**Introduction**

There is a growing interest in natural triterpenoids, also known as phytosterols, due to their wide spectrum of biological activities.[1] Triterpenes are a widespread group of natural compounds with considerable practical significance and are produced by arrangement of squalene epoxide in a chair–chair–chair–boat arrangement followed by condensation.[2] Triterpenes are important structural components of plant membranes, and free triterpenes serve to stabilize phospholipid bilayers in plant cell membranes just as cholesterol does in animal cell membranes.[3] Most triterpenes contain 28 or 29 carbons and 1 or 2 carbon–carbon double bonds, typically one in the sterol nucleus and sometimes a second in the alkyl side chain.[3] Triterpenes are natural components of human diets. Pentacyclic triterpenes are all based on a 30-carbon skeleton comprising 5 six-membered rings (ursanes and lanostanes) or 4 six-membered rings and 1 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 yield 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)-secours-12,20(30)-dien-18 alpha H-3 beta-yl acetate, urs-20(30)-en-18 beta H-3 beta-yl acetate and 16(17)-secours-12,20(30) dien-18-alpha H-3 betaol, and a lupene formulated as lup-1,12-dien-3-on-21-ol.[4] There are at least 4000 known triterpenes, most of which occur freely but others occur as glycosides (saponins) or in special combined forms.[5] 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] [Figure 1] occurs across a multitude of taxonomically diverse genera. It is commonly found in plants, viz. Hieracium pilosella,[6] Tamarindus indica,[7] Arbutus unedo,[8] Tipuana tipu,[9] Betula platyphylla,[10] latex of Lepidodendron hastate,[11] roots of Anemone raddeana,[12] bark of Gossampinus malabarica,[13] Acacia mellifera,[14] etc. 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.[15] The quantification of lupeol in fruits and medicinal plants has been performed and is summarized in Table 1.

**ABSTRACT**

Pentacyclic lupane-type triterpenes, exemplified by lupeol [lup-20(29)-en-3b-ol], principally found in common fruit plants such as olive, mango, strawberry, grapes, etc., were reported to possess beneficial effects as a therapeutic and preventive agent for a range of disorders. Although lupeol exhibits an array of biological activities like anti-inflammatory and anti-arthritis activities both in *in vitro* and *in vivo* systems, extensive exploration to establish its role as a chemopreventive compound is warranted. Last 15 years have seen tremendous efforts by researchers worldwide to develop this wonderful molecule for its clinical use toward the treatment of a variety of disorders. These studies also provide insight into the mechanism of action of lupeol and suggest that it is a multi-target agent with immense anti-inflammatory potential targeting key molecular pathways which involve nuclear factor kappa B (NF-jB), cFLIP, Fas, Kras, phosphatidylinositol-3-kinase in a variety of cells. It is noteworthy that lupeol at its effective therapeutic doses exhibits no toxicity to normal cells and tissues. 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.

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Table 1: Content of lupeol in fruits and in plants

<table>
<thead>
<tr>
<th>Name of plant</th>
<th>Lupeol (g/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olive fruit</td>
<td>3 g/g of fruit</td>
</tr>
<tr>
<td>Mango fruit</td>
<td>180 g mango pulp</td>
</tr>
<tr>
<td>Aloe leaves</td>
<td>280 g dry leaf</td>
</tr>
<tr>
<td>Eim plant</td>
<td>880 g bark</td>
</tr>
<tr>
<td>Japanese pear</td>
<td>175 g twig bar</td>
</tr>
<tr>
<td>Ginseng oil</td>
<td>15.2 mg/100g of oil</td>
</tr>
</tbody>
</table>

