Induction of Apoptosis by Lupeol and Mango Extract in Mouse Prostate and LNCaP Cells

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Prostate cancer (PCA) is one of the most invasive malignancy and second leading cause of cancer-related deaths in United States and some other countries. Long latency period makes PCA an ideal disease for pharmacologic or nutritional chemoprevention. Lupeol, a triterpene present in mango and other fruits, has shown to possess anticancer properties in vivo and in vitro assays. Here, we recorded the apoptogenic activity in mouse prostate by lupeol and mango pulp extract (MPE). Testosterone was injected subcutaneously (5 mg/kg body weight) for 14 consecutive days to male Swiss albino mice. Lupeol/MPE supplementation resulted in arrest of prostate enlargement in testosterone-treated animals. In mouse prostate tissue, lupeol and MPE supplementation resulted in a significantly high percentage of apoptotic cells in the hypodiploid region. The induction of apoptosis in mouse prostate cells was preceded by the loss of mitochondrial transmembrane potential and DNA laddering. In testosterone-induced mouse prostate, upregulation of antiapoptotic B-cell non-Hodgkin lymphoma-2 and downregulation of proapoptotic Bcl-2-associated X protein and caspase-3 were also recorded. We further observed apoptogenic activities of lupeol in an in vitro model using human prostate cancer cells [lymph node carcinoma of the prostate (LNCaP)]. The apoptogenic response of lupeol-induced changes in LNCaP cells can be summarized as early increase of reactive oxygen species followed by induction of mitochondrial pathway leading to cell death. Thus, the results of this study demonstrate that lupeol/MPE is effective in combating testosterone-induced changes in mouse prostate as well as causing apoptosis by modulating cell-growth regulators.

INTRODUCTION
Prostate cancer (PCA) is one of the most invasive malignancy in males of the United States and many other countries and is the second leading cause of cancer deaths in American men and the men of some other countries (1,2). It is estimated that average annual PCA incidence rates in the United States are 110.4 to 180.9, whereas in India, it ranged from 5.0 to 9.1, per 100,000 (3). Epidemiological, experimental, and clinical data point toward an important role of androgens in the development and progression of this disease (4). An increasing incidence of the disease and failure of conventional therapy for advanced invasive PCA leads to the search for new approaches for management of the disease (5,6). Epidemiological evidences have shown an inverse association between consumption of vegetables and risk of PCA (7,8). Mango (Mangifera indica L.) is a worldwide consumed fruit in the tropical countries around the world. Mango extract has been shown to be antimutagenic in Salmonella typhimurium TA1538 (9). Chemical analysis of mango pulp extract (MPE) has shown that it contains vitamins, organic acids, carbohydrates, amino acids, polyphenols, and volatile compounds (10). Lupeol [Lup-20 (29)-en-3-β-ol; Fig. 1] is a naturally occurring pentacyclic triterpene present predominantly in mango. Lupeol has been shown to exhibit strong anti-inflammatory, antirheumatic, antimutagenic, and antimalarial activities (11–13). It has also been shown to possess antitumor-promoting effects in mouse skin carcinogenesis (14). The oral administration of lupeol changed the tissue redox system induced by cadmium exposure by scavenging the free radicals and by improving the antioxidant status of the rat liver (15).

Generally, cancer is a multifactorial disease, which requires modulation of multiple pathways and targets. These targets include the activation of apoptosis, suppression of growth factor expression, or signaling and downregulation of antiapoptotic proteins (16). Apoptosis is a well organized cell death process characterized by loss of plasma membrane phospholipid asymmetry, enzymatic cleavage of the DNA into oligonucleosomal fragments, and segmentation of the cells into membrane-bound apoptotic bodies (17). Genetic changes leading to loss of apoptosis or derangement of apoptosis-signaling pathways in the transformed cells are critical for neoplastic changes (18). The execution process of cells by apoptosis is mediated by caspase-3, one of a family of cysteine proteases (19,20). Activation of caspase-3 is tightly regulated by the formation of apoptosome (21), which is dependent on the cytochrome c release from the mitochondria into cytosol, which is thought to be regulated by B-cell non-Hodgkin lymphoma-2 (Bcl-2)-associated X protein (Bax) and Bcl-2. Proapoptotic Bax forms pores in the outer mitochondrial membrane, releasing cytochrome c, whereas antiprototic Bcl-2 prevents the opening of mitochondrial transition

