Available online at www.sciencedirect.com
ScienceDirect
Cancer Letters 263 (2008) 1–13
CANCER Letters
www.elsevier.com/locate/canlet

Mini-review

Lupeol: Connotations for chemoprevention

Pranav K. Chaturvedi, Kulpreet Bhui, Yogeshwer Shukla *

Proteomics Laboratory, Industrial Toxicology Research Centre, Environmental Carcinogenesis Division, P.O. Box 80, M.G. Marg, Lucknow 226001, India

Received 7 October 2007; received in revised form 19 January 2008; accepted 21 January 2008

Abstract

The perception of chemoprevention lies still in its infancy. Intervention, to slow down, arrest or reverse the process of carcinogenesis, by the use of either natural or synthetic substances individually or in combination therapy has emerged as a promising and pragmatic medical approach to reduce cancer risk. Pentacyclic lupane-type triterpenes exemplified by lupeol [lup-20(29)-en-3b-ol], are principally found in common fruit plants such as olive, mango, fig, etc. Although, lupeol exhibits an array of biological activities like anti-inflammatory, anti-arthritic, anti-mutagenic and anti-malarial activity both in *in vitro* and *in vivo* systems yet, extensive exploration in regard to establish its role as chemopreventive compound is warranted. Interest in developing lupeol based potent anti-neoplastic agents, has led to the discovery of a host of highly active derivatives exhibiting greater potencies and better therapeutic indices. This review asserts on the chemopreventive prospects of lupeol and reveals potential chemoprevention drug targets, central to which are the cell cycle regulatory pathway genes and tries to explain the mechanism operating behind its action.

Keywords: Lupeol; Chemoprevention; Anti-carcinogenic; Mango

1. Introduction

Cancer results from a multistage, multi-mechanism carcinogenesis process that involves mutagenic, cell death and epigenetic mechanisms, during the three distinguishable but closely allied stages: initiation, promotion, and progression. Because reducing the initiation phase to a zero level is impossible, the most effective intervention would be at the promotion phase to eliminate premalignant cells before they become malignant [1]. It takes several years for the normal cells to transform into malignant ones. Therefore, concept of delaying or preventing this transformation remains a viable and attainable goal for the future [2]. An offshoot of thinking is to explore the prospects to intrude in the process of cancer development. Foods, diet manipulation strategies, or nutraceuticals may be appropriate to delay or prevent carcinogenesis progression in healthy populations with genetic or epidemiologic evidence of risk for future transformation [2]. It has been anticipated that by these diet manipulation strategies, more than two-thirds of human cancers can be prevented [3]. But, although for prevention of cancer, both the scientific community and general

* Corresponding author. Tel.: +91 522 2620207/2613786; fax: +91 522 2628227.
E-mail addresses: yogeshwer_shukla@hotmail.com, shukla_y@rediffmail.com (Y. Shukla).

0304-3835/$ - see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.canlet.2008.01.047

public relies on consumption of fruits and vegetables, their active ingredients (at the molecular level) and their mechanisms of action yet remain inexplicable. Interdisciplinary research endeavors are now solely directed at understanding the molecular mechanisms involved in chemoprevention. Completion of the human genome sequence and the advent of DNA microarrays using cDNAs have also enhanced the detection and identification of hundreds of differentially expressed genes in response to anticancer drugs or chemopreventive agents [4]. Epidemiological and experimental evidences emphasize that specific compounds may positively inhibit carcinogenesis at various sites, including the oral cavity, esophagus, stomach, colon/rectum, lung, breast, and prostate, but at the same time, another compelling body of evidences, together with the data from animal and *in vitro* studies, strongly supports the relationship between dietary constituents and the risk of cancer development [5]. Vegetables, fruits, dietary fiber, and certain micronutrients apparently seem to be protective against cancer, whereas fat, excessive calories and alcohol seem to increase the cancer risk. Over the past few decades, many chemopreventive agents have been developed empirically and recent advances in the molecular biology of carcinogenesis and drug designing continue to perk up the mechanistic approach in development of such agents. Molecular targeted approaches are designed to retain (or enhance) the preventive effects while reducing the already known toxic effects. Toxicity reduction already is being achieved in clinical trials, as agents working along more precise, or selective, molecular pathways are being developed. Of late, bioactive triterpene, lupeol commonly found in fruits like fig, mango, etc. has attracted interest in context to chemoprevention attributable in large part to its antioxidant [6], apoptosis inducing and antiproliferative [7], anti-mutagenic, anti-inflammatory [8] properties as well as its efficacy in inhibition of *in vivo* and *in vitro* cancer growth [9]. The purpose of this review is to briefly highlight the significant, though little, accomplishments made in regard to chemoprevention with lupeol and to present course for future research endeavors.

2. Distribution, isolation and pharmacokinetics of lupeol

Triterpenes represent a varied class of natural products. Thousands of structures have been reported till date with hundreds of new derivatives discovered each year. Pentacyclic triterpenes are all based on a 30-carbon skeleton comprising five, six-membered rings (ursanes and lanostanes) or four, six-membered rings and one, five-membered ring (lupanes and hopanes). Pentacyclic triterpenes are produced by arrangement of squalene epoxide molecules. These compounds occur commonly and are found in fruits, vegetables and other parts of several medicinal plants including the roots of *Hemidesmus indicus*, which yields six pentacyclic triterpenes including two oleanenes identified as olean-12-en-21 beta-yl acetate, and olean-12-en-3 alpha-yl acetate, three ursenes characterized as 16(17)-seco-urs-12,20(30)-dien-18 alpha H-3 beta-yl actetate, urs-20(30)-en-18 beta H-3 beta-yl acetate and 16(17)-seco-urs-12,20(30) dien-18-alpha H-3 beta-ol and a lupene formulated as lup-1,12-dien-3-on-21-ol [10]. There are at least 4000 known triterpenes most of which occur freely but others occur as glycosides (saponins) or in special combined forms [11]. Pentacyclic triterpenes have a wide spectrum of biological activities and some of them may be useful even in medicines. These include the pentacyclic lupane-type triterpenes which are represented by a diverse assemblage of bioactive natural products. Among this class of compounds, lupeol [lup-20(29)-en-3b-ol] (Fig. 1) occurs across a multitude of taxonomically diverse genera**.** It is commonly found in plants viz. *Hieracium pilosella* [12], *Tamarindus indica* [13], *Crataeva nurvala* (Buch Ham) [14], *Arbutus unedo* [15], *Tipuana tipu* [16], *Betula platyphylla* [17], latex of *Leptadenia hastate* [18], roots of *Anemone raddeana* [19], bark of *Gossampinus malabarica* [20] and *Acacia mellifera* [21], etc. Besides that, lupeol is also found in various edible plants such as olive, fig, mango, strawberry, red

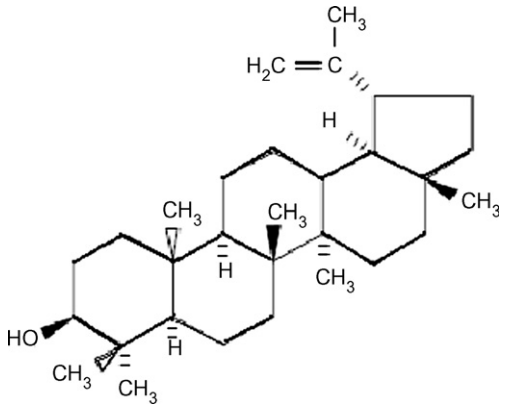

Fig. 1. Structure of lupeol.