Lupeol and anti-inflammatory activity

Lupeol has been shown to exhibit various pharmacological activities under in vitro and in vivo conditions. These include its beneficial activity against inflammation, cancer, arthritis, diabetes, heart diseases, renal toxicity and hepatic toxicity. Lupeol has been extensively studied for its inhibitory effects on inflammation under in vitro conditions and in animal models of inflammation. A comprehensive study conducted by Fernandez et al. showed that topical application of lupeol (0.5 and 1 mg/ear) alleviated 12-O-tetradecanoyl-phorbol acetate (TPA)-induced inflammation in an ear mouse model. This study showed that topical application of lupeol decreases myeloperoxidase levels (neutrophil specific marker), thus causing reduction in cell infiltration into inflamed tissues in mice. The anti-inflammatory potential of lupeol could be assessed from the observation that lupeol pretreatment significantly reduced prostaglandin E2 (PGE2) production in A23187-stimulated macrophages. Geetha et al. reported for the first time the utility of lupeol to treat or reduce inflammation in a mouse model of arthritis, which is an inflammation associated disease. The beneficial effect of lupeol in treating inflammation in arthritic mice was shown to be associated with its potential to modulate immune system and the generation of inflammatory factors. Lupeol was reported to suppress and modulate the phagocytic activity of macrophages and T lymphocytes, and suppresses CD4+ T cell mediated cytokine generation in a mouse model. Oral administration of lupeol (12.5–200 mg/kg) resulted in the significant reduction in CD4+ T and CD8+ T cell counts and the level of cytokines (IL-2, IFN-gamma and IL-4) in arthritic mice. A similar report by Latha et al. showed that increased activities of lysosomal enzymes and glycoproteins associated with decreased collagen in arthritic animals were significantly altered by lupeol (50 mg/kg) feeding. Another major development in establishing the anti-inflammatory potential of lupeol was a recent study by Vasconcelos et al., where lupeol was tested for the treatment of inflammation in a mouse model of bronchial asthma. It is well established that asthma is a chronic inflammatory disease of the airways, associated with a Th2 immune response. This study showed that lupeol administration causes a significant reduction in cellularity and eosinophil levels in the broncho-alveolar fluid. Treatment of lupeol was also found to reduce the production of mucus and overall inflammation in the lungs. The anti-inflammatory effect of lupeol was observed to be equal to dexamethasone, a well-known anti-inflammatory agent. The latex from Himatanthus succuuba is used in popular Amazonian medicine as an anti-inflammatory remedy and lupeol was observed to be an active constituent of this anti-inflammatory plant which at a dose of 100 mg/kg (p.o.) inhibited the edema and the abdominal constrictions by 50–40% and 57.9%, respectively, in animals. 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. 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 a strong potential for cancer prevention and therapy. Since nuclear factor kappa B (NF-kB) 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-kB 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 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. An ester derivative of lupeol, lupeol linoleate, also possesses marked anti-inflammatory activity,

as shown by oral or intraperitoneal administration of lupeol at a dose of 25–200 mg/kg body weight in acute and chronic inflammation in rats and mice. Lupeol, along with its acetate and palmitate esters, was found to be the main anti-inflammatory constituent in the croton oil induced ear edema test. Furthermore, lupeol hemisuccinate, synthesized from lupeol, exhibited a stronger activity than lupeol. Lupeol from *Crataeva religiosa* bark has been evaluated for its anti-inflammatory, analgesic, antipyretic effect on rat and mice. It was seen to exert significant dose-dependent effect on acute and chronic inflammatory processes.\(^{26}\) With lupeol established as a potent anti-inflammatory compound, it 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 as listed. Lupeol and lupeol linolate 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.\(^{27}\) Another lupane-type terpenoid, 3b,25-epoxy-3a-hydroxylop-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.\(^{28}\) Several studies were carried out to understand the molecular mechanism through which lupeol inhibits or abrogates the inflammatory processes under *in vitro* and *in vivo* situations and such studies provided several mechanistic facets of anti-inflammatory action of lupeol. Lupeol was reported to modulate several molecules which directly or indirectly play a role in inflammatory process. Lupeol was shown to inhibit the activity of soybean lipoxgenase-1 (15-sLO) with an IC50 equal to 35 nM.\(^{29}\) Lupeol treatment (10–100 IM) is also shown to decrease the generation of pro-inflammatory cytokines such as tumor necrosis factor-a (TNF-a) and interleukin-b (IL-b) in lipopolysaccharide-treated macrophages.\(^{17}\) Recent report by Yamashita et al. suggested that superoxide generation induced by arachidonic acid (AA) is suppressed by lupeol in N-formyl-methionyl-leucyl-phenylalanine (FMLP) treated human neutrophils.\(^{30}\) Further, lupeol treatment was observed to cause a reduction in the inflammation by decreasing the levels of type II cytokines, IL-4, IL-5 and IL-13, in a bronchial asthma mouse model.\(^{27}\) Recently, lupeol was reported to exhibit significantly high wound healing potential in a dead space wound healing mouse model.\(^{31}\) This study showed that lupeol exerts its wound healing effect by decreasing the level of monocytes and docking with GSK3b protein.\(^{32}\) The activation domain of GSK3b consists of Tyr216, with residues Asn64, Gly65, Ser66, Phe67, Gly68, Val70, Lys85, Leu132, Val135, Asp181 in the active pocket, docked with lupeol at the torsional degree of freedom 0.5 units.\(^{32}\) Taken together, these compelling evidences suggest that the therapeutic usefulness of lupeol for inflammatory conditions is attractive and warrants further investigation.

