Original Article

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.apjtb.org

doi: 10.4103/2221-1691.281465

Impact Factor: 1.59

Piperlongumine inhibits cell growth and enhances TRAIL-induced apoptosis in prostate cancer cells

Gorkem Kismali[1]✉, Ahmet Ceylan[2], Ogunc Meral[1], Merve Alpay[1,3], Funda Kosova[4], Dilek Ulker Cakir[5], Begum Yurdakok-Dikmen[6], Neslihan Tascene[7], Tevhide Sel[1]

[1]*Ankara University, Faculty of Veterinary Medicine, Department of Biochemistry, Ankara, Turkey*

[2]*Ankara University, Faculty of Veterinary Medicine, Department of Histology and Embryology, Ankara, Turkey*

[3]*Duzce University, Faculty of Medicine, Department of Biochemistry, Duzce, Turkey*

[4]*Celal Bayar University, School of Health Services, Department of Biochemistry, Manisa,Turkey*

[5]*Canakkale Onsekiz Mart University, Faculty of Medicine, Department of Medical Biochemistry, Canakkale, Turkey*

[6]*Ankara University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Ankara, Turkey*

[7]*Republic of Turkey Ministry of Agriculture and Forestry, Ankara, Turkey*

ABSTRACT

Objective: To investigate whether piperlongumine can sensitize prostate cancer cells to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and trigger apoptosis in prostate cells.

Methods: Human prostate cancer cell lines PC3, LNCaP, and VCaP were cultured with piperlongumine and TRAIL. Then, cell proliferation, migration, caspase activation, apoptotic protein expressions, and death receptor expressions were measured.

Results: Piperlongumine inhibited cell proliferation at low doses (<10 μM) alone and in combination with TRAIL (25 ng/mL), induced apoptosis, and suppressed cyclooxygenase activation. Additionally, piperlongumine induced expression of death receptors which potentiated TRAIL-induced apoptosis in cancer cells but did not affect decoy receptors. Piperlongumine also downregulated tumor cell-survival pathways, inhibited colony formation and migration of cancer cells alone or in combination with TRAIL. The combination of piperlongumine with TRAIL was found to be synergistic.

Conclusions: Our findings indicate that piperlongumine can sensitize cancer cells to TRAIL through the upregulation of death receptors and can trigger apoptosis with the downregulation of anti-apoptotic proteins.

KEYWORDS: Piperlongumine; Prostate cancer; Apoptosis; Tumor necrosis factor-related apoptosis-inducing ligand

1. Introduction

As one of the most common cancers in males, more than 1.2 million new cases of prostate cancer occur worldwide. It is aggressive and could metastasize rapidly. Prostate cancer is a major health problem in the western world due to its high incidence[1]. The high mortality is partly attributed to unspecific symptoms which are frequently misdiagnosed[2]. Age is the most important risk factor for prostate cancer development. Prostate cancer shows the steepest age-incidence curve among all cancers in unscreened populations with a rapid rise in the seventh decade. Before the age of 65, only 25% of patients with prostate cancer in Europe are diagnosed[3]. Various forms of vitamin E tend to have different effects on prostate cancer, with alpha-tocopherol potentially increasing and gamma-tocopherol potentially reducing the risk of prostate cancer. There is no convincing evidence of a beneficial effect on selenium, vitamin C, or beta-carotene, while

✉To whom correspondence may be addressed. E-mail: gorkemkismali@yahoo.com, gorkemkismali@ankara.edu.tr

How to cite this article: Kismali G, Ceylan A, Meral O, Alpay M, Kosova F, Cakir DU, et al. Piperlongumine inhibits cell growth and enhances TRAIL-induced apoptosis in prostate cancer cells. Asian Pac J Trop Biomed 2020; 10(5): 216-223.

Article history: Received 18 May 2019; Revision 23 July 2019; Accepted 20 February 2020; Available online 6 April 2020

lycopene appears to be negatively associated with the risk of disease[4]. A retrospective case-control trial showed that aspirin, the most effective drug, induced a small but significant reduction in disease incidence by about 10 percent[5]. However, many prostate cancer prevention studies are currently ongoing and must be completed before a full conclusion can be reached[6]. Androgen deprivation therapy (ADT) has been recently proven as the standard treatment for systemic prostate cancer. The growth of prostate cancer can be retarded effectively by ADT. However, once ADT is initiated, it should continue throughout patients' entire life. For elderly patients, the depletion of testosterone triggers a number of side effects. Even after discontinuation of ADT, testosterone level still can not return to the pre-ADT level[7].