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pore by binding with Bax (22). Reactive oxygen species (ROS) have been suggested to act as an upstream signal for caspase-3 activation (23).

Recently, a number of reports have shown that lupeol has chemopreventive properties (14,24,25); however, there are no reports on the effects of MPE on PCA. Thus, this study was aimed at evaluating the effect of pretreatment with lupeol/MPE on testosterone-induced changes in mouse prostate. Further, we studied the mechanism of antiproliferative activities of lupeol in androgen sensitive lymph node carcinoma of the prostate (LNCaP) cells. The alterations in levels of ROS, membrane potential, shift in Bcl-2:Bax ratio, and Caspase-3 were used as intermediate biomarkers for lupeol induced chemoprevention.

MATERIALS AND METHODS

Chemicals

Human prostate cancer cells LNCaP were obtained from the National Centre for Cell Science (Pune, India) and cultured in RPMI 1640 in a humified atmosphere of 95% air and 5% CO² at 37°C. Testosterone, lupeol, dichlorodihydrofluorescein diacetate dye (DCFH-DA), rhodamine 123, propidium iodide, and β-actin (clone AC-74) were purchased from Sigma (St. Louis, MO). The Bcl-2 [polyclonal anti-rabbit immunoglobulin G (IgG)], Bax (polyclonal anti-rabbit IgG), and caspase-3 (anti-mouse IgG) antibody were procured from Oncogene Research Products (Cambridge, MA). The anti-mouse and anti-rabbit horseradish-peroxidase conjugate secondary antibodies were obtained from Banglore Genei (Bangalore, India). The polyvinylidene fluoride membrane was obtained from Millipore (Bedford, MA). The rest of the chemicals were of analytical grade of purity and procured locally.

Preparation MPE

Fresh mango pulp (20 g) of ripened mango fruit was homogenized with 100 ml of 0.1 M (pH 7.0) phosphate-buffered saline (PBS). The resulting homogenate was filtered through 4-layered muslin cloth and then centrifuged at 6,000 g for 20 min at room temperature. The MPE was prepared fresh every time.

Quantification of Lupeol in MPE

The MPE was concentrated by lyophilization and then cleaned up using anhydrous sodium sulphate and charcoal. High-performance liquid chromatography (HPLC; Water Inc., MA, USA) was carried out using a C₁₈ column (4.6 × 200 mm) with 90% CH₃CN (aq) as a solvent [flow rate 1.0 ml/min, detection ultraviolet (UV) 210 nm] at 25°C (retention time = 7 min).

Animals and Treatment

Male, Swiss albino mice [25 ± 2 gm body weight (b. wt.)] were taken from the Industrial Toxicology Research Centre (Lucknow, India) animal colony and acclimatized properly. The animals were randomly divided into 6 groups, each comprising 10 animals. Animals were kept under standard condition (25 ± 2°C, relative humidity 57 ± 2%, and 12/12 hours light/dark phase) and were fed with synthetic pellet diet (M/S Ashirwad, Chandigharh, India) and water ad libitum. Mice in untreated control Group 1 were fed with drinking water, whereas animals in Group 3 and 5 were given lupeol (1 mg/mouse dissolved in ethanol and diluted in corn oil) orally through gavage for 14 consecutive days. Animals of Group 4 and 6 were given 1.0 ml MPE orally through gavage for 14 consecutive days. Testosterone (5 mg/kg b. wt. dissolved in corn oil) was given to animals of Group 2, 3, and 4 subcutaneously (26). The feeding regimen was followed for 2 wk. Animals from all the groups were examined every day for gross morphological changes during the entire study period. On the 15th day, all the animals were sacrificed humanly by cervical dislocation. Prostate from each animal were excised, weighed, and immediately washed with ice cold saline and stored at –80°C until further analysis.