grapes and medicinal plants used by native people in North America, Japan, China, Latin America and Caribbean islands [22–24]. Stem bark of *C. nurvala*, Buch Ham (Capparidaceae), is of particular interest as it is endowed with an excellent yield of lupeol and exhibits a continuum of biological activities [25]. A multitude of extraction and isolation schemes have been employed for the procurement of lupeol and other related triterpenoids. Typically, dry plant material is extracted with $CHCl_3$ (for aglycons), [26] MeOH (for both aglycons and glycosylated derivatives) [26,27], or even H_2O [28]. The plant may be defatted with hexane prior to extraction to remove non-polar materials [29]. The resultant extracts can be dried and further extracted with other solvents or directly subjected to column chromatography. Besides a wide distribution, the toxicity levels of lupeol are also known to be very low. Lupeol administered orally in a dose of 2 g/kg body weight has been reported to produce no adverse effects in rats and mice, and no mortality after 96 h of observation [30]. Lupeol and one of its ester derivatives viz. lupeol linoleate possess a wide range of medicinal properties. Isolated from *C. nurvala* stem bark, they both have protective effect against cyclophosphamide (CP) induced cardiotoxicity in rat which is associated with alterations of electrolytes both in serum and cardiac tissue. Their supplementation also restores CP-induced levels of urea, uric acid and creatinine in serum [31]. Both lupeol and lupeol linoleate also have pharmacological efficacy against CP-induced mitochondrial-cardiomyopathy. Their treatment in Wistar rats restores CP-induced decrease in the activities of tricarboxylic acid (TCA) cycle enzymes such as succinate dehydrogenase, malate dehydrogenase and isocitrate dehydrogenase. Simultaneously, they also increase the activities of mitochondrial complexes of electron transport chain [32]. In another study, lupeol isolated from *C. nurvala* stem bark has shown activity against free radical-induced toxicity in experimental urolithiasis. Lupeol administration induced a remarkable decrease in kidney oxalate level and also was effective in counteracting the free radical toxicity by bringing about a significant decrease in peroxidase level and increase in antioxidant status. [33]. Lupeol and lupeol linoleate treatment has also been observed to decrease the lipid peroxidation levels and increase enzymatic and nonenzymatic antioxidants which highlight the beneficial effects of lupeol and its linoleate ester derivative, in ameliorating the lipidemic-oxidative abnormalities in the early stage of hypercholesterolemic atherosclerosis [14]. The distribution of betulinic acid, a derivative of lupeol has been reported in tissues at 24 h post intraperitoneal administration. The distribution decreases in descending order as: perirenal fat, ovary, spleen, mammary gland, uterus, bladder, lymph node, liver, small intestine, caecum, lung, thymus, colon, kidney, skin, heart and brain [34].

3. Anti-inflammatory and anti-carcinogenic effects of lupeol

Not much has been documented on the anti-carcinogenic implications of lupeol although it has been reported to possess anti-mutagenic and anti-inflammatory effects. Inflammation, which orchestrates the tumor-supporting microenvironment, is a critical component of both tumor promotion and tumor progression and is an indispensable participant in the neoplastic process. It has been established that cancer can be promoted and/or exacerbated by inflammation and infections [35]. The hypothesis that aberrant induction of cyclooxygenase-2 (COX-2), a conventional marker of inflammation, and up-regulation of the prostaglandin cascade play a significant role in carcinogenesis is consolidated by accumulating body of evidences from molecular, animal, and human investigations and reciprocally, blockade of the process has strong potential for cancer prevention and therapy. Since NF-κB becomes activated in response to inflammatory stimuli and its constitutive activation has been associated with cancer, in addition to selective modulation of cytokine signaling, interfering with NF-κB activation in tumor cells can further expedite the prevention strategy and may render the cancer cells to elimination by pro-apoptotic cytokines.

Lupeol afforded significant inhibition, in a time- and dose-dependent manner, against 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-mediated increase in (i) skin edema and hyperplasia, (ii) epidermal ornithine decarboxylase (ODC) activity, and (iii) protein expression of ODC, COX-2, and nitric oxide synthase [8]. An ester derivative of lupeol, lupeol linoleate also possesses a marked anti-inflammatory activity [36] as shown by oral or intraperitonial administration of lupeol at a dose of 25–200 mg/kg body weight in acute and chronic inflammation in rats and mice [30]. Lupeol, along with its acetate and palmitate esters was found to be the main anti-inflammatory constituents in the croton oil-induced ear edema test [18]. Furthermore, lupeol hemisuccinate, synthesized

from lupeol, exhibited a stronger activity than lupeol [18]. Lupeol from *Crataeva religiosa* bark has been evaluated for anti-inflammatory, analgesic, antipyretic effect on rat and mice. It was seen to exert significant dose-dependent effect on acute and chronic inflammatory processes [37].

With lupeol established as a potent anti-inflammatory compound, is also thought to possess anticarcinogenic attributes. It inhibits cancer growth *in vitro* and *in vivo* and ameliorates inefficiency of cancer cells to undergo apoptosis [9] as listed in Tables 1 and 2. Lupeol and lupeol linoleate were also investigated for their possible hepatoprotective effect against cadmium-induced toxicity in rats and it was elucidated that the possible mechanism under clad is scavenging of peroxyl radicals by bolstering the levels of antioxidants and antioxidant enzyme system [38]. Another lupane-type terpenoid, 3b,25-epoxy-3a-hydroxylup-20(29)-en-28-oic acid exhibited the strongest inhibitory effect on tumor initiating activity in mouse models initiated with ultraviolet-B (UV-B) and promoted with TPA [39].

Emerging body of data from *in vitro* studies has evidently conceived the notion that lupeol and related terpenoids exhibit varied cytotoxicity against several cancer cell types. Lupeol has been found to induce differentiation and inhibit the cell growth of mouse melanoma and human leukemia cells [40,20]. Besides lupeol, lupenone which is obtained from *A. mellifera* has been shown to exhibit significant cytotoxicity against NSCLC-N6 (non-small-cell lung carcinoma) cell line [21]. In another study, lupeol, betulin, and methyl betulinate, glycosides (β-D-glucosides, α-L-rhamnosides, and α-D-arabinosides) were synthesized and tested *in vitro* for cytotoxicity against three cancerous cell lines: human lung carcinoma (A-549), human colon adenocarcinoma (DLD-1) and mice melanoma (B16-F1) [41]. Lupane-type terpenoids also exhibited cytotoxicity against human hepatocellular carcinoma (Hep-G2) and human epidermoid carcinoma (A-431), while did not affect the growth of tumor cell lines such as human melanoma (MEL-2), human lung carcinoma (A-549), and murine melanoma (B16-F10) [42]. Lupeol has been reported as a differentiation-inducing compound in B16 2F2 cells, up-regulating the melanogenesis of these cells. The cytotoxicity profiles of lupane triterpene against human cancer cells showed that its cytotoxic effect against lung cancer cell lines is strongest while it is very weak against osteosarcoma, breast cancer and urinary bladder cancer cells [43]. Synergistic cytotoxic effects of lupeol with chemotherapy drug, cisplatin have been observed *in vitro*, resulting in chemosensitization of head and neck squamous cell carcinoma (HNSCC) cell lines with high NF-κB activity [44].

