Original Article Open Access

Archives Of Breast Cancer

DOI: 10.32768/abc.2024114408-417

Effects of Lupeol on Estrogen and Androgen Receptor-Positive Breast and Prostate Cancer Cells

Mahdieh Nezami Majd[a], Arash Alizadeh*[b], Elham Zadeh Hashem[c]

[a]*DVM Graduate Student, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*

[b]*Division of Pharmacology and Toxicology, Department of Basic Science, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*

[c]*Division of Pharmacology and Toxicology, Department of Basic Science, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*

ARTICLE INFO

Received:
23 July 2024
Revised:
21 September 2024
Accepted:
24 September 2024

Keywords:
Lupeol, MCF-7, LNCaP, Estrogen receptors, Androgen receptor, Antioxidant capacity

ABSTRACT

Background: Different reports have shown that prostate and breast cancers are the most common cancers worldwide. Lupeol, a dietary triterpene, provides various beneficial effects including anti-cancer properties. The current study aims to investigate the anti-proliferative and antioxidant effects of lupeol, in line with the effects of lupeol on the expression of estrogen and androgen receptors in breast (MCF-7) and prostate (LNCaP) cancer cell lines.

Methods: MCF-7 and LNCaP cells were incubated with increasing concentrations of the lupeol (1, 10, and 100 µM) for 24h. The cytotoxicity of the lupeol was assessed by MTT and Neutral Red assays. Moreover, TAC (total antioxidant capacity), and gene expression of androgen and estrogen receptors were measured by spectrophotometric and qPCR methods, respectively. Overall, 17 beta-estradiol (E2) (9 nM) and dehydroepiandrosterone (DHEA) (5 µM) were selected as positive controls.

Result: The highest concentration of the lupeol induced cytotoxic effects on MCF-7 and LNCaP cells. Various levels of lupeol at specified time intervals increased TAC levels in comparison with the control group. Moreover, the expression levels of estrogen receptors (α and β) and androgen receptors were negatively affected by lupeol.

Conclusion: The findings of our study indicate that lupeol could serve as a promising, and accessible multi-functional anti-tumor agent against hormone-positive breast and prostate cancers.

INTRODUCTION

Epidemiological data suggested that triterpene-enriched diets could show beneficial effects on sex hormone-dependent cancers including breast, prostate, ovarian and endometrium cancers.[1] Lupeol (Lpl) is a multi-source natural triterpene gaining more and more attention nowadays, due to its beneficial effects on different conditions such as infectious diseases, renal, cardiovascular, and inflammatory disorders, diabetes, hepatic toxicity, microbial arthritis, and cancer.[2] Cancer, as one of the most aggressive diseases and the second cause of mortality, is a hyperproliferative disorder that involves various circumstances including transformation, dysregulation of apoptosis, proliferation, invasion, angiogenesis, and metastasis.[3,4]

***Address for correspondence:**
Arash Alizadeh,
Division of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Urmia University, Urmia, West Azerbaijan, Iran. P.O. Box 1177.
Email: dr.arash.alizadeh@gmail.com

It has been revealed that Lpl can act as a sensitizer and chemotherapeutic agent in combination with conventional anti-neoplastic medicine through its modulatory effects on pivotal signaling pathways in cancer etiology and progression such as the PI3K/AKT/mTOR and nuclear factor kappa B (NF-κB), downregulation of Bcl-2 and Bcl-Xl, MMP-9 as well as upregulation of caspase-3 that leads to apoptosis of apoptosis.[5–7] Lupeol has the potency to induce G2/M cell cycle arrest by inhibiting the cyclin-regulated signaling pathway in cancer cells.[8] The structural similarity of Lupeol with androgenic hormones makes it a potential candidate for modulating androgen receptor signaling cascade.[9] Previous investigations indicated different characteristics for lupeol including inhibition of cell migration, decrease of cell proliferation, and induction of apoptosis.[10] In addition, other mechanisms such as chemosensitization of tumor cells and inhibition of androgen receptor have also been reported as possible mechanisms of action for lupeol.[10]

The estrogen effects are associated with binding to the ERs: estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) while the stimulation or inhibition of the underlined signaling pathway in the target organ depends on the balance between ERα and ERβ activities.[13] Based on previous investigations, ERα is a transcriptional factor expressed in more than 70 percent of breast cancer patients and induces the proliferation of breast cancer cells,[14] but there is limited information about the role of ERβ in the treatment and biology of breast cancer.[15]

Concerning prostate cancer, epidemiological studies have shown that the incidence and mortality of prostate cancer are among the top five important malignancies on a worldwide scale.[16] Due to the fact that androgen receptor plays an important role in the progression of prostate cancer; therefore, successful treatment can be achieved by the modulation of the androgen receptor and associated pathways.[17]

Various limitations have been documented concerning the standard therapeutical protocols in cancer therapy such as surgery, chemotherapy, radiation, immune therapy, targeted therapy, and hormone therapy.[18]

Herbal medicines and nature-based medicinal approaches have provided a significant opportunity for the improvement of the applied treatment protocols because of broader beneficial effects with limited side effects in the prevention and treatment of cancer.[19,20]

Therefore, the purpose of this study was to investigate the anti-proliferation, antioxidant, and pro-apoptotic effects of lupeol on two distinct types of cancer cells (MCF-7 as the ER-positive human breast cancer cell line and LNCaP as the androgen-sensitive human prostate adenocarcinoma cell line). Moreover, the possible effect of lupeol on gene expression of estrogen and androgen receptor was evaluated to open new venues in cancer therapy and improve the quality of life in cancer patients.

METHODS

Cell culture

This is an invitro study on MCF-7 cells as the ER-positive human breast cancer cell line and LNCaP cells as the androgen-sensitive human prostate adenocarcinoma cell line were seeded in DMEM (Dulbecco's Modified Eagle Medium) and RPMI 1640 (Roswell Park Memorial Institute Medium) (Sigma-Aldrich, St. Louis, MO, USA), respectively. Both of the cell culture mediums were supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) penicillin-streptomycin (100IU/mL and 100µg/mL). The cells were kept in a 5% CO_2 and 37°C incubator as optimal conditions. The subculture of the cells was performed after reaching 70-80% confluency according to the standard protocols.[21] Cytotoxicity evaluations were conducted by 96 well plates with optimal density based on previous studies while the samples for qPCR analysis were prepared by seeding the cells in 6 well plates. Lupeol (L 5632) was purchased from Sigma (Sigma-Aldrich, St. Louis, MO, USA) and the stock solution was prepared with dimethyl sulfoxide (DMSO, Merck, Germany) while the final concentrations of Lpl (1, 10 and 100µM) were used as experimental concentrations, and untreated cells were utilized as control cells (0.5% DMSO). Dehydroepiandrosterone (DHEA, D 4000) 5µM and 17 beta-estradiol (E2) 9nM (Sigma-Aldrich, St. Louis, MO, USA) were utilized as associated controls and the stock solutions were prepared in DMSO.