Cell Viability Assay

The cleavage of the tetrazolium salt MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetraizolumbromide) into a blue colored formazan by the mitochondrial enzyme succinate dehydrogenase was used for assaying cell survival and proliferation (27). The effect of lupeol on the viability of LNCaP cells was determined by using this MTT assay. The cells were plated at 1 × 10⁴ cells per well in 200 µl of complete culture medium and treated with 0, 10, 20, 40, 60, 75, 100, and 125 µM concentrations of lupeol in 96-well microtiter plates. Lupeol stock solutions prepared at 1-mM concentration were mixed with fresh medium to achieve the desired final concentration. After incubation for 24, 48, and 72 h at 37°C in a humidified incubator, cell viability was determined. MTT (5 mg/ml in PBS) was added to each well and incubated for 5 h, after which the
FIG. 2. Effects of lupeol and mango pulp extract (MPE) on prostate enlargement. Testosterone treatment increased the mean prostate weight, which was decreased by supplementation of lupeol/MPE. C, untreated control; T, testosterone alone; L + T, lupeol + testosterone; M + T, MPE + testosterone; L, lupeol alone. Data are ± SE of 10 animals. *, values are significantly different over untreated control group, \( P < 0.01 \). #, values are significantly different over testosterone-treated group, \( P < 0.05 \).

plate was centrifuged at 1,800 rpm for 5 min at 4°C. The supernatant was removed from the wells by aspiration. After careful removal of the medium, 0.1 ml of buffered dimethyl sulfoxide (DMSO) was added to each well, and plates were shaken. The absorbance was recorded on a microplate reader at the wavelength of 530 nm. The effect of lupeol on growth inhibition was assessed as percent cell viability in which vehicle-treated cells were taken as 100% viable.

**Treatment of cells.** Lupeol stock (1 mM) was prepared by dissolving lupeol in minimal amount of ethanol and suitably diluted in DMSO. For dose dependent studies, the LNCaP cells (50% confluent) were exposed with lupeol (75 \( \mu \)M) for 48 h in complete cell medium. Cells that served as vehicle controls were incubated with ethanol + DMSO alone, whereas no treatment was given in the untreated control. The final concentration of DMSO and ethanol was 0.25% and 0.075%, respectively, in all treatment protocols. The cells were harvested by trypsinization, washed twice with cold PBS, and stored at –80°C until further analysis.

**Flow cytometric analysis of apoptosis.** Single cell suspension of prostate tissues was prepared by using madimachine (Becton-Dickinson, San Jose, CA). These single-cell suspensions of mouse prostate and LNCaP cells from each treated and untreated control were centrifuged at 2,000 rpm for 10 min at 4°C. The cell pellet of both in vivo and in vitro was resuspended separately in 50 \( \mu \)l cold PBS and fixed in 2 ml of 70% ice-cold ethanol. Cells were centrifuged and treated with 0.1% Triton X-100 for 5 min. After incubation, cells were centrifuged again and resuspended in 1 ml of PBS, ribonuclease (100 \( \mu \)g/ml) was
added, and the cells were incubated at 37°C for 30 min. After further centrifugation, cells were resuspended in 1 ml of PBS and 50 μg/ml propidium iodide (PI) and incubated for 30 min at 4°C. The data were acquired and analyzed excluding cellular debris on a flow cell cytometer (Becton-Dickinson LSR II, San Jose, CA) using Cell Quest 2.0 software (28).