DNA topoisomerases (Topos) are ubiquitous enzymes that play a crucial role in many aspects of DNA metabolism such as replication, transcription, recombination and chromosome segregation during mitosis [45]. Topos have therefore been identified for anticancer chemotherapeutic drug devel-

Table 2
In vivo studies of lupeol

Cancer type	Animal model	Reference
Skin	Rats and mice	[36]
Skin	Mouse	[39]
Skin	Mouse	[70]
Skin	CD-1 mice	[8]
Liver	Swiss albino mice	[71]
Liver	Rats	[72]

Table 1
In vitro studies of lupeol

Cancer cell type	Mechanism of prevention	Reference
B16 2F2 mouse melanoma cells	Up-regulation of melanogenesis through activation of the p38 MAPK pathway	[65]
Leukemia HL60, U937, K562 melanoma G36; SK-MEL-28 neuroblastoma GOTO, NB-1	Induction of apoptosis	[64]
Human leukemia cells HL-60	Induction of apoptosis	[20]
Non-small-cell lung carcinoma NSCLC-N6	Induction of apoptosis	[21]
Pancreatic cells AsPC-1	Aberration of Ras oncoprotein	[9]
Human prostate cancer LnCaP	Induction of Fas receptor and its adopter protein	[63]
Human lung carcinoma A-549, human colon adenocarcinoma DLD-1, human normal fibroblast WS1, mice melanoma B16-F1	Induction of apoptosis	[41]
Human hepatocellular carcinoma Hep G2, human epidermoid carcinoma A-431	Induction of apoptosis	[42]

opment [46]. Topo II inhibitors are mainly characterized into two groups according to their inhibitory mechanism. One group termed 'poisonous', stabilizes covalent intermediates named cleavable complexes, and the other one referred to as 'catalytic inhibitors' targets some other step during the catalytic cycle without formation of cleavable complexes [47,48]. Topo-II is an essential enzyme in the DNA replication process and is the primary cellular target for many of the widely used and effective anticancer agents. Naturally occurring lupane-type triterpenoids isolated from the bark of *Phyllanthus flexuosus* were screened for human Topos I and II inhibitory activities. It revealed that lupeol and betulin are selective catalytic inhibitors of human Topo II activity with IC_{50} values in the range of 10–39 μM [49].

3.1. Pancreatic cancer and lupeol

Pancreatic cancer is the most fatal of all cancers and the fifth most common cause of cancer-related deaths among both men and women in the western countries. Since the mortality from pancreatic cancer compares strikingly with its incidence, it has become a significant public health concern [50]. Long-term survival of patients with organ-confined disease is only 15%, and in the majority of cases with invasive metastasis survival is only 4%. Despite these disappointing statistics and the increase in incidence of pancreatic cancer over the past several decades, the molecular and biochemical determinants of the disease remain poorly understood and no effective therapeutic regimens to significantly ameliorate the clinical course or prognosis of this disease are apparent. Its treatment has largely been unsuccessful owing to higher resistance offered by pancreatic cancer cells to conventional approaches such as surgery, radiation and/or chemotherapy. In more than 90% of pancreatic cancers, the *ras* oncogene has been shown to be mutated [51]. This mutation is considered as an early genetic event in the development of pancreatic cancer and results in constitutive activation of an intracellular pathway leading to cellular proliferation. Several *ras*-induced signaling pathways, such as the phosphatidylinositol 3-kinase (PI3K)/Akt (protein kinase B pathway) mitogen activated protein kinases (MAPKs) and NF-κB pathways have been linked to chemo-resistance of the pancreatic carcinoma cells [51–54]. These findings suggest that Ras oncoprotein could be an important target for developing agents against pancreatic cancer. The aberration of Ras oncoprotein has been linked to the induction of multiple signaling pathways and to the resistance offered by pancreatic cancer cells to apoptosis. Lupeol can adopt a multi-prong strategy to target multiple signaling pathways leading to induction of apoptosis and inhibition of growth of pancreatic cancer cells. It caused a dose-dependent inhibition of cell growth as assessed by MTT assay and induction of apoptosis as assessed by flow cytometry, fluorescence microscopy and Western blotting [9]. Lupeol treatment to cells has been found to significantly reduce the expression of Ras oncoprotein and modulate the protein expression of various signaling molecules involved in protein kinase C alpha (PKCa)/ODC, PI3K/Akt and MAPKs pathways along with a significant reduction in the activation of NF-κB signaling pathway [9]. Lupeol-induced apoptosis of pancreatic cells is mediated through the activation of caspases-3, -8 and -9. The observation that expression levels of procaspase-3 did not exhibit any significant change upon lupeol treatment could be explained through a possible involvement of a feedback mechanism, which restores depleted procaspase-3 levels at intracellular level [9].

3.2. Prostate cancer and lupeol

Prostate cancer has become a major public health concern and is a leading cause of cancer-related deaths among males in the United States [50]. From a practical perspective, the most effective means of controlling prostate cancer or the morbidity associated with its treatment is to establish effective chemopreventive strategies to block, reverse or delay the process of carcinogenesis [5]. Prostate cancer is an excellent candidate disease for chemoprevention because it is a unique malignancy, which generally grows very slowly, likely for decades, before symptoms arises and the diagnosis is eventually established. It is typically diagnosed in elderly men and therefore, even a modest delay in the neoplastic development achieved through pharmacological or therapeutical intervention could consequently result in substantial reduction in the incidence of the clinically detectable disease [55,56]. This assumption has perked up the hunt for novel and potent chemopreventive agents as well as molecular targets for prostate cancer chemoprevention. Recent studies have shown that the prostatic regression in animal models is linked to expression of Fas receptor (also known as CD95/APO-1), a cell surface protein that

is also expressed in a variety of normal cells, including prostate epithelial cells, as well as in neoplastic cells [57–60]. Fas-mediated signaling has been reported to play a significant role in the hormonal regulation of the normal and differentiated prostatic epithelium and mutations in the *Fas* gene have been shown to be closely associated with the pathogenesis of prostatic intraepithelial neoplasia and concurrent carcinomas [61,62]. Lupeol induced apoptosis of prostate cancer cells has been concomitant with the induction of Fas receptor and its adaptor protein (i.e., FADD) and proceeds via death receptor-dependent apoptotic pathways, because no change in the expression of mitochondrial-dependent apoptotic signaling molecules, such as Bcl-2 and Bax, have been observed [63]. Further, among various death receptor pathways, lupeol has been observed to adopt Fas-associated apoptotic pathway, which is evident, as its supplementation did not cause any change in death receptor proteins, such as tumor necrosis factor receptor-1 (TNFR1), death receptor-3 (DR-3), and DR-5, as well as death receptor adaptor protein-TNFR associated death domain protein (TRADD) [63]. The end result of Fas receptor activation process is the unmasking of the proteolytic activity of caspase-8, which is then recruited to Fas-associated apoptotic pathway and triggers self-activation of the caspase cascade [59]. Initiator caspase-8 and caspase-9 and effector caspase-6 mediate lupeol-induced Fas signaling in prostate cancer cells, whereas no alteration is observed in the expression of caspase-3.

3.3. Skin cancer and lupeol

Human skin is recurrently exposed to various stressful and damaging conditions and is therefore vulnerable to skin cancer. Not surprisingly, more than one million new cases of skin cancer are diagnosed every year in the United States, accounting for 40% of all cancer cases. Epidemiologic studies implicate ultraviolet radiation from sunlight as an etiologic agent for the pathogenesis of the disease. Lupeol and its derivatives induce the differentiation of B16 2F2 mouse melanoma cells *in vitro*, and up-regulate melanogenesis through activation of the p38 MAPK pathway as they induce the formation of dendrites in these cells [64,65]. The remodeling of cytoskeletal components contributes to the dendricity of the melanoma cells. But, Western blotting revealing the effects of lupeol on the remodeling of cytoplasmic filaments showed no change in the levels of actin and tubulin. Instead of promoting the remodeling of microtubular networks, it rather attenuated the stress fiber assembly. Further studies carried out by Hata et al. [66] have reported the activation of cofilin, an actin-depolymerizing factor, in lupeol-treated B16 2F2 cells by Western blotting, whereas, the level of phospho-cofilin was found to decrease in a time dependent manner.

Besides the aforementioned activities, lupeol also possesses antitumor-promoting activity in mouse skin tumorigenesis model (CD-1) mice as shown by modulation of TPA-induced conventional markers and other novel markers of skin tumor promotion by topical application of lupeol (1–2 mg/mouse 30 min prior to TPA), elucidating an inevitable role of NF-κB and PI3K/Akt signaling in tumor promotion. It resulted in the inhibition of TPA-induced (i) activation of PI3K, (ii) phosphorylation of Akt at Thr^{308}, (iii) activation of NF-κB and IKKα, and (iv) degradation and phosphorylation of IκBα [8]. The animals pretreated with lupeol showed significantly reduced tumor incidence, lower tumor body burden and a significant delay in the latency period for tumor appearance.