Cytotoxicity assays (MTT)

Cell viability was quantified by the colorimetric MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. MTT assay is based on the assessment of the capacity of living cells to reduce the yellow water-soluble substrate tetrazolium salt into a purple water-insoluble formazan product, which is considered as an indicator of cell viability (22). MCF-7 and LNCaP cells were treated in 96-well plates (5×10^3 cells/well) with DMEM and RPMI culture medium, respectively. To this end, the cells were exposed to different concentrations of Lpl (1, 10 and 100µM), DHEA (5µM) and 17β-estradiol (9nM) for 24, 48 and 72 h. All the procedures were conducted based on the protocol of previous studies.[23] Cell viability

percentage was calculated by the following formula (Equation 1):

$$\text{Cell viability (\%)} = \frac{Mean\ OD\ (sample)}{Mean\ OD\ (blank)} \times 100 \quad (1)$$

Neutral red (NRU) assay

Neutral red is a vital dye, which is preferentially absorbed and endocytosed by viable cells and internalized inside lysosome; therefore, it can be considered as an indicator of lysosome and cell integrity.[22] MCF-7 and LNCaP cells were treated in 96-well plates (5×10^3 cells/well) containing 100µl of DMEM and RPMI medium, respectively, by a concentration range of 1-100µM of Lpl, 5µM of DHEA and 9nM of E2 for 24, 48 and 72 hours. Then, 5µl of Neutral red solution (4mg/ml) was added to the cells for 3h based on the previous protocol. As the final step, the absorbance was recorded at 540nm.

TAC assay

Total antioxidant capacity (TAC) was assessed in the supernatant of MCF-7 and LNCaP cells with different concentrations of Lpl (1, 10 and 100 µM) at time points of 24, 48 and 72 hours by the method described by Koracevic, et al.[24] In brief, 490µL of PBS solution was added to 10µL of the sample. Additionally, sodium benzoate, acetic acid, Fe-EDTA and H_2O_2 were added to the tubes, respectively. The tubes were then immersed at 37 °C for 60 minutes, and finally, after adding the thiobarbituric acid solution and placing the tubes in boiling water (10 minutes), the absorption of the samples was read at 532nm. A suitable solution ($FeSO_4.7H_2O$) of Fe^{2+} and ascorbic acid was used as a blank and standard solution.

RNA isolation and cDNA synthesis

The RNA isolation was performed by the standard TRIZOL method.[25] The RNA amount was determined spectrophotometrically and RNA purity was determined by NanoDrop 2000 (Thermo Scientific, Waltham, MA, USA) with expected values between 1.8 and 2. The samples were stored at −70°C for cDNA synthesis. The 20µL reaction mixture containing l µL RNA, 10µL 2 × reaction buffers, 2 µL enzyme mix and 7 µL RNase-Free water were prepared according to the instructions of Pars Tools Company. The cycling protocol for the 20µL reaction mixture was 10 min at 25°C, followed by 60 min at 47°C, and 5 min at 85°C to terminate the reaction.

Real-Time Polymerase Chain Reaction

The PCR reaction was carried out in a total volume of 20 µl, containing PCR master mix10 µl, FWD and REV specific primers (each 1 µl) and cDNA as a template (1 µl) and nuclease free water (7 µl). PCR conditions were run as follows: denaturation at 95 °C for 10 min, 1 cycle, followed by 45 cycles at 95 °C for 20 seconds. The annealing temperature (45 °C to 65 °C) was 20 to 40 seconds while elongation was 72 °C for 30 seconds and 72 °C for 10 min. Data were analyzed using the ddCt method and expression values were normalized to the expression levels of the b-actin gene. Primer pairs for Real-Time PCR are depicted in Table 1.

Table 1. Pair of Real-time PCR primer.

Target genes	Primer sequences	
Androgen receptor	F: CTGGCTTCCGCAACTTACAC	R: TCATTCGGACACACTGGCT
ERα	F: TCCTGATGATTGGTCTCGTCT	R: TCTGGAAGAGAAGGAACCATATCC
ERß	F: GCTCAATTCCAGTATGTACC	R: GGACCACATTTTTGCACT
ß-actin	F: CTGGAACGGTGAAGGTGACA	R: TGGGGTGGCTTTTAGGATGG

Statistical analysis

For statistical analyses, mean and standard deviation of the measured parameters were calculated. All data are reported as the mean ± SD of triplicate experiments. The results were analyzed using Graph Pad Prism software (Version 8.02. GraphPad Software Inc. San Diego, California, USA). The comparisons between groups were made by analysis of variance (One-Way ANOVA) followed by the Bonferroni post-hoc test. The significant difference between the control and the treatment group is marked with an asterisk symbol (∗) in the results section. A P-value less than 0.05 was considered significant.

RESULTS

Cell viability

The MTT method was used to evaluate the effects of Lpl with increasing concentrations on breast (MCF-7) and prostate (LNCaP) cancer cell viability. The results of the MTT test showed that 100µM Lpl significantly reduced the cell viability starting from 24h incubation and lasting for 72h ($P<0.05$). It should be noted that the decrease in the survival of LNCaP cells with the highest concentration of Lpl was not statistically significant at 24h incubation period but longer exposure resulted in a significant decrease in cell viability. As depicted in Figure 1, the control treatments with DHEA and E2 have shown no effect on cell viability.