Potential natural compounds, both in natural and synthetic forms, have been applied extensively to treatments of diseases. Many studies have revealed a wide range of bioactivities, chemical structures and analogues of such compounds showing the high potential in commerce. In recent years, natural molecules called phytochemicals have been extracted from several sources. They have been explored as potential therapies of cancer according to their effects, accessibility, long-term uses, and proficiencies in targeting cancer cell signaling pathways[8,9]. Phytochemicals commonly act as antioxidants due to their eliminating activity against free radicals. Many antioxidants derived from dietary and medicinal plants have been found to have the cellular antioxidant capacity or facilitate detoxification of carcinogens and other toxins. Phytochemicals also display prooxidant activity which may cause reactive oxygen species (ROS) production in the cell. The malignant transformation could make cells more sensitive than normal cells to ROS. Therefore, innovative ROS homeostasis therapy is a promising approach for cancer treatment like prostate cancer[10,11].

Piperlongumine (PL) is a lipid-soluble alkaloid amide isolated from the long pepper (*Piper longum* L.). *Piper longum* L. (Piperaceae) is a woody root in the aromatic region. *Piper longum* extract contains an alkaloid with anti-inflammatory, anti-angiogenic, anti-lipidemic, anti-fungal potency[12]. Raj *et al*. disclosed a natural compound, PL, which killed cancer cells of various origins in a highly specific manner[13]. It was reported that PL is a selective inducer of cancer cell death. Studies on the mode of action reveal PL could inhibit the potential of glutathione S-transferase P (GSTP1) as an anticancer agent, especially through the formation of ROS. The researchers suggest that PL leads to apoptotic cell death; this would be contrary to some other substances that generate ROS[13,14].

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a very well-known cytokine that induces apoptosis in many cancer cells through its interaction with death receptor 4 (DR4) and death receptor 5 (DR5)[15]. Even though TRAIL is a very selective and effective protein to trigger apoptosis, studies showed that repeated application of TRAIL-induced resistance through multiple pathways[16].

This study aimed to investigate the *in vitro* antitumor effects of PL on prostate cancer and to explore whether PL can modulate TRAIL-mediated apoptosis.

2. Materials and methods

2.1. Reagents

5,6-dihydro-1-[1-oxo-3-(3,4,5-trimethoxyphenyl)-*trans*-2-propenyl]-2(1H)-pyridinone (PL) was purchased from Sigma-Aldrich (SML0221-5MG). The stock solution was prepared in dimethylsulfoxide (150 mM) and kept at –20 ℃ as small aliquots and diluted as required in the media of cell culture. Soluble recombinant human TRAIL-Apo2L was purchased from Millipore (2707833).

2.2. Cell culture

Prostate cancer cell lines PC3 (ATCC® CRL-1435™), VCaP (ATCC® CRL-2876™) and LNCaP clone FGC (ATCC® CRL-1740™) were cultured to confluence at 37 ℃ in DMEM F12 (Sigma-Aldrich) basal media supplemented with 10% fetal calf serum in a 5% CO_2 atmosphere and 50 μg/mL gentamicin sulfate solution (Millipore). Cells were grown in 25 cm^2 or/and 75 cm^2 vented cap flasks (BD Falcon). The cells were subcultured at a 1:4 ratio with trypsin-EDTA (Sigma-Aldrich) solution and the media were changed every two days.

2.3. Cell viability assay

The effect of PL and TRAIL on the cytotoxicity of human prostate cancer cells was determined by the measurement of mitochondrial dehydrogenase activity using methyl thiazolyl tetrazolium (MTT) as a substrate. PC3, VCaP and LNCaP cells were grown in 96-well plates at a density of 5×10^5 cells/well. Cells were treated with either PL alone (30 μM, 15 μM, 7.5 μM, 3.75 μM, 1.9 μM, 0.93 μM, 0.47 μM and 0.23 μM) or PL+TRAIL (25 ng/mL) for 24 h. After incubation, cells were washed with fresh medium, 100 μL of MTT (5 mg/mL) was added into wells. Sodium dodecyl sulfate (10%, 100 μL) was used to solubilize the formazan salt formed after 4 hours of incubation and a microplate reader (Sunrise, Tecan GmbH, Austria) was used to quantify the amount of formazan salts at 570 nm. Each experiment was performed in triplicate.