Ultraviolet light is the main factor responsible for majority of non-melanoma skin cancers, of which, effects of ultraviolet light B (UVB: 290–320 nm) are primarily thought to contribute to skin photo carcinogenesis. Lupeol derivative viz. 3b,25-epoxy-3a-hydroxylup-20(29)-en-28-oic acid have shown the strongest inhibitory effect on mouse models initiated with UVB and promoted with TPA [39]. Another effective cutaneous tumor promoter, benzoyl peroxide (BPO) acts through the generation of oxidative stress, the induction of ODC activity and the enhancement of DNA synthesis [67]. BPO treatment increases cutaneous microsomal lipid peroxidation and hydrogen peroxide generation. Also, the activity of the cutaneous antioxidant enzymes, namely catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glucose 6-phosphate dehydrogenase (G6PD) and glutathione *S*-transferase (GST), is decreased and levels of cutaneous glutathione are depleted. BPO treatment also enhances [^{3}H]thymidine uptake in DNA synthesis [68]. The topical application of lupeol prior to BPO treatment results in a significant inhibition of BPO-induced cutaneous ODC activity and a marked reduction in BPO-enhanced [^{3}H]thymidine uptake in cutaneous DNA in a dose-dependent manner [22]. This sharp decrease in BPO-mediated induction of cutaneous ODC activity and enhanced

[^{3}H]thymidine incorporation in DNA with the pretreatment of lupeol suggests the antitumor-promoting potential of lupeol in murine skin. It has also been observed that lupeol reversed BPO-mediated inhibition of the activities of several antioxidant enzymes such as CAT, GPx, GR, G6PD and GST [22].

7,12-Dimethylbenz[*a*]anthracene (DMBA), a polycyclic aromatic hydrocarbon (PAH), is a potent carcinogen and is known to generate DNA-reactive species, which may enhance oxidative stress in cells, during its metabolism. There occurs formation of mutagen–DNA adducts, e.g. DMBA–DNA that have been analyzed, both, *in vitro* and *in vivo* in mouse skin, and found that 99% of the DMBA–DNA adducts are depurinating adducts formed by one-electron oxidation, in which the 12-methyl group of DMBA reacts with the N-7 of adenine or guanine in a 4:1 ratio, respectively. Only a fraction of the 1% of stable adducts corresponds to diol epoxide products [69]. In one of our recent studies, lupeol was demonstrated to inhibit the DMBA induced DNA strand breaks in Swiss albino mice [70], possibly due to its anti-oxidative property via which it curbs the free radicals generated [71].

3.4. Lupeol as a hepatoprotective agent

Aflatoxins are potent hepatotoxic and hepatocarcinogenic agents. Reactive oxygen species (ROS) generation and consequent peroxidative damage caused by aflatoxins is considered to be the main mechanism leading to hepatotoxicity [72]. The aflatoxin B1 (AFB1)-induced decrease in the liver enzymes was significantly inhibited by lupeol pretreatment in a manner similar to that observed with silymarin, a known hepatoprotectant. The protection rendered by lupeol may be due to its antioxidant effect and ability to act as a radical scavenger, thereby protecting membrane permeability. The restoration of intracellular reduced glutathione (GSH) content and GST activity to normal levels by lupeol pretreatment indicates that they play a vital role in mitigating AFB1-induced oxidative stress and subsequent damage to the liver. Also, lupeol elevates the GSH status culminating in an increase in the superoxide dismutase (SOD) activity thereby encumbering the deleterious effects of superoxide radicals [71]. Thus, lupeol indirectly influences the activities of SOD and CAT. The raised ascorbate and GSH content in the lupeol pretreated animals regenerates vitamin E and establishes a synergistic effect among them thereby enhancing the antioxidant protection [73].

Due to the poor handling and excretion of heavy metals, the likelihood of serious damage increases with their continued exposure. Heavy metal toxicity has been shown to affect almost every organ system of the body including liver [38]. Cadmium being a very toxic transition metal has been recently designated as a human carcinogen. It exerts its toxic effect by causing specific cell membrane lesions. Cadmium exposure leads to a hepatotoxic condition resulted by elevated levels of malondialdehyde (basal and induced) and decreased levels of antioxidants and antioxidising enzymes in the liver. Lupeol and lupeol linoleate have been reported to regenerate the glutathione pool in cadmium-induced hepatotoxicity along with an elevation in the activities of the antioxidising enzymes and antioxidants. The concomitant increase in SOD and catalase activities effectively eliminates the superoxides and peroxides produced by cadmium chloride [38].

DMBA induces hepatic alterations in ROS generation, lipid peroxidation, status of the antioxidant enzymes, mitochondrial membrane potential (MMP) and events involved in apoptosis such as modulation of Bcl-2, Bax and caspase-3 as well as DNA fragmentation in Swiss albino mice. The inhibition of apoptosis in DMBA induced mouse hepatocytes is consistent with the notion that lupeol/mango pulp extract (MPE) deactivates pre-existing apoptotic machinery. Treatment with lupeol/MPE in an earlier study conducted by us, resulted in an induction of Bcl-2 and suppression of Bax and caspase-3, coupled with a decrease in the fragmentation of nuclear DNA, which inhibit the DMBA-induced onset of apoptosis [71]. Lupeol/MPE was seen to restore the DMBA-induced reduced MMP and prevent normal cell death. It has been consistently shown that the *Bcl-2* family genes play an essential regulatory role in apoptosis, either as activator (Bax) or as inhibitor (Bcl-2) [74–76]. Inhibition of DMBA-induced cell death by lupeol/MPE in mouse liver has been allied with repressed levels of Bcl-2 and elevated levels of Bax and caspase-3. Analysis of the data indicates that lupeol/MPE possibly disturbs Bcl-2 and Bax ratio, thereby culminating in subdued apoptosis. There has been a significant decline in the activities of SOD and CAT after DMBA administration, indicative of oxidative stress. These decreased antioxidant enzyme activities in liver were restored by the supplementation with lupeol/MPE. The protection provided by

lupeol/MPE is due in large part to the scavenging of superoxide and peroxyl radicals. DMBA exposure caused a marked oxidative impact and increase in lipid peroxides might have resulted from an increased production of free radicals and a decrease in antioxidant status. Lupeol/MPE significantly lowered lipid peroxidation and the values were comparable with that of the control animals.

3.5. Anti leukemia activity of lupeol and derivatives

Lupeol and its derivatives are cytotoxic against human leukemias, melanomas, neuroblastomas and normal fibroblast cells. Lupane triterpenes with a carbonyl group at C-17 demonstrated discernible inhibitory effects on leukemia, melanoma and neuroblastoma cell growth. Lup-28-al-20(29)-en-3-one markedly inhibited leukemic cell growth to a larger extent as compared to other human cancers and normal lung fibroblast cells. Its treatment on K562 (human leukemia cells)-derived adriamycin (ADM)-resistant (K562/ADM) and vincristine (VCR)-resistant (K562/VCR) cells exhibited strong cytotoxic effect. Drug-resistant K562 cells showed cross-resistance to both drugs. However, this lupeol derivative inhibited K562/ADM (IC_{50} – 1.7 mM) and K562/VCR (IC_{50} – 2.0 mM) cell growth to the same extent as K562 (IC_{50} – 1.8 mM) cell growth (0.9- and 1.1-fold to K562 cells), respectively [43]. The morphological observations of leukemia nuclei and the gel electrophoresis analysis of DNA extracted from lupeol and its derivatives treated leukemia cells revealed that lupeol induces apoptosis in these cells.

