies showed that oral administration of squalene effectively inhibits chemically induced colon, lung and mammary gland tumorigenesis [95-97]. In addition to its chemoprevention ability, Squalene has been shown to overcome chemotherapy-induced side effects [98]. This protective effect of squalene against chemotherapy-induced side effects is attributed to its free radicals scavenging ability. Squalene has been suggested to inhibit chemically-induced cancer by following mechanisms [93,94].

1. Squalene has been proposed to inhibit cancer cell proliferation by inhibiting Ras oncoprotein farnesylation by decreasing the level of Farnesyl pyrophosphate as well as inhibiting the conversion of HMG CoA to mevalonate by negative feedback regulation of HMG CoA reductase. Thus reducing the level of mevalonate in cells which is necessary for DNA synthesis and cell proliferation.
2. A second mechanism by which squalene may inhibit tumorigenesis is by modulating the biosynthesis and function of xenobiotic metabolizing enzymes, thus altering the metabolic activation of carcinogens.

3. The 3rd possible cancer chemopreventive mechanism of squalene is attributed to its free radical scavenging ability. Antioxidant as well as protective effect of ROS-induced DNA damage by squalene has been tested [92].

**Oleanolic Acid**

Oleanolic acid is one of the most common pentacyclic triterpenoids present in a large number of plant species. The concentration of oleanolic acid in olive oil ranges from 4 mg/kg-79mg/kg [99]. Very recently, Ziberna et al., reviewed the anticancer activity, cellular targets and molecular mechanism of oleanolic acid [100]. Oleanolic acid has been extensively studied and its anticancer effects have been evaluated in various human cancer cell lines of multiple origin including liver cancer, lung cancer, breast cancer, colon cancer, bladder cancer, prostate cancer, pancreatic cancer, gastric cancer, gallbladder cancer, glioblastoma, osteosarcoma and hematological malignancies, e.g., leukemia [100]. Cancer preventive effects of oleanolic acid have also been investigated in TPA-induced skin cancer and colon cancer in animal models [101,102]. Further in
in vivo studies showed the anti-metastatic effects of oleanolic acid [103- 105].

Collective data from extensive in vitro studies conducted to evaluate the antitumor effect of oleanolic acid in various human cancers indicate that it induces apoptosis and cell cycle arrest in cancer cells by interfering with various cellular targets and multiple signaling pathways important for cell survival and growth. It has been shown to induce apoptosis by both intrinsic and extrinsic pathways. The induction of apoptosis was found to be associated with Bcl-2 family proteins modulation, mitochondrial dysfunction, ROS generation, caspases activation and PARP cleavage. In addition to apoptosis induction, oleanolic acid has also been reported to induce autophagy as evidenced by up-regulation of Atg5 and beclin and increased LC3II/LC3I ratio. Interestingly, induction of apoptosis as well as autophagy by oleanolic acid was primarily found to be linked with ROS generation. In addition, oleanolic acid disrupted Warburg effect by inducing pyruvate kinase muscle (PKM) isoforms switch from PKM2 to PKM1 through suppression of phosphorylated mTOR in various cancer cells. Other studies reported it as Topoisomerase I and II inhibitor. Oleanolic acid has been shown to inhibit multiple survival pathways including PI3K/AKT/mTOR, ERK/JNK/p38MAPK, NF-κB and STAT3 pathways in various cancer cells [100].
Melatonin

In mammals, melatonin is predominantly produced by pineal gland at night [16]. Besides its well-known hormonal and sleep-inducing properties, several preclinical and clinical studies have documented its role as a powerful antioxidant and tumor suppressor. In addition to the general protective effect against a wide range of tumors, the oncostatic effect of melatonin against hormone-dependent tumors has been well documented. Melatonin exerts its oncostatic action by directly protecting cells from the effect of free radicals and indirectly by stimulating the activation of antioxidant enzymes in general and inhibiting the secretion of hormones such as estrogen in hormone-dependent cancers such as breast and ovarian cancers specifically. Mounting evidence from multitudinous studies have reported that melatonin could effectively inhibit growth, induces cell cycle arrest and apoptosis, inhibits cancer invasion and metastasis through multiple mechanisms, thus highlighting its therapeutic significance [106]. The level of melatonin decreases as one ages [107]. Moreover, as a result of industrial revolution, artificial light at night decreased the length of sleep in dark at night. Artificial light at night has been inversely associated with melatonin biosynthesis in pineal gland [16]. Therefore, exogenous intake of melatonin is suggested as an effective strategy to overcome tumorigenesis associated with endogenous melatonin deficiency. In recent year, melatonin has been identified in olive oil. The contents of me-
Melatonin in EVOO were found to be two times more than refined olive oil. The contents of melatonin in EVOO have been detected in a range of 71-119pg/mL [16]. Although not abundant, the collective data suggest that exogenous intake of melatonin in EVOO may promote tumor suppressive effects as evidenced by low cancer incidence in Mediterranean populations.

**Conclusion**

In this book chapter, we have summarized and discussed the potential role of EVOO in cancer chemoprevention. Collective data from various epidemiological, *in vitro* and *in vivo* animal models studies demonstrated an inverse relationship between EVOO intake and cancer risk. Although, the exact molecular mechanism of cancer chemoprevention by EVOO is not yet known, the scientific evidence discussed here demonstrates a clear protective role of EVOO against cancer. In addition to providing excellent antioxidant activity as raw ingredients in Mediterranean diet, that play a key role in prevention of cancer by neutralizing ROS, EVOO improves the nutritional value of food and inhibits the formation of potentially toxic and carcinogenic compounds such as acrylamide, PHAs, HAs and hydroxy-alkenals during cooking. EVOO contains huge amount of MUFAs, hydrophilic polyphenols, squalene and melatonin which make it unique from other seed oils. Indeed, cancer preventive and oncostatic potential of these molecules have been well established in a huge number of preclinical studies. The above mentioned
bioactive components of EVOO have been shown to inhibit cancer initiation as well as cancer progression by interfering with multiple cellular targets and mechanisms which are central to cancer development and progression as shown in figure 2.

**Figure 2:** EVOO components cancer initiation and progression by interacting with multiple mechanisms.
Acknowledgments

This work was supported by a research grant from National Natural Science Foundation of China (NSFC) to Muhammad Khan (81650110526).

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