2.4. Assessment of cell injury

Cell damage was quantified by analyzing the release from perturbed cells into the bathing medium of lactate dehydrogenase (LDH). PC3 cells (5×10^5 cells/well) were seeded in 96-well plates and incubated with PL (30 μM, 15 μM, 7.5 μM, 3.75 μM, 1.9 μM, 0.93 μM, 0.47 μM and 0.23 μM). Concentrations of supernatant LDH levels were measured using a colorimetric assay kit (TML Medical, Ankara, Turkey). An experiment was performed using a Clinical Chemistry Analyzer (ERBA XL 600, Meinheim, Germany). For each experiment, the complete release of LDH leading to maximum cell

death was determined. The experiment was conducted three times.

2.5. Wound healing assay

PC3 and LNCaP cells were maintained in DMEM F12 supplemented with 10% fetal calf serum, then trypsinized and seeded in 6-well tissue culture plates to obtain a density of ~70%-80% confluence following 24 hours of incubation. The monolayer cells underwent a scratch with a 1 mL pipette tip across the center of the well. Cells were gently washed with medium twice. Fresh medium was added with two concentrations of either PL (5 μM and 10 μM) alone or 5 μM PL + 25 ng/mL TRAIL and 10 μM PL + 25 ng/mL TRAIL combinations. Cells were incubated for 24 h under cell culture conditions. Cells were rinsed three times with 1×phosphate buffer (PBS) and fixed for 30 min with 3.7% paraformaldehyde, then stained with 1% crystal violet in 2% ethanol for 30 min. Gap sizes were measured with Leica Application Suit software. The area of the wound was measured for two independent experiment in every group and the percentage of the scratch area was compared with the control.

2.6. Clonogenic assay

PC3 cells were treated for 24 h with 1.5 μM, 3.5 μM and 10 μM PL. Since there was a significant effect observed in 3.5 μM PL, this dose was selected for combination treatment. Cells were treated with combination of PL+TRAIL (3.5 μM PL + 25 ng/mL TRAIL) as well. The medium was changed and allowed to form colonies. After 9 days, the colonies were stained with 0.3% crystal violet and the total number of colonies was counted. Each experiment was conducted in triplicate.

2.7. Western blot analysis

PC3 and LNCaP cells were treated with PL (3.5-30 μM). After treatment, PC3 and LNCaP cells were harvested with a cell scraper and washed 3 times with PBS. Using the cell lysis buffer, whole-cell lysates were collected (Bio-Rad Laboratories, USA). Total protein concentration was determined by using Pierce™ 660nm Protein Assay Reagent (Thermo, USA). The protein expressions of XIAP, survivin, Bcl-xL, Bax, DR4, DR5, DcR1 end DcR2 were determined by semi-quantitative Western blotting. β-actin was used as housekeeping control. The proteins were separated in 10% sodium dodecyl sulphate poly acrylamide gel electrophoresis. An equal amount (20 μg) of protein obtained from the whole-cell lysates was applied for each sample. Following electrophoresis, the proteins were transferred to nitrocellulose membrane. The membranes were blocked with 5% dry-milk in TBS-Tween 20 and probed with primary antibodies. Biotin-xx anti-mouse IgG or Biotin-xx anti-rabbit (Invitrogen, NY, USA) was used as a secondary antibody. Gel Logic 200 Imaging System (Kodak, NY, USA) was used to scan the nitrocellulose membranes (Kodak, NY, USA).

2.8. Cyclooxygenase (COX)-2 analysis

PC3 and LNCaP cells were plated in 75 cm^2 flasks, they were treated with fresh media containing low, intermediate and high (5 μM, 10 μM and 15 μM) concentrations of PL for 24 h. Cells were harvested with lysis buffer. COX-2 levels in cell lysates were measured with the ELISA method using the Pierce 660 nm Protein Assay Kit. and equalized at 300 μg of protein (Cell Signaling, USA).