4. Mechanism of action of lupeol based chemoprevention

Elucidation of critical events associated with carcinogenesis provides an opportunity for bioactive compounds like lupeol and its derivatives to impede cancer development via multi-prong strategy commencing with modulation of a plethora of biomolecules and their cross talk encompassing their signaling pathways, and culminating in induction of apoptosis. Of particular note is that, despite envisaging the properties of lupeol and derivatives pertaining in regard to its chemopreventive potential, a mechanism to offer a molecular basis of intervention as of role of chemoprevention still remains obscure.

Apoptosis, a form of programmed cell death, is a pivotal defense system against the occurrence of cancer and is essential in metazoans to maintain tissue homeostasis [77]. Phenotypically and morphologically, apoptosis is characterized by chromatin condensation, nuclear fragmentation into mono and oligonucleosomal units, cell shrinkage and plasma membrane blebbing [78]. It is a complex process taking place via either intrinsic (mitochondrial mediated) or extrinsic (death receptor mediated) pathway, enveloping numerous specific targets within each arm. Emerging body of evidences indicate that lupeol and its derivatives may trigger apoptosis via copius molecular targets (Fig. 2). Intrinsic mitochondrial pathway is characterized by alterations in the mitochondrial polarization and release of mitochondrial proteins, including cytochrome c, endonuclease G, secondary mitochondria-derived activator of caspase (Smac)/direct inhibitor of apoptosis protein (IAP) binding protein with low pI (DIABLO), Omi/HtrA2, apoptosis-inducing factor (AIF), and its homolog AIF-homologous mitochondrion-associated inducer of death [79]. Lupeol mediated release of cytochrome c can then trigger caspase activation and ultimately execution of apoptosis [71,74–76].

The extrinsic pathway is triggered by members of the TNF receptor super family, which comprises of almost 20 members of cytokine receptors, such as TNFR1, Fas, and TNF-related apoptosis inducing ligand (TRAIL) receptors [80,81]. These receptors share in common a protein domain on the intracellular region known as the death domain and are activated by ligands such as tumor necrosis factor α (TNFα), TRAIL or FasL (CD95L). These ligands can vary in the response they produce, but in general activation of the death receptors results in oligomerization of the receptor [82]. The receptors recruit adapter proteins, including FADD, to their cytosolic death domains, with subsequent binding to procaspases, particularly caspase-8, which contains a protein interaction motif (the death effector domain, or DED), which binds a complementary domain in FADD. Next, intracellular recruitment of the death-inducing signaling complex (DISC) occurs by means of protein/protein interactions involving death domains [83]. This event then allows the recruitment of other signaling proteins to the DISC, the precise composition of which determines the fate of the cell. DISC plays a central role in the extrinsic pathway by activating the initiator caspases-8 and -10 [84]. Recruitment of caspase-8 to the DISC typically leads to the initiation of the caspase cascade and cell death. As one would expect, DED-

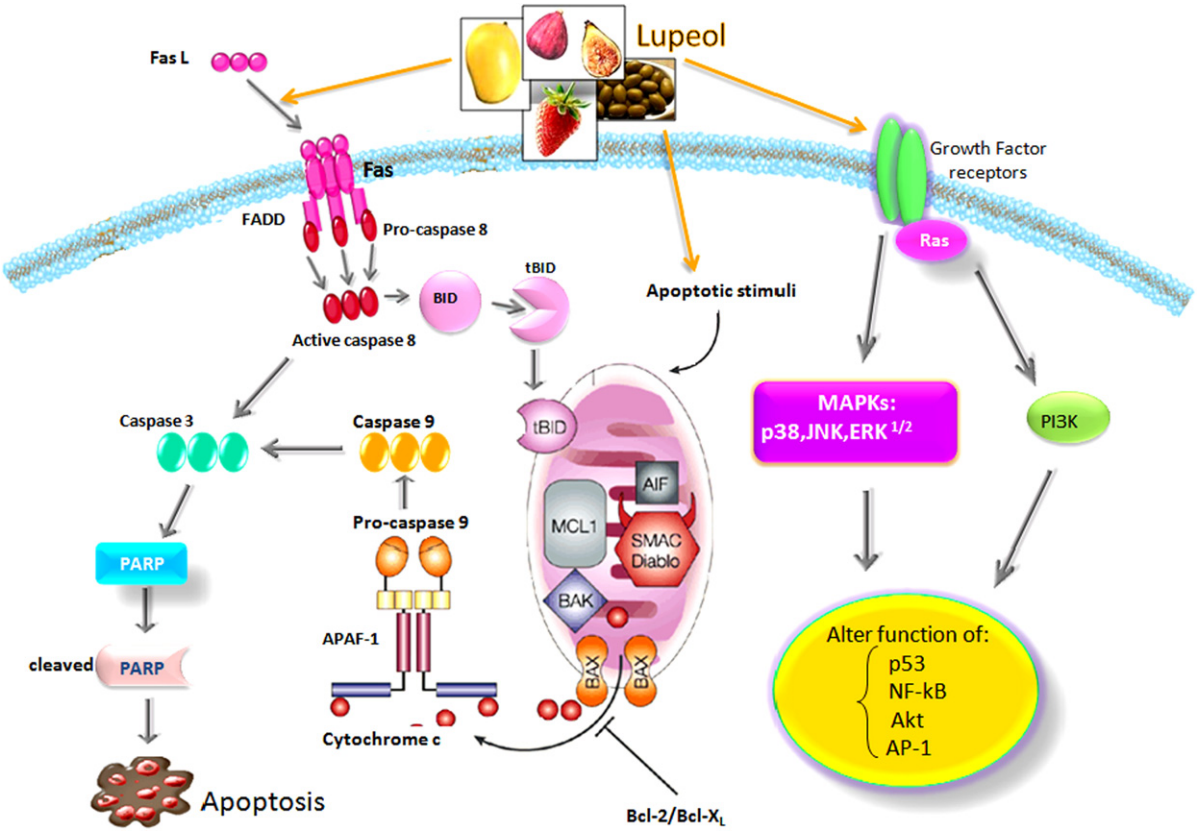

Fig. 2. Multiple signaling pathways involved in lupeol endowed chemoprevention.

containing antagonists of Fas and procaspase-8, such as FADD-like interleukin-1β-converting enzyme inhibitory protein (FLIP), suppresses this pathway. Modulation of the afore-mentioned proteins and their effectors could be one of the intervention itinerary lupeol undertakes as suggested by a number of studies [9,59].

Besides following the intrinsic and extrinsic pathways for apoptosis, lupeol mediated apoptosis is essentially manifested by the cleavage of poly (ADP-ribose) polymerase (PARP). Since poly (ADP-ribosylation) is a post-translational modification of proteins which plays a crucial role in DNA repair and cell death, PARP, a DNA nick sensor, serves as one of the best-known biomarkers of apoptosis. During apoptosis, PARP protein is cleaved into an 85 kDa C-terminal fragment, with a reduced catalytic activity, and a 24 kDa N-terminal peptide, which retains the DNA binding domains. Studies elucidating the effect of lupeol (30–50 μM) on PARP cleavage reveal that the full-size PARP (116 kDa) protein is cleaved to yield an 85 kDa fragment after treatment of cells with lupeol at 48 h post-treatment [9].

Other contributing molecular changes in cancer progression include activation of oncogenes and inactivation of tumor suppressor genes. The mutation in *ras*, an oncogene, results in the accumulation of Ras oncoprotein, and constitutive activation of various intricate intracellular signaling pathways, leading to cellular proliferation. Targeting the *ras* gene by lupeol has been shown to inhibit the growth of metastatic cells [9].