Measurement of ROS generation. ROS production was monitored by flow cytometry (Becton-Dickinson LSR II, San Jose, CA) using DCFH-DA dye as described by Esposti and McLennan (29). Briefly, single-cell suspension of prostate was prepared as described previously in PBS. Single-cell pellets of both prostate and LNCaP were dissolved in PBS supplemented with 50 mM glucose and incubated with 10 μM DCFH-DA at 37°C for 1 h. The fluorescence increased due to the hydrolysis of DCFH-DA to DCFH by some nonspecific cellular esterases, and its subsequent oxidation by peroxides was measured. Values were given in terms of mean fluorescence intensity (MFI).

Mitochondrial membrane potential analysis. The untreated control and treated single-cell suspension of prostate and LNCaP cells were incubated with rhodamine 123 (5 μg/ml) for 60 min in dark at 4°C, harvested, and suspended in PBS. The mitochondrial membrane potential was measured using flow cytometry by the fluorescence intensity (FL-1) of 10,000 cells (30).

DNA fragmentation. The DNA fragmentation pattern (DNA ladder) was carried out by agarose gel electrophoresis (31). Untreated control and treated cells of prostate and LNCaP were pelleted by centrifugation at 200 g for 10 min and the pellet lysed with 0.5 ml lysis buffer (10 mM Tris-HCl, pH 7.5, 20 mM ethylenediamine tetraacetic acid (EDTA), 0.5% Triton X-100)
on ice for 30 min. The DNA in lysed solution was extracted with phenol/chloroform and precipitated with 3 M sodium acetate (pH 5.2) and cold ethanol. The purity of DNA was determined by measuring optical density (OD) at 260 and 280 nm and absorbance ratio obtained between 1.7 and 1.9. DNA (2 µg) was then loaded on 0.8% agarose gel and electrophoresis carried out. The bands were visualized by ethidium bromide staining under UV light.

**Total cell lysate preparation.** In untreated control and treated LNCaP cells, ice-cold lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 1 mM ethylene glycol-bis-aminoethylther-N,N,N′,N′-tetraacetic acid, 1 mM EDTA, 20 mM NaF, 100 mM Na3VO4, 0.5% NP-40, 1% Triton X-100, 1 mM phenylmethylsulfonylfluoride, 10 µg/ml aprotinin, 10 µg/ml leupeptin, pH 7.4) was added to the plates, which were then placed over ice for 30 min. The cells were scraped, and the lysate was collected in a microfuge tube and passed through a 21 G needle to break up the cell aggregates. The lysate was cleared by centrifugation at 14,000 g for 15 min at 4°C, and the supernatant (total cell lysate) was either used immediately or stored at –80°C.

**Western blotting.** Western blotting was carried out as described earlier (32) in the prostate tissue homogenate and LNCaP cells. Protein content was estimated by the method of Lowry et al. (33). Proteins (30 µg) were resolved on 10% gel followed by electro transfer onto an immobile polyvinylidene fluoride membrane using semidy transfer (Bio-Rad Laboratories, Hercules, CA). The membranes were blocked overnight with 5% nonfat dry milk and probed with primary antibody of Bcl-2/Bax/caspase-3 at dilutions recommended by the suppliers. Equal loading was confirmed by stripping the immunoblot and reprobing it for β-actin. Finally, the intensity of the bands was quantitated using Easy Win 32 software on Gel Documentation System (Herolab, GmbH, Wiesloch, Germany). Densitometry measurements of the scanned band were performed using digitized scientific software program UNSCAN. Data were expressed as mean values ± SE of 3 separate sets of experiment.