2.9. Determination of cleaved caspase-3 and caspase 9

PC3 cells were treated with PL alone (5 μM, 10 μM and 15 μM) or with TRAIL (25 ng/mL) for 24 h. Whole-cell extracts were prepared with RIPA lysis buffer (Thermo Scientific, USA) and analyzed for caspase 3 and caspase 9. PathScan® Cleaved Caspase-3 (Asp175) Sandwich ELISA Kit (Cell Signaling, USA) was used to determine cleaved caspase-3 levels. Solid-phase enzyme-linked immunosorbent assay (ELISA) was used to measure endogenous levels of cleaved caspase-3 protein according to product protocol. Caspase 9 activity was determined by the chromogenic method using Caspase-9 Colorimetric Activity Assay Kits (Millipore, USA) according to product protocols.

2.10. DAPI staining

PC3 cells were treated with PL alone (10 μM) and combination with TRAIL (25 ng/mL) for 24 h, washed with ice-cold PBS and fixed through incubation with 4% formaldehyde for 10 min at room temperature. Following incubation with Triton X-100 (0.1% PBS) for 5 min, cells were incubated with 4',6-diamidino-2-phenylindole (DAPI) for 10 min at room temperature and observed under a fluorescence microscope (Leica DMI 4000, Germany).

2.11. Data analysis

GraphPad Prism Software (Inc., La Jolla, CA, USA) was used. All measurement data with normal distribution were expressed as mean ± SD, and analyzed by one-way ANOVA followed by unpaired Student *t*-test. The significance level was set as $\alpha = 0.05$.

3. Results

3.1. Effect of PL and TRAIL on cell proliferation and cell injury

PL suppressed the proliferation of cell lines in a dose-dependent

manner. In addition, TRAIL (25 ng/mL) enhanced the inhibitory effect of PL in prostate cancer cell lines PC3, LNCaP, and VCaP (Figure 1A-C). These results indicated that PL had potent antiproliferative activity against prostate cancer cells.

To confirm the results observed by the MTT assay, the LDH assay was conducted in culture media. Increased LDH levels demonstrate cell injury (broken cell membrane). Significant increases were observed in LDH levels with PL treatment at 1.9 μM and above compared to the control as shown in Figure 1D (P<0.05).

3.2. Effect of PL on cell migration and colony formation

Wound healing assay showed that the horizontal migration capacity, represented as the wounded area, of PC3 and LNCaP cells was decreased after treated by 5 μM and 10 μM PL for 24 h. TRAIL (25 ng/mL) synergistically inhibited cell migration with PL (Figure 2). In addition, PL significantly inhibited colony formation (Figure 3).

3.3. Effect of PL on death receptors, apoptotic proteins and cell survival proteins expression

Treatment with PL resulted in increased expression of Bax protein. XIAP and survivin protein expressions were downregulated after 15 μM PL treatment. No significant change in Bcl-xL was observed.

PL induced DR5 and DR4 expression in a dose-dependent manner in PC3 cells. A slight decrease of DR5 was observed in LNCaP cells treated with 3.5 μM PL. However, no significant change of Decoy receptor 1 and 2 (DcR1 and DcR2) levels was observed (Figure