The MAPK pathway, in addition to NF-κB and Akt pathways, has received increasing attention as a target molecule for cancer prevention and therapy. The MAPK cascades include extracellular signal-regulated protein kinases (ERKs), c-Jun N-terminal kinases/stress-activated protein kinases (JNKs/SAPKs), and p38 kinases. ERKs are believed to be strongly activated and to play a critical role in transmitting signals initiated by growth-inducing tumor promoters, including TPA, epidermal growth factor (EGF), and platelet-derived growth factor (PDGF) [85]. On the other hand, stress-related tumor promoters, such as ultraviolet (UV) irradiation and arsenic, potently activate JNKs/SAPKs

and p38 kinases [86]. The MAPK pathway consists of a cascade in which a MAP3K activates a MAP2K that activates a MAPK (ERK, JNK, and p38), resulting in the activation of NF-κB, cell growth, and cell survival [87]. Melanogenesis being one of the hallmarks of melanoma cell differentiation has already been shown to be regulated by lupeol via activation of the p38 MAPK signaling [88]. Henceforth, other than the pathways executing cell death as well as those activating the oncogenes, the mechanisms of lupeol endowed inhibition may be due to the blockade of the mitogenic and differentiating signals through modulating MAPK and Ras-MAPK kinase-MAPK cascades. Also, as of role of NF-κB signaling in carcinogenesis, lupeol could afford intrusion in the pathway via activation of PI3K, phosphorylation of Akt at Thr(308), activation of NF-κB and IKKα, and degradation and phosphorylation of IκBα [8]. The suppression of various tumor biomarkers including growth factor receptor tyrosine kinases, PI3K and Ras could also be one of the dimensions of its activity.

5. Conclusion and future prospects

It is now evident from the above dialogue that a surfeit of naturally occurring bioactive agents in fruits and vegetables have the knack to interfere with multiple cell-signaling pathways. These agents can be used either in their natural form for prevention in general and perhaps in their pure form for the therapy, where large doses may be desired [87]. Drug-based strategies for chemoprevention may predominantly rely upon targeted therapies with tolerable but defined toxicities for treatment. Multi-targeting agents capable of intervening with a number of critical pathways responsible for tumorigenesis will have a merit over other single-targeting agents [8]. But still, further clinical trials are warranted to validate the pragmatism of these agents either alone or in combination with existing therapy. The concept of chemoprevention with lupeol is still in its infancy. Lupeol can be harnessed as an effective molecular targeted chemopreventive agent that can be used to reduce several cancer risks in prospective cohorts as well as surrogate endpoints in clinical studies. It appears that numerous molecular targets exist *in vivo* and collectively converge on vital signaling pathways. This affords the probability of using molecular targeted dietary agents like lupeol and its derivatives (natural products or their synthetic analogs) in view of the fact that their protective effects prove to be proficient enough in bringing chemoprevention to fruition. They are believed to function by modulating processes associated with xenobiotic biotransformation, with the protection of cellular elements from oxidative damage, or with the promotion of a more differentiated phenotype in target cells. However, they have been shown to stimulate apoptosis by intervening in several signaling pathways or signaling cascades. The discussion portends that lupeol and its derivatives can be exploited as prospective targets for development of novel chemotherapeutic agents for the prevention and/or treatment of cancer. While encouraging, there are many considerations that remain, such as the issue of its appropriate dose, appropriate timing and duration of exposure, importance of cell type specificity, its relative bioavailability, and potentially adverse side effects and interactions. Its interaction with the components of the diet still entails more focus and research. Chemoprevention with lupeol, though being relatively new avenue of oncology, offers great assurance in the toil against cancer by inhibiting the process of carcinogenesis through the regulation of cell defensive and cell death machineries, yet there is a vast saga to explore and much needs to be extricated out of it, in the near future.

References

[1] J.E. Trosko, The role of stem cells and gap junctions as targets for cancer chemoprevention and chemotherapy, Biomed. Pharmacother. 59 (2005) 326–331.
[2] D.E. Brenner, A.J. Gescher, Cancer chemoprevention: lessons learned and future directions, Br. J. Cancer 93 (2005) 735–739.
[3] Y.J. Surh, Cancer chemoprevention with dietary phytochemicals, Nat. Rev. Cancer 3 (2003) 768–780.
[4] B.A. Narayanan, Chemopreventive agents alters global gene expression pattern: predicting their mode of action and targets, Curr. Cancer Drug Targets 6 (2006) 711–727.
[5] M.B. Sporn, N. Suh, Chemoprevention of Cancer, Carcinogenesis 21 (2000) 525–530.
[6] M. Nagaraj, S. Sunitha, P. Varalakshmi, Effect of lupeol, a pentacyclic triterpene, on the lipid peroxidation and antioxidant status in rat kidney after chronic cadmium exposure, J. Appl. Toxicol. 20 (2000) 413–417.
[7] M. Laszczyk, S. Jäger, B. Simon-Haarhaus, A. Scheffler, C.M. Schempp, Physical, chemical and pharmacological characterization of a new oleogel-forming triterpene extract from the outer bark of birch (betulae cortex), Planta Med. 72 (2006) 1389–1395.
[8] M. Saleem, F. Afaq, V.M. Adhami, H. Mukhtar, Lupeol modulates NF-kappaB and PI3K/Akt pathways and inhibits skin cancer in CD-1 mice, Oncogene 23 (2004) 5203–5214.
[9] M. Saleem, S. Kaur, M. Kweon, V.M. Adhami, F. Afaq, H. Mukhtar, Lupeol, a fruit and vegetable based triterpene,