**Lupeol and cancer**

Recent studies have shown that diets rich in phytochemicals can significantly reduce cancer risk by as much as 20%\(^{2,21,32}\). Epidemiological data suggest that the phytosterol content of the diet is associated with a reduction in common cancers including cancers of the colon, breast, and prostate.\(^{2,31,32}\) Data emanating from molecular studies with various tumorigenic models suggest that phytosteros modulate host systems potentially enabling more robust antitumor responses such as enhancing immune recognition of tumor cells, altering hormone-dependent growth of endocrine tumors and modulating sterol biosynthesis.\(^{33}\) Reports suggest that the decreased risk for various cancers associated with high olive oil consumption may be associated with its rich triterpene content.\(^{34}\) A number of triterpenoids have shown promise as antineoplastic agents and exhibit antiproliferative activity when tested against various cancer cell lines.\(^{2,31,32}\) These triterpenoids include members belonging to the cytochrome, lupane, friedelane, dammarane, ursane, oleanane, limonoid and curcurbitacin family.\(^{2,23-44}\) Recent reports have shown that triterpenes directly inhibit tumor growth, cell cycle progression, and induce the apoptosis of tumor cells under *in vitro* and *in vivo* situations.\(^{32}\) Mutations that occur through DNA strand breaks have been shown to form the precursors of cancer development, and cells harboring mutations are at high risk to transform into neoplastic phenotype.\(^{45-48}\) During the course of tumorigenesis, mutations get accumulated, thus transforming neoplastic cell into malignant carcinomas.\(^{45-48}\) It is noteworthy that lupeol was reported to exhibit strong anti-mutagenic activity under *in vitro* and *in vivo* systems\(^{49,50}\) and references therein. Earlier reports have shown that lupeol inhibits the chemically induced DNA damage under *in vitro* conditions.\(^{51}\) Study by Nigam et al. showed that topical application of lupeol (200 lg/mouse) prevents 7,12-dimethylbenza(anthracene (DMBA)-induced DNA damage (DNA strand breaks) in murine skin.\(^{51}\) Recently, lupeol was shown to inhibit benzo(a)pyrene (B(a)P) toxicity, which is a well-known mutagen-induced genotoxicity in a mouse model.\(^{52}\) This study showed that pretreatment with lupeol (1 mg/animal) for 7 days prior to B(a)P administration significantly decreased B(a)P-induced clastogenicity and caused an increase in mitotic index.\(^{52}\) Lupeol was observed to significantly inhibit the activity of ODC protein which is a well-known biomarker of tumor promotion.\(^{53,54}\) Lupeol was tested against human melanoma tumor cells *in vitro* and in a xenograft athymic nude mouse model.\(^{55}\) We showed that lupeol inhibits growth of highly metastatic tumors of human melanoma origin by modulating the ratio of Bcl-2 and Bax protein levels *in vitro* and *in vivo*.\(^{56}\) The most important observation in this study was that no toxic effect on normal human melanocytes was observed at a dose at which lupeol kills malignant melanoma cells. Recently, studies have been carried out to investigate the structure–activity relationships of lupeol in various human cancer cell lines.\(^{56-58}\) A study conducted by Aratanechemuge et al. showed that lupeol induces apoptosis of human promyelocytic HL-60 leukemia cells.\(^{58}\) This study showed that lupeol induces the formation of hypodiploid nuclei and fragmentation of DNA (a characteristic of apoptosis) in a dose- and time-dependent manner.\(^{58}\) Lupeol has been found to induce differentiation and inhibits the cell growth of mouse melanoma and human leukemia cells.\(^{59,60}\) Besides lupeol, lupenone, which is obtained from *A. meliffera*, has been shown to exhibit significant cytotoxicity against non–small-cell lung carcinoma-N6 (NSCLC-N6) cell line.\(^{61}\) In another study, lupeol, betulin, methyl betulinate, and glycosides (b-d-glucosides, a-L-rhamnosides, and a-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).\(^{62}\) Lupane-type terpenoids also exhibited cytotoxicity against human hepatocellular carcinoma (Hep-G2) and human epidermoid carcinoma (A-431), while they 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). Lupeol has been reported as a differentiation-inducing compound in B16 2F2 cells, up-regulating the melanogenesis of these cells. The cytotoxicity profiles of lupeol triterpene against human cancer cells showed that its cytotoxic effect against lung cancer cell lines is the strongest, while it is very weak against osteosarcoma, breast cancer and urinary bladder cancer cells. 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-kB activity. DNA topoisomerases (Topos) are ubiquitous enzymes that play a crucial role in many aspects of DNA metabolism such as replication, transcription on, recombination and chromosome segregation during mitosis. Topos have therefore been identified for anticancer chemotherapeutic drug development. 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. Topo-II is an essential enzyme in the DNA replication process and is the primary cellular target or many of the widely used and effective anticancer agents. Naturally occurring lupeane-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 IC50 values in the range of 10–39 nM.