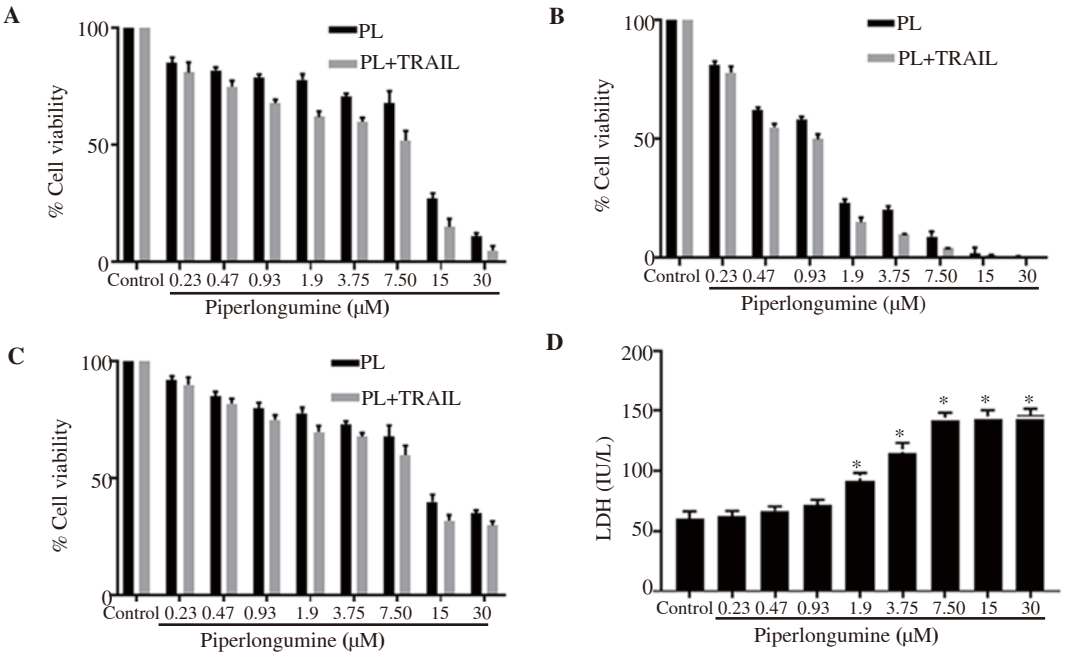

Figure 1. Effect of piperlongumine (PL) and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) on cell proliferation and cell injury. A: LNCaP; B: VCaP; C: PC3; D: LDH. Data are shown as mean ± SD, $^{*}P$<0.05 *vs.* the control group.

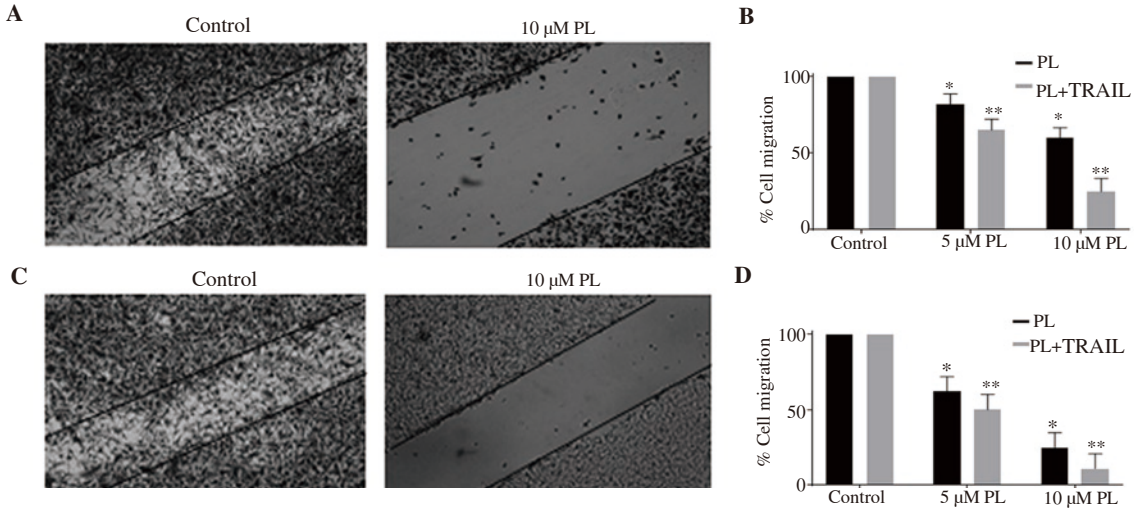

Figure 2. Wound healing assay result. A, B: PC3; C, D: LNCaP. Data are shown as mean ± SD, $^{*}P$<0.05, $^{**}P$<0.01 *vs*. the control group. PL: piperlongumine, TRAIL: tumor necrosis factor-related apoptosis-inducing ligand.

4). COX-2 was identified mainly in solid tumors to modulate cell proliferation and apoptosis. As shown in Figure 5, PL suppressed COX-2 activity in PC3 and LNCaP cells.

3.4. Effect of PL on caspase 9 and cleaved caspase-3

Our results showed that caspase 9 was increased after treatment with 15 μM PL (P<0.05). In addition, PL also significantly increased cleaved caspase-3 level (P<0.05), which is a well-known key molecule of apoptosis, in a dose-dependent manner (Figure 6).