induces apoptotic death of human pancreatic adenocarcinoma cells via inhibition of Ras signaling pathway, Carcinogenesis 26 (2005) 1956–1964.
[10] S.K. Roy, M. Ali, M.P. Sharma, R. Ramachandram, New pentacyclic triterpenes from the roots of *Hemidesmus indicus*, Pharmazie 56 (2001) 244–246.
[11] P. Jiří, Biologically active pentacyclic triterpenes and their current medicine, J. Appl. Biomed. 1 (2003) 7–12.
[12] M. Gawrońska-Grzywacz, T. Krzaczek, Identification and determination of triterpenoids in *Hieracium pilosella* L, J. Sep. Sci. 30 (2007) 746–750.
[13] S. Imam, I. Azhar, M.M. Hasan, M.S. Ali, S.W. Ahmed, Two triterpenes lupanone and lupeol isolated and identified from *Tamarindus indica* linn, Pak. J. Pharm. Sci. 20 (2007) 125–127.
[14] V. Sudhahar, S.A. Kumar, P. Varalakshmi, Role of lupeol and lupeol linoleate on lipemic–oxidative stress in experimental hypercholesterolemia, Life Sci. 78 (2006) 1329–1335.
[15] G.A. Karikas, M.R. Euerby, R.D. Waigh, Constituents of the stems of *Arbutus unedo*, Planta Med. 53 (1987) 223–224.
[16] A. dos Santos Pereira, F.R. de Aquino Neto, Chemical composition of *Tipuana tipu*, a source for tropical honey bee products, Z. Naturforsch. 58 (2003) 201–206.
[17] H. Zhang, M. Shibuya, S. Yokota, Y. Ebizuka, Oxidosqualene cyclases from cell suspension cultures of *Betula platyphylla* var. *japonica*: molecular evolution of oxidosqualene cyclases in higher plants, Biol. Pharm. Bull. 26 (2003) 642–650.
[18] J.B. Nikiéma, R. Vanhaelen-Fastré, M. Vanhaelen, J. Fontaine, C. De Graef, M. Heenen, Effects of antiinflammatory triterpenes isolated from *Leptadenia hastata* latex on keratinocyte proliferation, Phytother. Res. 15 (2001) 131–134.
[19] K. Yamashita, H. Lu, J. Lu, G. Chen, T. Yokoyama, Y. Sagara, M. Manabe, H. Kodama, Effect of three triterpenoids, lupeol, betulin, and betulinic acid on the stimulus-induced superoxide generation and tyrosyl phosphorylation of proteins in human neutrophils, Clin. Chim. Acta 325 (2002) 91–96.
[20] Y. Aratanechemuge, H. Hibasami, K. Sanpin, H. Katsuzaki, K. Imai, T. Komiya, Induction of apoptosis by lupeol isolated from mokumen (*Gossampinus malabarica* L. Merr) in human promyelotic leukemia HL-60 cells, Oncol. Rep. 11 (2004) 289–292.
[21] C. Mutai, D. Abatis, C. Vagias, D. Moreau, C. Roussakis, V. Roussis, Cytotoxic lupane-type triterpenoids from *Acacia mellifera*, Photochemistry 65 (2004) 1159–1164.
[22] M. Saleem, A. Alam, S. Arifin, M.S. Shah, B. Ahmed, S. Sultana, Lupeol, a triterpene, inhibits early responses of tumor promotion induced by benzoyl peroxide in murine skin, Pharmacol. Res. 43 (2001) 127–134.
[23] T.H. Beveridge, T.S. Li, J. C Drover, Phytosterol content in American ginseng seed oil, J. Agric. Food. Chem. 50 (2002) 744–750.
[24] R. Kakuda, T. Iijima, Y. Yaoita, K. Machida, M. Kikuchi, Triterpenoids from *Gentiana scabra*, Phytochemistry 59 (2002) 791–794.
[25] T. Geetha, P. Varalaxmi, R. Mary, Effect of triterpenes from *Crataeva nurvala* stem bark on lipid peroxidation in adjuvant induced arthritis in rats, Pharmacol. Res. 37 (1998) 3.
[26] K. Kinoshita, M. Akiba, M. Saitoh, Y. Ye, K. Koyama, K. Takahashi, N. Kondo, H. Yuasa, Antinociceptive effect of triterpenes from cacti, Pharm. Biol. 36 (1998) 50–57.
[27] S.Y. Ryu, C.K. Lee, C.O. Lee, H.S. Kim, O.P. Zee, Antiviral triterpenes from *Prunella vulgaris*, Arch. Pharm. Res. 15 (1992) 242–245.
[28] F.A. Macias, A.M. Simonet, J.C.G. Galindo, Natural products that influence plant growth and development from plants: the case of *Melilotus messanensis* (L.), All. Proc. Growth Regul. Soc. Am. 22 (1995) 53–57.
[29] M. Higa, K. Ogihara, S. Yogi, Bioactive naphthoquinone derivatives from *Diospyros maritime*, Blume. Chem. Pharm. Bull. 46 (1998) 1189–1193.
[30] T. Geetha, P. Varalakshmi, Anti-inflammatory activity of lupeol and lupeol linoleate in adjuvant-induced arthritis, Fitoterapia 69 (1998) 13–19.
[31] P.T. Sudharsan, Y. Mythili, E. Selvakumar, P. Varalakshmi, Cardioprotective effect of pentacyclic triterpene, lupeol and its ester on cyclophosphamide-induced oxidative stress, Hum. Exp. Toxicol. 24 (2005) 313–318.
[32] P.T. Sudharsan, Y. Mythili, E. Selvakumar, P. Varalakshmi, Lupeol and its ester exhibit protective role against cyclophosphamide-induced cardiac mitochondrial toxicity, J. Cardiovasc. Pharmacol. 47 (2006) 205–210.
[33] R. Baskar, M. Malini, P. Varalakshmi, K. balakrishnan, R. Bhima Rao, Effect of lupeol isolated from *Crataeva nurvala* stem bark against stem bark induced toxicity in experimental urolithiasis, Fitoterapia 67 (1996) 121–125.
[34] G.O. Udeani, G.M. Zhao, Y. Geun Shin, B.P. Cooke, J. Graham, C.W. Beecher, A.D. Kinghorn, J.M. Pezzuto, Pharmacokinetics and tissue distribution of betulinic acid in CD-1 mice, Biopharm. Drug Dispos. 20 (1999) 379–383.
[35] W-W Lin, M. Karin, A cytokine-mediated link between innate immunity, inflammation, and cancer, J. Clin. Invest. 117 (2007) 1175–1183.
[36] T. Geetha, P. Varalakshmi, Anti-inflammatory activity of lupeol and lupeol linoleate in rats, J. Ethnopharmacol. 76 (2001) 77–80.
[37] S. Singh, S. Bani, G.B. Singh, B.D. Gupta, S.K. Banerji, B. Singh, Anti-inflammatory activity of lupeol, Fitoterapia 68 (1997) 9–16.
[38] S. Sunitha, M. Nagaraj, P. Varalakshmi, Hepatoprotective effect of lupeol and lupeol linoleate on tissue antioxidant defence system in cadmium-induced hepatotoxicity in rats, Fitoterapia 72 (2001) 516–523.
[39] Y. Fukuda, K. Sakai, S. Matsunaga, H. Tokuda, R. Tanaka, Cancer chemopreventive effect of orally administrated lupane-type triterpenoid on ultraviolet light B induced photocarcinogenesis of hairless mouse, Cancer Lett. 240 (2006) 94–101.
[40] K. Hata, K. Hori, S. Takahashi, Differentiation- and apoptosis-inducing activities by pentacyclic triterpenes on a mouse melanoma cell line, J. Nat. Prod. 65 (2002) 645–648.
[41] C. Gauthier, J. Legault, M. Lebrun, P. Dufour, A. Pichette, Glycosidation of lupane-type triterpenoids as potent *in vitro* cytotoxic agents, Bioorg. Med. Chem. 14 (2006) 6713–6725.
[42] W. Liu, J.C.K. Ho, F.W.K. Cheung, B.P.L. Liu, W. Ye, C. Che, Apoptotic activity of betulinic acid derivatives on murine melanoma B16 cell line, Eur. J. Pharmacol. 498 (2004) 71–78.
[43] K. Hata, K. Hori, H. Ogasawara, S. Takahashi, Anti-leukemia activities of Lup-28-al-20(29)-en-3-one, a lupane triterpene, Toxicol. Lett. 143 (2003) 1–7.
[44] T.K. Lee, R.T. Poon, J.Y. Wo, S. Ma, X.Y. Guan, J.N. Myers, P. Altevogt, A.P. Yuen, Lupeol suppresses cisplatin-