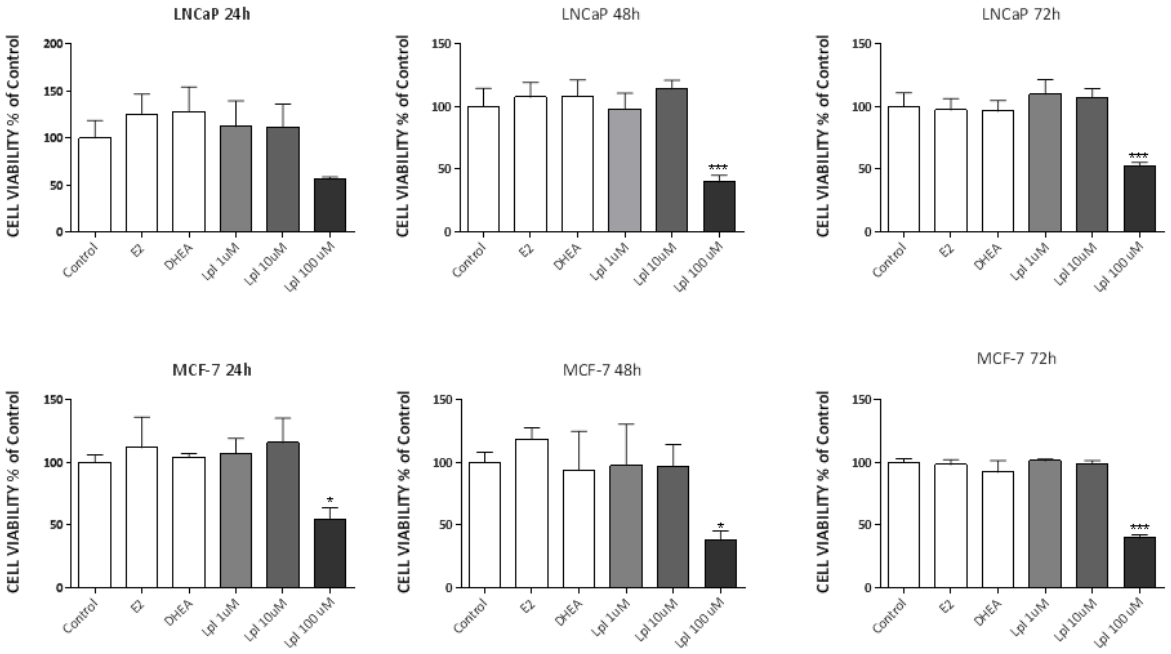

Figure 1. LNCaP and MCF-7 cells viability, exposed to different concentrations of Lpl (1, 10 and 100 µM) based on MTT test assay. $P \leq 0.01$*** and $P \leq 0.05$* were considered as significant changes in relation to control

Neutral red

NR method was used to evaluate the effects of Lpl with increasing concentrations on survival and Lysozyme activity of breast (MCF-7) and prostate (LNCaP) cancer cells. For this purpose, the cells were exposed to different concentrations of Lpl as well as single concentrations of E2 and DHEA for 24, 48 and 72 hours. As shown in Figure. 2, the highest concentration of Lpl in MCF-7 cells after 24 hours showed a significant effect ($P<0.05$).

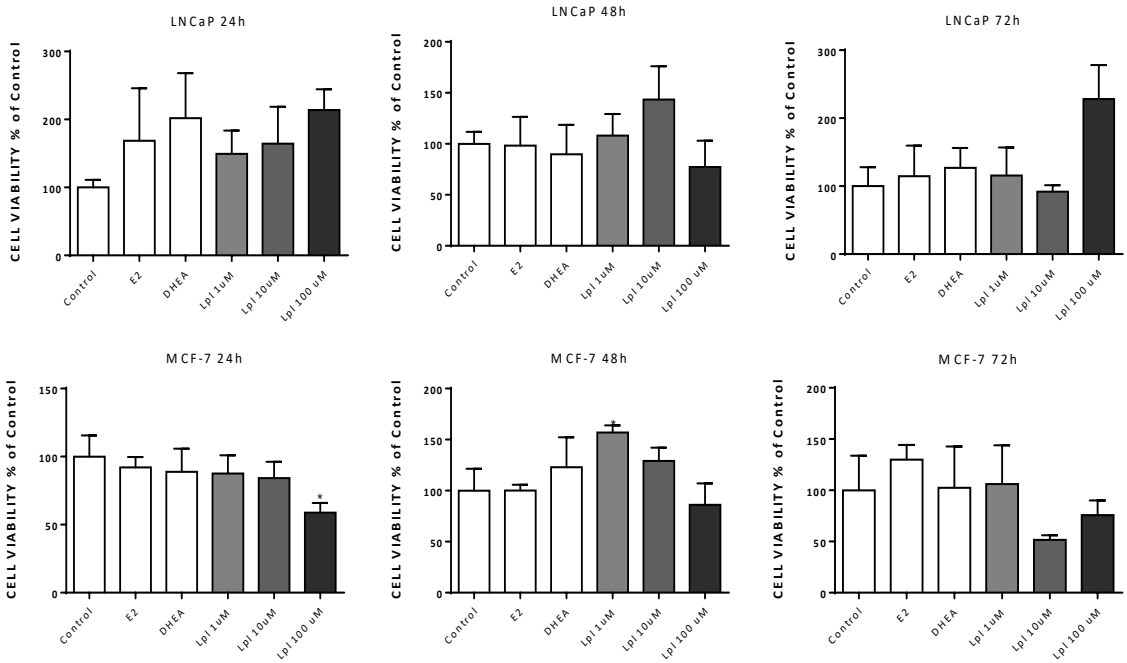

Figure 1. LNCaP and MCF-7 lysosomal enzymes activity exposed to different concentrations of Lpl (1, 10 and 100 µM) according to neutral red assay. $P \leq 0.05$* considered as significant changes in relation to control

Cell morphology of LNCaP cells

As shown in Figure. 3, the morphology of LNCaP cells following 24h incubation by E2, DHEA, and various concentrations of Lpl shows significant changes in the highest Lpl concentration.