3.5. Morphological changes

As shown in Figure 7, the hallmark of apoptosis *e.g.* cell condensation and chromatin margination were observed in treatment groups. These morphological features were seen more distinctively on PL+TRAIL treated group compared to other groups.

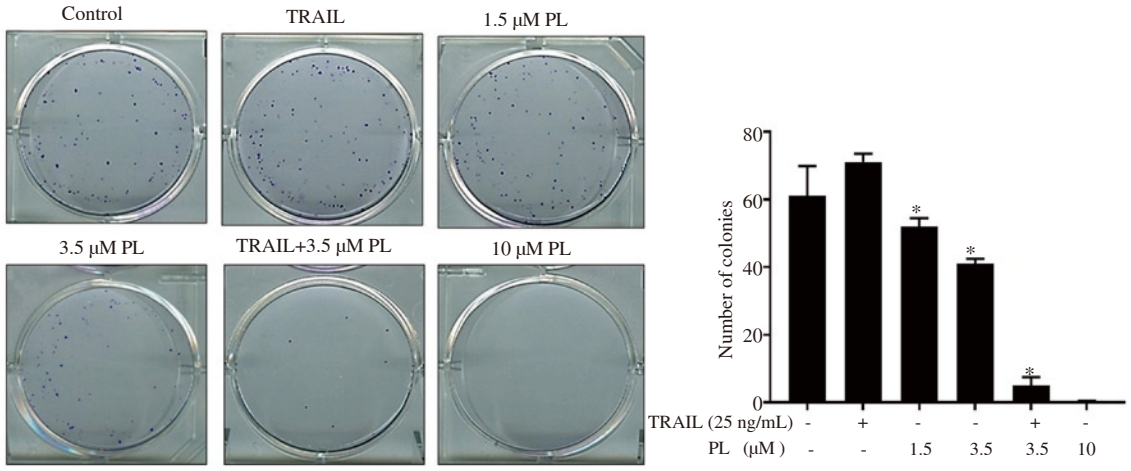

Figure 3. Colony-forming assay result. Data are shown as mean ± SD, $^{*}P$<0.05 *vs.* the control group. PL: piperlongumine, TRAIL: tumor necrosis factor-related apoptosis-inducing ligand.

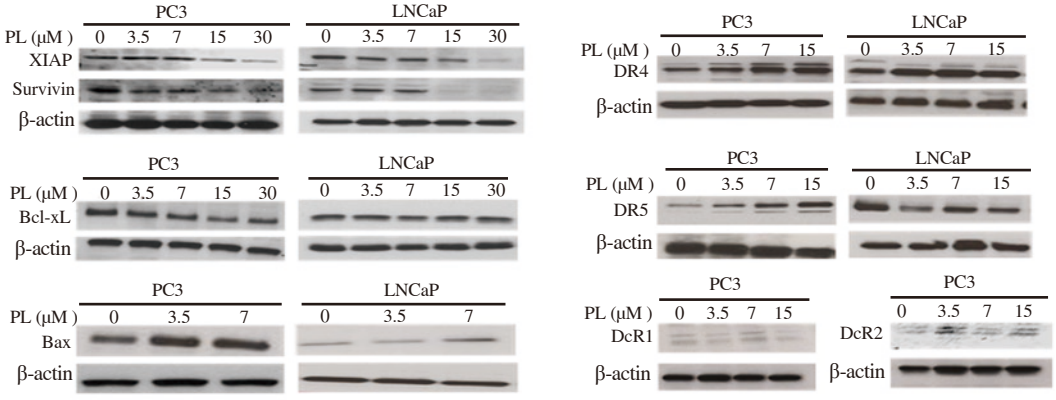

Figure 4. Effect of piperlongumine (PL) on marker proteins of apoptosis and death receptors using Western blotting. β-actin is used as housekeeping control.