induced nuclear factor-kappaB activation in head and neck squamous cell carcinoma and inhibits local invasion and nodal metastasis in an orthotopic nude mouse model, Cancer Res. 67 (2007) 8800–8809.
[45] J.M. Fortune, N. Osheroff, Topoisomerase II as a target for anticancer drugs: when enzymes stop being nice, Prog. Nucleic Acid Res. Mol. Biol. 64 (2000) 221–253.
[46] S.H. Kaufmann, Cell death induced by topoisomerase-targeted drugs: ore questions than answers, Biochim. Biophys. Acta 1400 (1998) 195–211.
[47] C.A. Austin, K.L. Marsh, Eukaryotic DNA topoisomerase II beta, Bioessays 20 (1998) 215–226.
[48] F.H. Drake, G.A. Hofmann, S.M. Mong, J.O. Bartus, R.P. Hertzberg, R.K. Johnson, M.R. Mattern, C.K. Mirabelli, *In vitro* and intracellular inhibition of topoisomerase II by the antitumor agent merbarone, Cancer Res. 49 (1989) 2578–2583.
[49] S. Wada, A. Iida, R. Tanaka, Screening of triterpenoids isolated from *Phyllanthus flexuosus* for DNA topoisomerase inhibitory activity, J. Nat. Prod. 64 (2001) 1545–1547.
[50] A. Jemal, R. Siegel, E. Ward, T. Murray, J. Xu, M.J. Thun, Cancer statistics, CA Cancer J. Clin. 57 (2007) 43–66.
[51] M.T. Yip-Schneider, A. Lin, M.S. Marshall, Pancreatic tumor cells with mutant K-ras suppress ERK activity by MEK-dependent induction of MAP kinase phosphatase-2, Biochem. Biophys. Res. Commun. 280 (2001) 992–997.
[52] H.Q. Xiong, Molecular targeting therapy for pancreatic cancer, Cancer Chemother. Pharmacol. 54 (2004) S69–S77.
[53] T. Asano, Y. Yao, J. Zhu, D. Li, J.L. Abbruzzese, S.A. Reddy, The PI 3-kinase/Akt signaling pathway is activated due to aberrant Pten expression and targets transcription factors NF-kappaB and c-Myc in pancreatic cancer cells, Oncogene 23 (2004) 8571–8580.
[54] V. Stoll, V. Calleja, G. Vassaux, J. Downward, N.R. Lemoine, Dominant negative inhibitors of signalling through the phosphoinositol 3-kinase pathway for gene therapy of pancreatic cancer, Gut 54 (2005) 109–116.
[55] E.A. Klein, I.M. Thompson, Update on chemoprevention of prostate cancer, Curr. Opin. Urol. 14 (2004) 143–149.
[56] O.W. Brawley, H. Parnes, Prostate cancer prevention trials in the USA, Eur. J. Cancer 36 (2000) 1312–1315.
[57] M.E. Peter, P.H. Krammer, The CD95 (APO-1/Fas) DISC and beyond, Cell Death Differ. 10 (2003) 26–35.
[58] A. Ashkenazi, V.M. Dixit, Death receptors: signaling and modulation, Science 281 (1998) 1305–1308.
[59] X.X. Wu, Y. Mizutani, Y. Kakehi, O. Yoshida, O. Ogawa, Enhancement of Fas-mediated apoptosis in renal cell carcinoma cells by adriamycin, Cancer Res. 60 (2000) 2912–2918.
[60] A. Suzuki, A. Matsuzawa, T. Iguchi, Down regulation of Bcl-2 is the first step on Fas-mediated apoptosis of male reproductive tract, Oncogene 13 (1996) 31–37.
[61] T.E. Hedlund, R.C. Duke, M.S. Schleicher, G.J. Miller, Fas-mediated apoptosis in seven human prostate cancer cell lines: correlation with tumor stage, Prostate 36 (1998) 92–101.
[62] H. Takayama, T. Takakuwa, Z. Dong, Fas gene mutations in prostatic intraepithelial neoplasia and concurrent carcinoma: analysis of laser captures micro dissected specimens, Lab. Invest. 81 (2001) 283–288.
[63] M. Saleem, M. Kweon, J. Yun, V.M. Adhami, N. Khan, D.N. Syed, H. Mukhtar, A novel dietary triterpene lupeol induces Fas-mediated apoptotic death of androgen-sensitive prostate cancer cells and inhibits tumor growth in a xenograft model, Cancer Res. 65 (2005) 11203–11213.
[64] K. Hata, K. Ishikawa, K. Hori, T. Konishi, Differentiation-inducing activity of lupeol from Chinese dandelion root *(hokoueikon)* on mouse melanoma cell line, Biol. Pharm. Bull. 23 (2000) 962–967.
[65] K. Hata, K. Hori, S. Takahashi, Role of p38 MAPK in lupeol-induced B16 2F2 cell differentiation, J. Biochem. 134 (2003) 441–445.
[66] K. Hata, K. Hori, J. Murata, S. Takahashi, Remodeling of actin cytoskeleton in lupeol-induced B16 2F2 cell differentiation, J. Biochem. 138 (2005) 467–472.
[67] T.J. Slaga, A.J.P. Klein-Szanto, L.L. Triplett, L.P. Yotti, Skin tumor promoting activity of benzoyl peroxide, a widely used free radical-generating compound, Science 213 (1981) 1023–1025.
[68] H. Rezazadeh, M. Athar, Effect of iron overload on the benzoyl peroxide-mediated tumor promotion in mouse skin, Cancer Lett. 126 (1998) 135–142.
[69] D. Chakravarti, J.C. Pelling, E.L. Cavalieri, E.G. Rogan, Relating aromatic hydrocarbon-induced DNA adducts and c-H-ras mutations in mouse skin papillomas: the role of apurinic sites, Proc. Natl. Acad. Sci. 92 (1995) 10422–10426.
[70] N. Nigam, S. Prasad, Y. Shukla, Preventive effects of lupeol on DMBA induced DNA alkylation damage in mouse skin, Food Chem. Toxicol. 45 (2007) 2331–2335.
[71] S. Prasad, N. Kalra, Y. Shukla, Hepatoprotective effects of lupeol and mango pulp extract of carcinogen induced alteration in Swiss albino mice, Mol. Nutr. Food Res. 51 (2007) 352–359.
[72] S.P. Preetha, M. Kanniappan, E. Selvakumar, M. Nagaraj, P. Varalakshmi, Lupeol ameliorates aflatoxin B1-induced peroxidative hepatic damage in rats, Comp. Biochem. Physiol. 143 (2006) 333–339.
[73] I.M. Lee, Antioxidant vitamins in the prevention of cancer, Proc. Assoc. Am. Physicians 111 (1999) 10–15.
[74] Z. Liu, Q.F. Jiao, C. You, Y.J. Che, F.Z. Su, Effect of hyperbaric oxygen on cytochrome C, Bcl-2 and Bax expression after experimental traumatic brain injury in rats, Chin. J. Traumatol. 9 (2006) 168–174.
[75] D.R. Green, J.C. Reed, Mitochondria and apoptosis, Science 281 (1998) 1309–1312.
[76] J.M. Adams, S. Cory, The Bcl-2 protein family: arbiters of cell survival, Science 281 (1998) 1322–1326.
[77] K. Martin, Targeting apoptosis with dietary bioactive agents, Exp. Biol. Med. 231 (2006) 117–129.
[78] M. Rodriguez, J. Schaper, Apoptosis: measurement and technical issues, J. Mol. Cell. Cardiol. 38 (2005) 15–20.
[79] S. Sun, N. Hail, R. Lotan, Apoptosis as a novel target for cancer chemoprevention, J. Natl. Cancer Inst. 96 (2004) 662–672.
[80] S. Bouralexis, D. Findlay, A. Evdokiou, Death to the bad guys: targeting cancer via Apo2L/TRAIL, Apoptosis 10 (2005) 35–51.
[81] J. Reed, Dysregulation of apoptosis in cancer, J. Clin. Oncol. 17 (1999) 2941–2953.
[82] P.R. Dash, J. McCormick, M.J.C.B. Thomson, A.P. Johnstone, J.E. Cartwright, G.St.J. Whitley, Fas ligand-induced apoptosis is regulated by nitric oxide through the inhibition of fas receptor clustering and the nitrosylation of protein kinase Cε, Exp. Cell Res. 313 (2007) 3421–3431.

[83] U. Fischer, K. Schulze-Osthoff, Apoptosis-based therapies and drug targets, Cell Death Differ. 12 (2005) 942–961.
[84] P. Perik, E. de Vries, J. Gietema, W. van der Graaf, D. Sleijfer, A. Suurmeijer, D.V. Veldhuisen, The dilemma of the strive for apoptosis in oncology, Crit. Rev. Oncol. Hematol. 53 (2005) 101–113.
[85] S. Cowley, H. Paterson, P. Kemp, C.J. Marshall, Activation of MAP kinase kinase is necessary and sufficient for PC12 differentiation and for transformation of NIH 3T3 cells, Cell 77 (1994) 841–852.
[86] A.M. Bode, Z. Dong, Signal transduction pathways: targets for chemoprevention of skin cancer, Lancet Oncol. 1 (2000) 181–188.
[87] B.B. Aggarwal, S. Shishodia, Molecular targets of dietary agents for prevention and therapy of cancer, Biochem. Pharmacol. 71 (2006) 1397–1421.
[88] K. Hata, T. Mukaiyama, N. Tsujimura, Y. Satoykosaka, K. Sakamoto, K. Hori, Differentiation-inducing activity of lupane triterpene on a mouse melanoma cell line, Cytotechnology 52 (2006) 151–158.