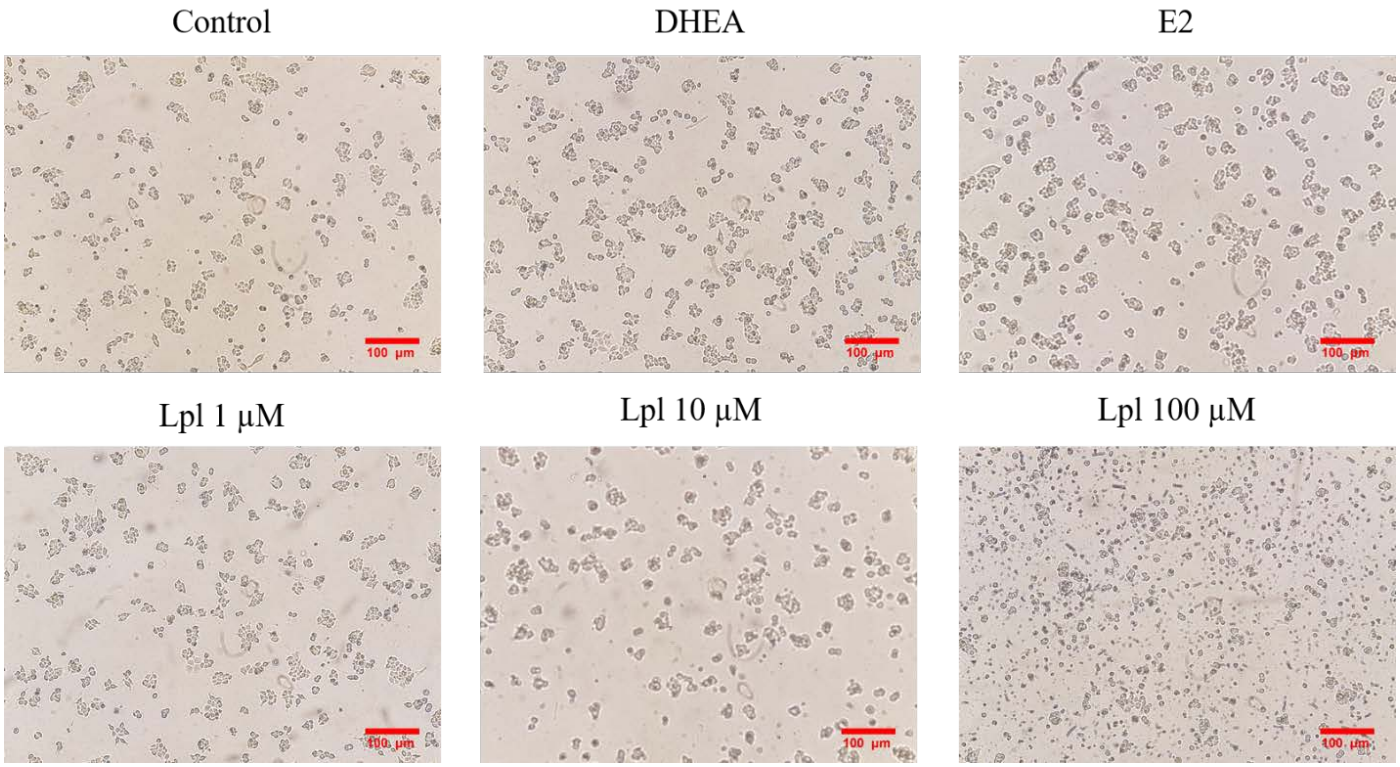

Figure 3. Morphology of LNCaP cells exposed to different concentrations of lupeol

Cell morphology of MCF-7 cells

As shown in Figure 4, the morphology of MCF-7 cells following 24h incubation by E2, DHEA and different concentrations of Lpl shows significant changes in the DHEA group and the highest Lpl concentration.

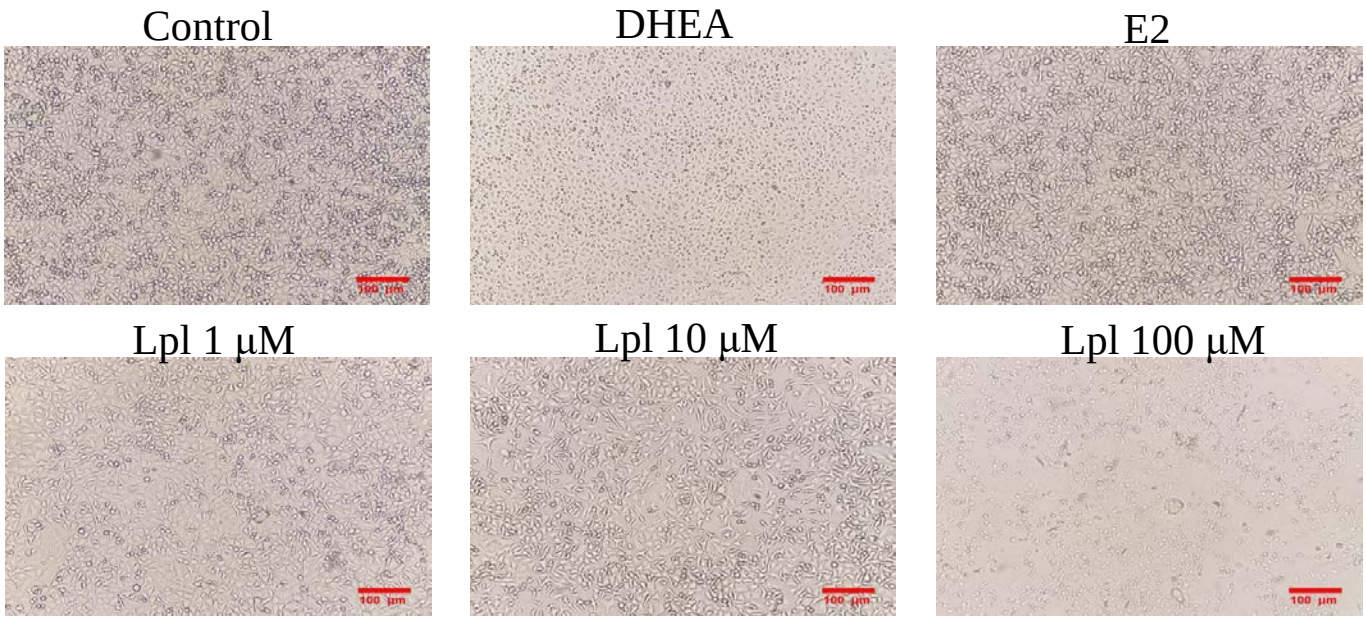

Figure 4. Morphology of MCF-7 cells exposed to different concentrations of lupeol

TAC

Table 2 shows the results of the TAC assay in MCF-7 and LNCaP cells. Compared to the control group, 1 µM and 10 µM of Lpl after 24, 48, and 72h and 100 µM of Lpl after 48h, in MCF-7 cells, and 1 µM and 10 µM of Lpl after 48 and 72h and 100 µM of Lpl after 72h, in LNCaP cells increased TAC level (The mean OD was measured at 532 nm using a spectrophotometer). Both E2 and DHEA increased TAC compared to the control group in 48 and 72h of treatments ($P<0.05$).