**Statistical Analysis**

Significance difference of variance in antioxidant level data between control and experimental groups was analyzed using Student t-test, and $P < 0.05$ and $P < 0.01$ were considered to be significant.
FIG. 5. A: A flow cytometric analysis showing testosterone-induced production of reactive oxygen species (ROS), which was quenched by lupeol and mango pulp extract (MPE) in mouse prostate. C: In the case of lymph node carcinoma of the prostate (LNCaP) cells, lupeol increases the ROS generation. The horizontal axis represents dichlorodihydrofluorescein diacetate dye fluorescence vs count in horizontal axis. The respective mean fluorescence intensity (MFI) of treated and untreated B: control mouse prostate and D: LNCaP cells are represented by bar diagram. C, untreated control; T, testosterone alone; L + T, lupeol + testosterone; M + T, MPE + testosterone; L, lupeol alone; V, vehicle, FL1-H, fluorescence intensity. Data are ± SE of 10 animals. *, values are significantly different over untreated control group, \( P < 0.05 \). #, values are significantly different over testosterone-treated group, \( P < 0.05 \).

FIG. 6. Effect of lupeol and mango pulp extract (MPE) on mitochondrial membrane potential. Single-cell suspension of both mouse prostate and lymph node carcinoma of the prostate (LNCaP) cells added with Rhodamine 123 and incubated for 60 min and its fluorescence measured using a flow cytometer with fluorescence intensity (FL)-1 filter. A: Results expressed as representative histogram overlay showing loss in membrane potential by lupeol/MPE treatment and not by testosterone treatment in mouse prostate. C: Similarly, lupeol resulted loss in mitochondrial membrane potential of LNCaP cells. The respective MFI of treated and untreated B: control mouse prostate and D: LNCaP cells are represented by bar diagram. C, untreated control; T, testosterone alone; L + T, lupeol + testosterone; M + T, MPE + testosterone; L, lupeol alone; V, vehicle; MFI, mean fluorescence intensity; FL1-H, fluorescence intensity. Data are ± SE of 10 animals. *, values are significantly different over untreated control group, \( P < 0.05 \). #, values are significantly different over testosterone-treated group, \( P < 0.05 \).
RESULTS

On the basis of the results obtained, we report that lupeol/MPE possess protective effects against testosterone-induced alterations in mouse prostate and exhibit their antiproliferative effect by ROS mediated apoptosis in an in vitro experimental model. In testosterone alone treated animals (Group 2), the mean prostate weight was significantly ($P < 0.05$) increased (48.2 mg) twice to normal animals (24.5 mg) having no treatment (Group 1; Fig. 2). Both lupeol (Group 3) and MPE (Group 4) significantly ($P < 0.05$) reduced the growth of prostate by (35.1 mg) $\approx 1.4$ and (37.3 mg) $\approx 1.3$ times, respectively, in comparison to testosterone alone treated Group 2 (Fig. 2). As expected, lupeol and MPE alone showed no significant ($P < 0.05$) alterations in any parameters studied when compared with untreated controls in vivo. Treatment of lupeol $(10–125 \mu M)$ to LNCaP cells resulted in dose-dependent inhibition of cell proliferation, and the extent of growth inhibition increased up to 78% as a function of time (24, 48, and 72 h) as observed by MTT assay. Inhibitory concentration of 50% (IC50) values, calculated from growth inhibition curves obtained 48 h after lupeol treatment, were at 75 $\mu M$ in LNCaP cells.

Quantification of Lupeol

The amount of lupeol was quantified by comparing the area of the sample with the area of standard as shown in an HPLC chromatogram (Fig. 3). The amount of lupeol in our study was found to be 0.18 mg/100 g of mango pulp.

Inhibition of Cell Death by Lupeol/MPE

We observed that lupeol and MPE possess the ability to protect cells from proliferation induced by testosterone. In mouse prostate, testosterone treatment in Group 2 increased the cell proliferation observed as a significant ($P < 0.05$) increase in S-phase by flow cytometry, whereas smaller S-phase was observed in untreated control (Fig. 4A). This hyperplasic condition of prostate cells was checked by inducing apoptosis (sub G1 peak) in both lupeol and MPE administrated animal. In in vitro assay, the lupeol treatment resulted in significant ($P < 0.05$) inhibition of cell proliferation in a dose-dependent (10–125 $\mu M$) as well as time-dependent (24, 48, 72 h) manner. Lupeol treatment at the concentration of 75 $\mu M$ (IC50) for 48 h of incubation time resulted in death of LNCaP cells by apoptosis (Fig. 4B).