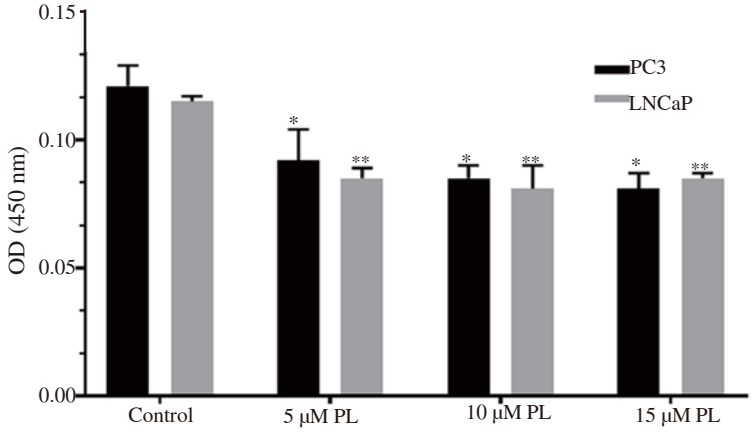

Figure 5. Effect of piperlongumine (PL) on cyclooxygenase-2. $^{*}P$<0.05, $^{**}P$<0.01 *vs.* the control group.

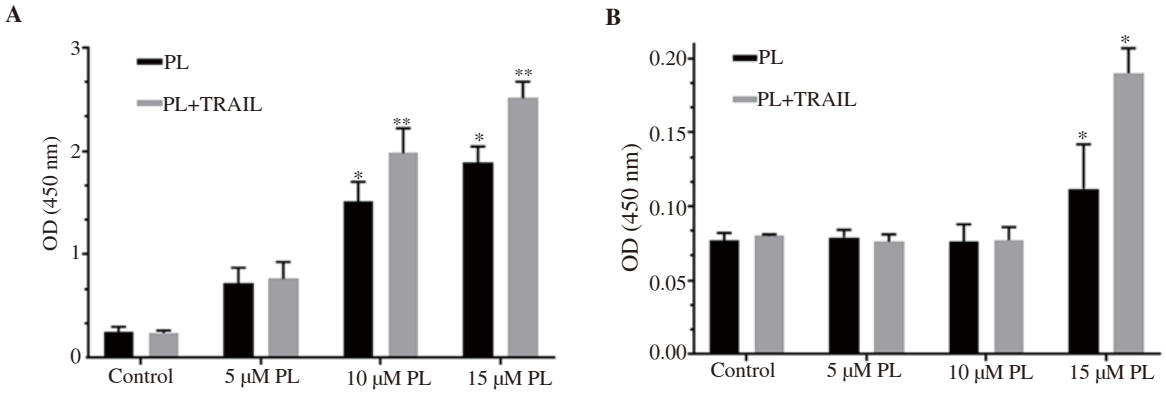

Figure 6. Effects of piperlongumine (PL) and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) on cleaved caspase 3 (A) and caspase 9 (B) activation. $^{*}P<0.05$, $^{**}P<0.01$ *vs.* the control group.

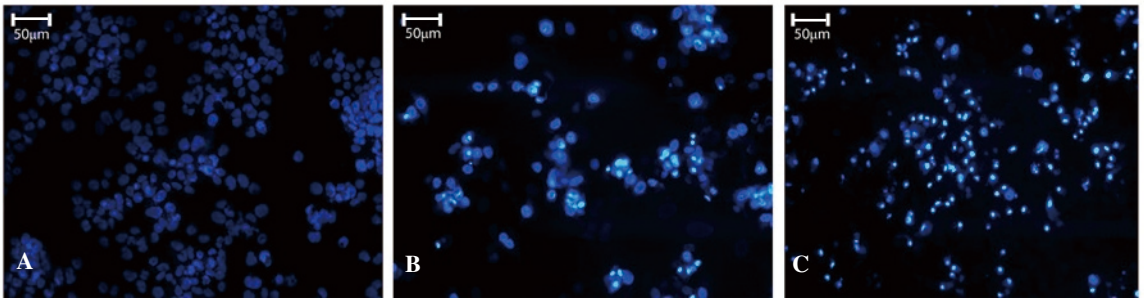

Figure 7. Effect of piperlongumine (PL) on apoptotic changes using DAPI nuclear staining. A: control; B: PL; C: PL+tumor necrosis factor-related apoptosis-inducing ligand.