Table 2. Total antioxidant capacity in different study groups at 24, 48, 72 hours

Time	24h		48h		72h	
Groups	MCF-7	LNCaP	MCF-7	LNCaP	MCF-7	LNCaP
Control	0.376 ± 0.019	0.557 ± 0.073	0.188 ± 0.010	0.319 ± 0.019	0.471 ± 0.016	0.345 ± 0.008
E2	0.336 ± 0.016	0.693 ± 0.004	0.860^{***} ± 0.077	0.546^{***} ± 0.017	0.822^{***} ± 0.051	0.900^{***} ± 0.038
DHEA	0.458^{***} ± 0.017	0.584 ± 0.109	0.533^{***} ± 0.013	0.439^{*} ± 0.021	0.851^{***} ± 0.043	0.804^{***} ± 0.091
Lpl 1µM	0.508^{***} ± 0.004	0.614 ± 0.094	0.695^{***} ± 0.012	0.456^{**} ± 0.015	0.875^{***} ± 0.022	0.867^{***} ± 0.055
Lpl 10µM	0.478^{***} ± 0.010	0.743^{*} ± 0.014	0.611^{***} ± 0.025	0.513^{***} ± 0.037	0.744^{***} ± 0.077	0.898^{***} ± 0.008
Lpl 100µM	0.307 ± 0.003	0.544 ± 0.080	0.463^{***} ± 0.022	0.373 ± 0.077	0.418 ± 0.071	0.515^{**} ± 0.060

LNCaP and MCF-7 cells exposed to different concentrations of Lpl (1, 10, 100 µM) and the total antioxidant capacity evaluated in the supernatant of the treated cells. P ≤ 0/05* P ≤ 0/01 **and P ≤ 0/001*** considered as significant changes compared to control

qPCR

qPCR analysis was used to evaluate the gene expression levels of the androgen receptor and alpha and beta estrogen receptors, As shown in Figure 5, the expression of alpha estrogen receptor genes at concentrations of 10 µM and 100 µM of Lpl was significantly reduced compared to the control group, but the decreasing effects of beta estrogen receptor gene expression were observed at 1 µM and 10 µM Lpl.

Figure 5. Effect of lupeol on gene expression of alpha and beta estrogen receptors relative to the ß-actin reference gene in MCF-7 cells. P ≤ 0/05 *, P ≤ 0/01** and P ≤ 0/001*** were considered as significant in relation to control.

It should be noted that DHEA (5µM) induced a decrease in gene expression levels of both receptors compared to the control group (P < 0.05).

As shown in Figure 6, the gene expression of androgen receptor at concentrations of 1 µM and 10 µM of Lpl was significantly reduced compared to the control group. However, DHEA showed decreasing effects and E2 showed increasing effects on the gene expression of the androgen receptor compared to the control group.

DISCUSSION

Breast and prostate cancers as hormone-dependent tumors are most common malignancies in women and men, respectively.[26,27] In men and women, increased levels of androgens/estrogens and mutations in their receptors have been shown to increase the risk of prostate and breast hormone positive (HR^{+}) cancers, respectively.[28,29]

The current study was set up to investigate the cytotoxic effects of Lpl on MCF-7 cells as a human breast cancer cellular model with estrogen receptors, and LNCaP cells as an androgen-sensitive human prostate adenocarcinoma cellular model.

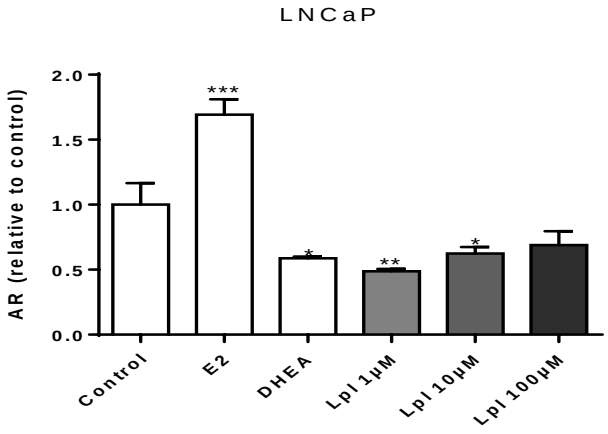

Figure 6. Effect of Lpl on gene expression of androgen receptor relative to the ß-actin reference gene in LNCaP cells P≤0/05 * P≤0/01 **and P≤0/001*** were considered as significant compared to control.

Then, the effects of Lpl on estrogen and androgen receptor expressions and the antioxidant capacity of Lpl-exposed cells were evaluated to shed more light on the potential beneficial effects of Lpl.

The obtained results from MTT assay showed that Lpl can reduce cell viability at the highest concentration (100 µM) MCF-7 and LNCaP cell lines after 24, 48 and 72h. These results are in line with findings from a study by Pitchai *et al.*[7] which showed that lupeol isolated from *Elephantopus scaber* plant has the ability to reduce the viability of MCF-7 cells with IC_{50} value of 80µM.

The results from the NR assay in the current study were not identical in association with time and concentrations. Results from a previous study that investigated the effect of betulinic acid (BA) and oleanolic acid (OA) as triterpenoids on the function and morphology of mitochondria and lysosomes in human skin keratinocyte (HaCaT) cells showed that despite the structural similarity of these triterpenoid compounds, betulinic acid was capable of damaging lysosomal and mitochondrial membranes but oleanolic acid was not capable of this function. This different function of these triterpenoids that was observed in this study can be related to the specific structure–activity relationships of these two compounds.[30] It seems that the chemical structure of lupeol as a triterpenoid compound can be an important factor concerning the NR results.