Levels of ROS

The intracellular ROS level was determined in terms of MFI of 2,7′-dichlorofluorescein. In mouse prostate, testosterone treatment (Group 2) significantly ($P < 0.05$) increased the ROS level (MFI = 84.12) over untreated control Group 1 (MFI = 40.94; Figs. 5A and 5B). The increased levels of ROS were reduced significantly ($P < 0.05$) by both lupeol (MFI = 57.40) and
MPE (MFI = 66.48) supplementation (Group 5) in the prostate (Figs. 5A and 5B). However, ROS levels were increased (MFI = 112.45) in lupeol-treated LNCaP cells over untreated control (MFI = 45.44) and vehicle (MFI = 52.56) treated cells (Figs. 5C and 5D).

**Restoration of Mitochondrial Membrane Potential (Δψm)**

During apoptosis, dying cells undergo an ordered series of events resulting in activation of proteases; accumulation of intracellular peroxidase; protein release; and importantly, alteration in plasma membrane. Here, we examined for changes in mitochondrial membrane potential (Δψm) during testosterone-induced changes. The mitochondrial membrane potential was determined in terms of MFI using flow cytometry. In mouse prostate, a significant (P < 0.05) loss of mitochondrial membrane potential (Figs. 6A and 6B) was observed by administration of lupeol/MPE (MFI = 42.36/46.34; P < 0.05) in comparison to testosterone-treated Group 2 (MFI = 185.38). However, with LNCaP cells, lupeol treatment decreased the membrane potential (MFI = 40.54) over untreated control (MFI = 103.63) and vehicle-treated (MFI = 98.87) cells (Figs. 6C and 6D).

**Analysis of DNA Fragmentation**

DNA fragmentation resulting in a ladder formation on agarose gel electrophoresis is a characteristic feature of apoptosis. The apoptotic effect of lupeol and MPE on mouse prostate and LNCaP cells was observed using agarose gel electrophoresis. In testosterone-exposed animals, lupeol/MPE treatment (Groups 3 and 4) resulted in multiple fragmentations of DNA (Fig. 7A). Lupeol (75 µM) treated LNCaP cells also showed DNA fragmentation (Fig. 7B).
FIG. 8. Continued

Lupeol/MPE Modified the Expression Level of Bcl-2:Bax Ratio and Caspase-3

The levels of Bcl-2:Bax ratio and caspase-3 were reported to play a crucial role in apoptotic response mediated by many agents (34). In this in vivo study, Western blot analysis revealed that testosterone (Group 2) induced significant ($P < 0.01$) up-regulation of Bcl-2 ($\approx$1.9-fold compared to untreated control; Fig. 8A) and significant ($P < 0.01$) downregulation of Bax and caspase activity ($\approx$1.5 and $\approx$1.6-fold compared to untreated control; Fig. 8A). Densitometry of the immunoblots showed that lupeol/MPE treatment significantly decreased the Bcl-2 ($\approx$1.3-fold/1.2-fold compared to the testosterone-treated group) and increased the Bax expression ($\approx$1.4-fold/1.3-fold compared to the testosterone-treated group). Lupeol/MPE supplementation further increased the caspase-3 levels ($\approx$1.3-fold/1.2-fold compared to the testosterone-treated group). The results of this study indicate that lupeol/MPE supplementation significantly ($P < 0.05$) decreased the ratio of Bcl-2:Bax in Groups 3 and 4 (Fig. 8A). However, lupeol/MPE alone (Groups 5 and 6) had no significant ($P < 0.05$) effect on the level of Bcl-2, Bax, and caspase-3 activity (Fig. 8A) in mouse prostate due to their non-toxicity. In in vitro study, immunoblotting exhibited a significant decrease ($P < 0.01$; $\approx$1.8-fold compared to untreated control) in the expression of Bcl-2 (Fig. 8B) and increased ($\approx$1.6-fold and $\approx$1.5-fold compared to untreated control) expression of Bax and caspase-3 (Fig. 8B) when LNCaP cells were treated with 75 µM of lupeol for 48 h. In the LNCaP cells, lupeol supplementation showed a lower Bcl-2:Bax ratio, thus supporting our hypothesis that a shift in the Bcl-2:Bax ratio (Fig. 8B) and increased expression of caspase-3 activated the apoptotic pathway in lupeol treated cells.