**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. 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. 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-kB pathways, have been linked to chemoresistance of the pancreatic carcinoma cells.

These findings suggest that Ras oncprotein could be an important target for the development of Ras oncprotein 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-pronged 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 [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] assay and induction of apoptosis as assessed by flow cytometry, fluorescence microscopy and Western blotting. Lupeol treatment to cells has been found to significantly reduce the expression of Ras oncprotein 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-jB signaling pathway. Lupeol-induced apoptosis of pancreatic cells is mediated through the activation of caspase-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.

**Anti-leukemia activity of lupeol and derivatives**

Lupeol and its derivatives are cytotoxic against human leukemias, melanomas, neuroblastomas and normal fibroblast cells. Lupeane triterpenes with a carbonyl group at C-17 demonstrated discernible inhibitory effects on leukemia, melanoma and neuroblastoma cell growth. Luf-28-al-20(29)-en-3-one markedly inhibited leukemia 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 produced a strong cytotoxic effect. Drug-resistant K562 cells showed cross-resistance to both drugs. The morphological observations of leukemia nuclei and the gel electrophoresis analysis of DNA extracted from leukemia cells treated with lupeol and its derivatives revealed that lupeol induces apoptosis in these cells.

**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. 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 glutathione-S-transferase (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.

**Prostate cancer and lupeol**

Prostate cancer has become a major public health concern and is a leading cause of cancer-related deaths among males. 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. 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 arise and the diagnosis is eventually established. It is typically diagnosed in elderly men, and therefore even a modest delay in the neoplastic development achieved consequently result in substantial reduction in the incidence of the clinically detectable disease. 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.\textsuperscript{30-33} 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 \textit{Fas} gene have been shown to be closely associated with the pathogenesis of prostatic intraepithelial neoplasia and concurrent carcinomas.\textsuperscript{34,35} 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 mitochondria-dependent apoptotic signaling molecules, such as Bcl-2 and Bax, has been observed.\textsuperscript{36} 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).\textsuperscript{37} 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.

\textbf{Lupeol as a cardioprotective agent}

Lupeol has been investigated for its cardioprotective effects and was demonstrated to provide 34.4\% protection against \textit{in vitro} low-density lipoprotein (LDL) oxidation.\textsuperscript{38,39} Lupeol and lupeol acetate have also shown hypotensive activity, which may make them possible preventative agents in this cardiac disorder and other consequent cardiovascular diseases.\textsuperscript{40} In addition, supplementation of lupeol or lupeol linoleate was effective against the cardiac oxidative injury caused by cyclophosphamide, a drug used in the treatment of cancer and autoimmune disorders. A study showed that lupeol and lupeol linoleate can ameliorate the lipidemic-oxidative abnormalities in the early stages of hypercholesterolemic atherosclerosis in rats.\textsuperscript{41} It revealed the triterpene’s mode of action by a restoration of several transmembrane enzymes, total cholesterol, triglycerides and phospholipids to normal levels, preventing hypertriglyceridemic cardiac histology. It also demonstrated lupeol’s antidyplasidipic activity in hamster at a dose of 100 mg/kg body weight. In addition, the authors synthesized 10 lupeol ester derivatives and found a nicotinic acid derivative that exhibited better lipid-lowering profile, at a dosage twice lower than lupeol, along with antihyperglycemic effect which revealed lupeol’s potential as a scaffold for developing drugs targeting coronary diseases and diabetes.