The current investigation demonstrated that Lpl has the ability to considerably alter the total antioxidant capacity (TAC) in time- and dose-dependent manner in MCF-7 and LNCaP cell lines, so that in 1 and 10µM concentrations it increases the TAC but decreases the TAC at 100 µM concentration. Analysis of the ethanolic extraction of *Ficus pseudopalma* plant showed that the plant contains significant amounts of Lpl with antioxidant properties.[31] It has been reported that antioxidant capacity of Lpl can be related to the free radicals/ROS species-scavenging activity and lipid peroxidation inhibitory effects of Lpl addressed by studies utilizing DPPH and ABTS assays.[32,33] Moreover, DHEA and E2 in the present study which were used to affect androgen and estrogen receptors also showed the ability to increase total antioxidant capacity (TAC) levels in sample supernatants at time points of 48 and 72 hours. The results are in line with previous studies confirming the ability of DHEA and E_2 to inhibit oxidative-stress responses and modulate the redox balance.[34,35]

A study by Ding *et al.*[36] showed that pretreatment of Leydig cells isolated from rats with DHEA could reduce oxidative stress and DNA injury induced by hydrogen peroxide (H_2O_2). In another study using mouse decidual endometrial stromal cells (ESCs), DHEA showed the potency to reduce intracellular reactive oxygen species (ROS) levels in dose-dependent manner.[34] Considering the ameliorative effects of E2 on TAC levels in sample supernatants, the results of the current study are in agreement with the results from investigations using E2 in colon epithelial cells (CCD841CoN cells), where E_2 at a dose of 8 nM could induce antioxidant enzymes including heme oxygenase-1 (HO-1) and NAD(P)H-quinone oxidoreductase-1 (NQO-1).[37] It should be noted that E_2 has the key role in augmentation of antioxidant capacity via activation of the Nrf_2 signaling pathway.[38]

In general, similar to drugs such as tamoxifen, phytoestrogenic bioactive compounds may act as selective estrogen receptor modulators (SERMs), exhibiting estrogen agonist or antagonist effects in a dose and target organ tissue-dependent manner through changes in the expression of particular co-activators or co-repressors of ERα and ERβ activity.[39,40] In this regard, a study by Thongon *et al.*[41] indicated that phytoestrogenic extract obtained from *Curcuma comosa* plant had estrogenic activity at low doses (0.1-1 µM) and anti-estrogenic activity at high doses (10-50 µM) on HEK-293T cells while showing antagonistic effects on estrogen receptors in MCF-7 cells. In an *in vitro* study, results of gene expression analysis indicated that lupeol could induce the expression of endogenous estrogen receptor at a concentration of 1µM in a way that the effects on ERβ were more pronounced than those on ERα, However, lupeol alone (10^{-9} and 10^{-8}µM) has been shown to serve as an antagonist in HEK293T-Erα.[42] Interestingly, co-administration of lupeol with an estrogen receptor antagonist (fulvestrant)) resulted in the lupeol-effect as an estrogen agonist in the tissue of vagina.[38]

The results of the present study showed that Lpl at high concentrations (10 and 100µM) has a decreasing effect on ERα expression levels, which is in line with previous reports.[42] It is known that DHEA and metabolites could act as ligands for estrogen and androgen receptor.[43] On the other hand, based on the function of aromatase enzymes, androgens can be converted to estrogens, which can also affect estrogen receptors. Therefore, under certain circumstances they can produce more estrogens (E2) as well as estrogenic effects.[44]

In the present study, the gene expression analysis showed that Lpl acts as an anti-androgen on androgen receptor. Based on the structural similarity of lupeol with androgens and its interactions with androgen receptor signaling function through competitive antagonism and reducing the expression level of prostate-specific antigen (PSA) as an androgen

receptor target, it can be concluded that a decrease in androgen receptor transcription is inevitable.[9,45]

The microscopic images of MCF-7 and LNCaP cells demonstrated that the highest concentration of Lpl (100µM) after 24 hours damages the cells. These results are in line with the findings of previous studies that have examined the cytotoxic effects of Lpl derived from different natural sources on different cell lines where the cytotoxic effects of Lpl on cancer cells were documented in changes in the expression of proteins involved in cell cycle (cyclin proteins and cyclin-dependent kinases), apoptosis, autophagy and epithelial–mesenchymal transition (EMT) processes.[46–48]

CONCLUSION

Based on the results obtained from this study, it can be concluded that lupeol has significant effects on the cell viability of ER-positive breast (MCF-7) and AR-positive prostate (LNCaP) cancer cells. Furthermore, lupeol exerted inhibitory effects on the expression levels of androgen and estrogen receptors, which might be an important factor in development of cancer cells. In addition to these effects, evaluating the TAC induced by lupeol can account for complementary beneficial effects of lupeol in cancer patients. The findings of our study indicate that lupeol could serve as a promising, and accessible multi-functional anti-tumor agent against hormone-positive breast and prostate cancers. Further studies are warranted to unravel the detailed mechanism of action behind beneficial effects of lupeol.

ACKNOWLEDGMENTS

The Research Deputy of Urmia University funded the present experiment. We are grateful to Dr. Hassan Malekinezhad for his kind assistance during the experiment.

CONFLICT OF INTEREST

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript

ETHICAL CONSIDERATIONS

Not applicable.

FUNDING

No specific grant from funding agencies in the public, commercial, or not-for-profit sectors was received for this study.

DATA AVAILABILITY

The data used in the current study are available from the corresponding author upon reasonable request.