DISCUSSION

Benign prostate hyperplasia (BPH) and PCA are considered problems of public health (35). The present study demonstrated that lupeol/MPE reduced prostate weight in adult male Swiss albino mice in which BPH has been induced by testosterone. Finasteride is an inhibitor of the enzyme 5-α-reductase 2. This enzyme converts testosterone to dihydrotestosterone, which is responsible for the proliferative action on prostate (36).
Finasteride is an elective drug for BPH but is associated with some side effects (37). Because of this, lupeol/MPE could become an important alternative for the treatment of BPH.

The Bcl-2 family proteins have emerged as critical regulators of the mitochondria-mediated apoptosis by functioning as either promoters (e.g., Bax) or inhibitors (e.g., Bcl-2) of the cell death process (38,39). Normal prostate cells do not express Bcl-2, whereas cancer cells resistant to hormone therapy show strong expression of Bcl-2 (40). Similar results were recorded in the present study in that Bcl-2:Bax ratio increased in testosterone-induced prostate tissue as well as in LNCaP cells. This indicates that it confers on the cancer the ability to grow without all of the factors normally associated with proliferation and require an increased ratio of Bcl-2:Bax. Bcl-2 generally expresses more strongly as malignant changes progress along the continuum from benign to preneoplastic to cancer (41,42). The supplementation of lupeol/MPE in mouse and LNCaP cells has resulted in an inhibition in proliferation and induction of apoptosis. The increase in MPE/lupeol-induced apoptosis was associated with an increase in the levels of Bax, which heterodimerizes with, and thereby inhibits, Bcl-2. Our results show that lupeol/MPE can regulate the Bcl-2:Bax ratio and therefore lead to induction of apoptosis in LNCaP cells and prostate tissue.

The study also confirms that the induction of apoptosis in lupeol/MPE-treated mouse prostate and in LNCaP cells is consistent with the view that lupeol/MPE possess an altering capacity of apoptosis machinery. Indeed, treatment with lupeol/MPE caused an induction of caspase-3, associated with the fragmentation of nuclear DNA, which preceded the onset of apoptosis in LNCaP and hyperplastic mouse prostate cells.

ROS have been shown to be mediators, triggers, or executioners of essential protective mechanisms such as apoptosis, phagocytosis, and detoxification reactions (43,44). Antioxidants present in fruits and vegetables reduced ROS level, restored mitochondrial membrane potential, and reduced tissue oxidative damage (45). Mitochondrial transmembrane potential is often employed as an indicator of cellular viability, and its disruption has been implicated in a variety of apoptosis phenomena (46). In the present study, lupeol/MPE resulted in loss of mitochondrial membrane potential both in vivo and in vitro, which can ultimately lead to apoptosis.

Taken together, the present study has demonstrated that lupeol/MPE has dual effects, that is, to prevent development of cancer and to eliminate cancer cells through induction of apoptosis. It is clear, however, that the central mechanism of apoptosis is evolutionarily conserved and that the Bcl2:Bax ratio and caspase activation is an essential step in this complex apoptotic pathway. The data also implies that antioxidant enzymes and mitochondrial cell death pathway can be used as targets for studies on prevention of different types of cancer and that lupeol/MPE merits further investigations for developing strategies for chemoprevention.

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