\textbf{Mechanism of action of lupeol-based chemoprevention}

As far as lupeol’s mechanism of action in cancer cells is concerned, the first understanding of lupeol’s cytotoxic activity was attributed to its ability to inhibit topoisomerase II (topo II),\textsuperscript{42} an essential enzyme in eukaryotic cell replication, whose role is to relax supercoiled DNA by catalyzing a transient break in double-stranded DNA. Therefore, lupeol was screened for its capacity for inhibiting the conversion of supercoiled plasmid DNA to relaxed DNA by topo II. It was found that lupeol selectively inhibited topo II catalytic reaction. Lupeol inhibited the farnesyltransferase enzyme, making it a potential anticancer agent in tumors where the \textit{ras} oncogene plays a role.\textsuperscript{43} Lupeol has also been demonstrated to induce the estrogen receptor (ER) expression, which may explain its growth inhibitory action in MDA-MB-231 breast cancer cells.\textsuperscript{44} Another mechanism lupeol has been proven to act through is angiogenic inhibition. Angiogenesis is the formation of new blood vessels from the pre-existing vessels and is known to play an important role in tumor growth and metastasis.\textsuperscript{45} Much focus has been placed on lupeol-induced apoptosis. The first evidence for apoptosis in cancer cells treated with lupeol was shown in human promyelocytic leukemia HL-60 cells, where apoptotic bodies were observed along with DNA fragments characteristic of apoptosis.\textsuperscript{11,46} This process known as “programmed cell death” is used to remove ineffective or irreparable damaged cells. 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.\textsuperscript{47} Phenotypically and morphologically, apoptosis is characterized by chromatin condensation, nuclear fragmentation into mono and oligonucleosomal units, cell shrinkage and plasma membrane blebbing.\textsuperscript{48} 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. An emerging body of evidences indicates that lupeol and its derivatives may trigger apoptosis via copious molecular targets. Lupeol mediated apoptosis is essentially manifested by the cleavage of poly (ADP-ribose) polymerase (PARP). Since poly (ADP-riboseylation) 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 lM) 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.\textsuperscript{49} Other contributing molecular changes in cancer progression include activation of oncogenes and inactivation of tumor suppressor genes. The mutation in \textit{ras}, an oncogene, results in the accumulation of Ras oncoprotein, and constitutive activation of various intrinsic intracellular signaling pathways, leading to cellular proliferation. Targeting the \textit{ras} gene by lupeol has been shown to inhibit the growth of metastatic cells.\textsuperscript{50} The MAPK pathway, in addition to NF-\textit{\beta} 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-promoting tumor promoters, including TPA, epidermal growth factor (EGF), and platelet-derived growth factor (PDGF).\textsuperscript{51} On the other hand, stress-related tumor promoters, such as ultraviolet (UV) irradiation and arsenic, potently activate JNKs/SAPKs and p38 kinases.\textsuperscript{52} Melanogenesis, being one of the hallmark s of melanoma cell differentiation, has already been shown to be regulated by lupeol via activation of the p38 MAPK signaling. Hence, 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 cascade. Also, as of the role of NF-\textit{\beta} signaling in carcinogenesis, lupeol could afford intrusion in the pathway via activation of PI3K, phosphorylation of Akt at Thr(308), activation of NF-\textit{\beta} and I\textit{\beta}k, and degradation and phosphorylation of I\textit{\beta}a. 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. Inhibitors of this lyase activity might be expected to sensitize cancer cells to DNA-damaging agents and to potentiate their cytotoxicity, being regarded as promising adjuvant drugs to anticancer therapy.\(^{[90]}\)

**Conclusion**

It is now evident from the above discussion that a surfeit of naturally occurring bioactive agents in fruits and vegetables has 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.\(^{[99]}\) As this review demonstrates, lupeol is a potent agent to anticancer therapy.\(^{[98]}\) These drugs to anticancer therapy.\(^{[98]}\) potentially potentiating their cytotoxicity, being regarded as promising adjuvant agents to anticancer therapy.\(^{[98]}\) 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. Inhibitors of this lyase activity might be expected to sensitize cancer cells to DNA-damaging agents and to potentiate their cytotoxicity, being regarded as promising adjuvant drugs to anticancer therapy.\(^{[90]}\)

**References**


10. Dos Santos-Pereira A, de Aquino-Neto FR. Chemical composition of...


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