REFERENCES

1. Șoica C, Voicu M, Ghiulai R, Dehelean C, Racoviceanu R, Trandafirescu C, et al. Natural compounds in sex hormone-dependent cancers: The role of triterpenes as therapeutic agents. *Front Endocrinol (Lausanne).* 2021;11:612396. doi:10.3389/fendo.2020.612396.
2. Siddique HR, Saleem M. Beneficial health effects of lupeol triterpene: a review of preclinical studies. *Life Sci.* 2011;88(7–8):285–293. doi:10.1016/j.lfs.2010.11.020.
3. Hanahan D. Hallmarks of cancer: new dimensions. *Cancer Discov*. 2022;12(1):31–46. doi: 10.1158/2159-8290.CD-21-1059.
4. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA Cancer J Clin.* 2022;72(1):7–33. doi:10.3322/caac.21708.
5. Malekinejad F, Kheradmand F, Khadem-Ansari MH, Malekinejad H. Lupeol synergizes with doxorubicin to induce anti-proliferative and apoptotic effects on breast cancer cells. *DARU, J Pharm Sci*. 2022;30(1):103–115. doi:10.1007/s40199-022-00436-w.
6. Fatma H, Jameel M, Siddiqui AJ, Kuddus M, Buali NS, Bahrini I, et al. Chemotherapeutic Potential of Lupeol Against Cancer in Pre-Clinical Model: A Systematic Review and Meta-Analysis. *Phytomedicine*. 2024;155777. doi:10.1016/j.phymed.2024.155777.
7. Pitchai D, Roy A, Ignatius C. In vitro evaluation of anticancer potentials of lupeol isolated from Elephantopus scaber L. on MCF-7 cell line. *J Adv Pharm Technol Res*. 2014;5(4):179–184. doi:10.4103/2231-4040.143037.
8. Prasad S, Nigam N, Kalra N, Shukla Y. Regulation of signaling pathways involved in lupeol induced inhibition of proliferation and induction of apoptosis in human prostate cancer cells. *Mol Carcinog*. 2008;47(12):916–224. doi:10.1002/mc.20442.
9. Siddique HR, Mishra SK, Karnes RJ, Saleem M. Lupeol, a novel androgen receptor inhibitor: implications in prostate cancer therapy. *Clin Cancer Res*. 2011;17(16):5379–5391. doi:10.1158/1078-0432.CCR-11-0916.
10. Liu K, Zhang X, Xie L, Deng M, Chen H, Song J, et al. Lupeol and its derivatives as anticancer and anti-inflammatory agents: Molecular mechanisms and therapeutic efficacy. *Pharmacol Res.* 2021;164:105373. doi:10.1016/j.phrs.2020.105373.
11. Mennini FS, Aurilio MT, Gazzillo S, Nardone C, Sciattella P, Marcellusi A, et al. An analysis of the social and economic costs of breast cancer in Italy. *Int J Environ Res Public Health*. 2021;18(17). doi:10.3390/ijerph18179005.
12. Sorensen S V., Goh JW, Pan F, Chen C, Yardley D, Martín M, et al. Incidence-based cost-of-illness model

for metastatic breast cancer in the United States. *Int J Technol Assess Health Care*. 2012;28(1):12–21. doi:10.1017/S026646231100064X.
13. Welboren W-J, Sweep FCGJ, Span PN, Stunnenberg HG. Genomic actions of estrogen receptor?: what are the targets and how are they regulated? *Endocr Relat Cancer*. 2009;16(4):1073. doi:10.1677/ERC-09-0086.
14. Clusan L, Le Goff P, Flouriot G, Pakdel F. A closer look at estrogen receptor mutations in breast cancer and their implications for estrogen and antiestrogen responses. *Int J Mol Sci*. 2021;22(2):756. doi:10.3390/ijms22020756.
15. Gruvberger S. Estrogen receptor alpha and beta in breast cancer-gene expression profiles and clinical implications. *Department of Oncology, Clinical Sciences, Lund University*; 2005. 96 p. doi:10.1016/j.ejso.2004.02.010.
16. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71(3):209–249. doi:10.3322/caac.21660.
17. Kim TJ, Lee YH, Koo KC. Current status and future perspectives of androgen receptor inhibition therapy for prostate cancer: a comprehensive review. *Biomolecules*. 2021;11(4):492. doi:10.3390/biom11040492.
18. Amini P, Moazamiyanfar R, Dakkali MS, Khani A, Jafarzadeh E, Mouludi K, et al. Resveratrol in cancer therapy: from stimulation of genomic stability to adjuvant cancer therapy: a comprehensive review. *Curr Top Med Chem*. 2023;23(8):629–648. doi:10.2174/1568026623666221014152759.
19. Ahmed M, Khan MI, Khan MR, Muhammad N, Khan AU, Khan RA. Role of medicinal plants in oxidative stress and cancer. *Sci Rep*. 2013;2(1):641–644. doi:10.4172/scientificreports.641.
20. Amini P, Moazamiyanfar R, Dakkali MS, Jafarzadeh E, Ganjizadeh M, Rastegar-Pouyani N, et al. Induction of cancer cell death by apigenin: a review on different cell death pathways. *Mini Rev Med Chem*. 2023;23(14):1461–1478. doi:10.2174/1389557523666230119110744.
21. ECACC. Fundamental Techniques in Cell Culture. SIGMA Lab. 2008;1–61.
22. Kamiloğlu Beştepe S, Sarı G, Özdal T, Çapanoğlu Güven E. Guidelines for cell viability assays. *FOOD Front*. 2020;1(3). doi:10.1002/fft2.44.
23. Varasteh S, Braber S, Kraneveld AD, Garssen J, Fink-Gremmels J. l-Arginine supplementation prevents intestinal epithelial barrier breakdown under heat stress conditions by promoting nitric oxide synthesis. *Nutr Res*. 2018 Sep;57:45–55. doi:10.1016/j.nutres.2018.05.007.
24. Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. Method for the measurement of antioxidant activity in human fluids. *J Clin Pathol*. 2001;54(5):356–61. doi:10.1136/jcp.54.5.356.
25. Chomczynski P, Sacchi N. The single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction: twenty-something years on. *Nat Protoc*. 2006;1(2):581–585. doi:10.1038/nprot.2006.83.
26. Harbeck N, Penault-Llorca F, Cortes J, Gnant M, Houssami N, Poortmans P, et al. Breast cancer. Vol. 5, *Nature Reviews Disease Primers*. 2019. doi:10.1038/s41572-019-0111-2.
27. Lawhn-Heath C, Salavati A, Behr SC, Rowe SP, Calais J, Fendler WP, et al. Prostate-specific Membrane Antigen PET in Prostate Cancer. *Radiology*. 2021 May;299(2):248–260. doi:10.1148/radiol.2021202771.
28. Barbieri CE, Tomlins SA. The prostate cancer genome: Perspectives and potential. *Urol Oncol Semin Orig Investig*. 2014;32(1):53.e15-53.e22. doi:10.1016/j.urolonc.2013.08.025.
29. Sloan M, Cancer K, City NY, City NY. Estrogen Receptor-Positive Breast Cancer : Exploiting Signaling Pathways Implicated in Endocrine Resistance. 2018;528–539. doi:10.1634/theoncologist.2017-0423.
30. Martins WK, Costa ÉT, Cruz MC, Stolf BS, Miotto R, Cordeiro RM, et al. Parallel damage in mitochondrial and lysosomal compartments promotes efficient cell death with autophagy : The case of the pentacyclic triterpenoids. *Nat Publ Gr*. 2015;(June):1–17. doi:10.1038/srep12425.
31. Santiago LA, Mayor ABR. L upeol : A n antioxidant triterpene in F icus pseudopalma B lanco. *Asian Pac J Trop Biomed*. 2014;4(2):109–118. doi:10.1016/S2221-1691(14)60218-5.
32. Extract L, Crateva OF, Oliv A, Tchimene MK, Nwaehujor CO, Ezenwali M, et al. Free Radical Scavenging Activity of Lupeol Isolated from the Methanol Leaf Extract of Crateva adansonii Oliv . (Capparidaceae). *International Journal of Pharmacognosy and Phytochemical Research*. 2016; 8(3); 419-426.
33. Pérez-González MZ, Nieto-Trujillo A, Gutiérrez-Rebolledo GA, García-Martínez I, Estrada-Zúñiga ME, Bernabé-Antonio A, et al. Lupeol acetate production and antioxidant activity of a cell suspension culture from Cnidoscolus chayamansa leaves. *South African J Bot*. 2019;125:30–38. doi:10.1016/j.sajb.2019.06.030.
34. Qin A, Qin J, Jin Y, Xie W, Fan L, Jiang L, et al. DHEA improves the antioxidant capacity of endometrial stromal cells and improves endometrium receptivity via androgen receptor. *Eur J Obstet Gynecol Reprod Biol*. 2016;198:120–126. doi:10.1016/j.ejogrb.2016.01.016 .
35. Jin LY, Lv ZD, Wang K, Qian L, Song XX, Li XF, et al. Estradiol alleviates intervertebral disc degeneration through modulating the antioxidant enzymes and inhibiting autophagy in the model of menopause rats. *Oxid Med Cell Longev*. 2018;2018:7890291. doi:.org/10.1155/2018/7890291.
36. Ding X, Yu L, Ge C, Ma H. Protective effect of DHEA on hydrogen peroxide-induced oxidative damage and apoptosis in primary rat Leydig cells. 2017;8(10):16158–16169. doi:10.18632%2Foncotarget.15300.
37. Son HJ, Kim N, Song C, Lee SM, Lee H, Surh Y. 17 β -Estradiol reduces inflammation and modulates antioxidant enzymes in colonic epithelial cells. *The Korean Journal of Internal Medicine*. 2020 Mar;35(2):310. doi:10.3904/kjim.2018.098.

38. Gorrini C, Gang BP, Bassi C, Wakeham A, Pegah S, Hao Z. Estrogen controls the survival of BRCA1-deficient cells via a PI3K – NRF2-regulated pathway. Proceedings of the National Academy of Sciences. 2014 Mar 25;111(12):4472-4477. doi:10.1073/pnas.1324136111.
39. Abiramasundari G, Mohan Gowda CM, Sreepriya M. Selective Estrogen Receptor Modulator and prostimulatory effects of phytoestrogen β-ecdysone in Tinospora cordifolia on osteoblast cells. *J Ayurveda Integr Med*. 2018;9(3):161–168. doi:10.1016/j.jaim.2017.04.003.
40. Wardell SE, Nelson ER, McDonnell DP. From empirical to mechanism-based discovery of clinically useful Selective Estrogen Receptor Modulators (SERMs). *Steroids*. 2014;90:30–38. doi:10.1016/j.steroids.2014.07.013.
41. Thongon N, Boonmuen N, Suksen K, Wichit P, Chairoungdua A, Tuchinda P, et al. Selective Estrogen Receptor Modulator (SERM)-like Activities of Diarylheptanoid, a Phytoestrogen from Curcuma comosa, in Breast Cancer Cells, Pre-osteoblast Cells, and Rat Uterine Tissues. *J Agric Food Chem*. 2017;65(17):3490–3496. doi:abs/10.1021/acs.jafc.7b00769.
42. Zingue S, Ntsa DM, Magne Nde CB, Michel T, Ndinteh DT, Clyne C, et al. Lupeol, the major compound of the dichloromethane extract of Millettia macrophylla Benth (Fabaceae), displays estrogenic effects in ovariectomized rats. *Phyther Res.* 2019;33(4):949–957. doi:10.1002/ptr.6288.
43. Clark BJ, Prough RA, Klinge CM. Mechanisms of Action of Dehydroepiandrosterone. 1st ed. Vol. 108, Vitamins and Hormones. *Elsevier Inc*.; 2018. 29–73 p. doi:10.1016/bs.vh.2018.02.003.
44. Capellino S, Straub RH, Cutolo M. Aromatase and regulation of the estrogen-to-androgen ratio in synovial tissue inflammation : common pathway in both sexes. 2014;1317:24–31. doi:10.1111/nyas.12398.
45. Khan MA, Singh D, Fatma H, Akhtar K, Arjmand F, Maurya S, et al. Antiandrogen enzalutamide induced genetic, cellular, and hepatic damages: amelioration by triterpene Lupeol. *Drug Chem Toxicol*. 2022;1–12. doi:10.1080/01480545.2022.2040528
46. Bhatt M, Patel M, Adnan M, Reddy MN. Anti-metastatic effects of lupeol via the inhibition of MAPK/ERK pathway in lung cancer. *Anti-Cancer Agents Med Chem (Formerly Curr Med Chem Agents)*. 2021;21(2):201–206. doi:10.2174/1871520620666200424131548.
47. Saleem M, Murtaza I, Tarapore RS, Suh Y, Adhami VM, Johnson JJ, et al. Lupeol inhibits proliferation of human prostate cancer cells by targeting β-catenin signaling. *Carcinogenesis*. 2009;30(5):808–817. doi:10.1093/carcin/bgp044.
48. Zhang X, Gao Z, Chen K, Zhuo Q, Chen M, Wang J, et al. Lupeol inhibits the proliferation and migration of MDA-MB-231 breast cancer cells via a novel crosstalk mechanism between autophagy and the EMT. *Food Funct*. 2022;13(9):4967–4976. doi:10.1039/D2FO00483F.

How to Cite This Article

Nezami Majd M, Alizadeh A, Zadeh Hashem E.Effects of Lupeol on Estrogen and Androgen Receptor-Positive Breast and Prostate Cancer Cells. Arch Breast Cancer. 2024; 11(4):408-17.
Available from: https://www.archbreastcancer.com/index.php/abc/article/view/999