4. Discussion

Treatment of prostate cancer has been improved significantly over the last ten years. Finding appropriate treatment of localized, recurrent and advanced prostate cancer has been a challenge for physicians[17]. Patients develop resistance to chemotherapy or hormone therapy treatments; thus, new approaches are needed to change or complement ongoing therapies. In the present study, we examined whether PL, an alkaloid isolated from the plant, can apply anticancer activity against prostate cancer *via* the apoptotic pathway and chemosensitizing effect on TRAIL.

Our results show that PL inhibited proliferation and induced cell death in prostate cancer cells. PC3 cells were found more resistant to PL than other cell lines. The antiproliferative and apoptosis-inducing effects of PL are similar to other researches on leukemia[18], head and neck cancer[19], breast cancer[20], ovarian cancer[21], kidney[22] and glioblastoma multiforme[23] cells.

Disturbance of the apoptosis process is associated with the development of a large number of fatal diseases and disorders including cancer. Selective induction of tumor cell apoptosis may become the fundamental strategy for malignancy treatment[24]. Our result showed that PL inhibited prostate cancer cell growth *via* activation of caspase-3 and -9. Initiator caspases such as caspase 9 activate executioner caspases that subsequently coordinate their activities to demolish key structural proteins and activate other enzymes[25,26]. Mitochondria play a key role in apoptosis control. The mitochondrial membranes are permeabilized in response to the triggers of death, leading to the release of cytochrome c in the cytosol. Cytochrome c facilitates apoptosis by binding and activating factor-1 (Apaf-1)-caspase-9 complex apoptotic protease activation, which forms an apoptosome acting as a processing/activation center for the downstream caspase-3. In addition to extracellular signals, intracellular accumulation of ROS can result in the release of apoptosis factors from the mitochondria into the cytosol, causing caspase-dependent or caspase-independent cytosolic events[27–29]. Cancer cells have an increase in spatially located ROS production compared to normal cells due to failed proper redox control[30]. Raj *et al*. disclosed PL kills cancer cells of various origins in a highly specific manner. PL could modestly inhibit the enzymatic activity of recombinant GSTP1 which plays the key role in ROS detoxification *in vitro*. It is consistent with the idea that GSTP1 is a target of PL[13,14]. In the present study, we found that PL initiated apoptotic pathway *via* caspase 9 activation and caspase 3 cleavages. This finding may be related to ROS accumulation.

The overexpression of COX-2 which is related to cell survival and cell proliferation leads to tumor formation. Downregulation of the expression of COX-2 may lead to the decreased growth of prostate cancer cells. Over-expression of COX-2 is associated with tumorigenic capacity with resistance to apoptosis in certain cell types[31]. For prostate cancer, the rise of prostate-specific antigen became slow after the administration of COX-2 inhibitor in patients with recurrent prostate cancer[32]. Bieniek *et al*. suggested that compounds with more selective COX-2 inhibitors than celecoxib or the EP1 receptor antagonists may

also be required for the COX-2 signaling targeted therapy of prostate cancer[33]. PL downregulates COX-2 expression in prostate cancer cells. Further research is needed to explain the distinctive role of COX-2 inhibition in the treatment of prostate cancer and the control of signaling pathways involved in its effects.

It is important to know the way in which cell death occurs to understand the mechanism of cell damage. Induction of apoptosis has been considered as the main mechanism of anticancer drugs[34–37]. In the present study, after damage to the plasma membrane, LDH, a stable cytoplasmic enzyme present in cells, was released into the culture medium. LDH levels were increased in bathing media as cell proliferation decreased. Although the release of LDH in cell culture does not explicitly mean necrosis, damage to the plasma membrane is a characteristic of necrotic cells that could help to assess necrosis among other criteria. However, morphological observations displayed that very few necrotic cells-mostly chromatin condensed apoptotic-like cells were formed. Because apoptotic cells can not undergo rapid phagocytosis under conditions of *in vitro* cell culture, but they would undergo phagocytosis *in vivo* in the intact tissue, the release of LDH may also be a characteristic of late apoptotic cells[38].

In this research, we observed that apoptosis induced by TRAIL was potentiated by the PL. We investigated further how PL could enhance the sensitivity of prostate cancer cells to TRAIL-induced apoptosis. We observed that PL significantly up-regulated the expression of the TRAIL receptor DR4 but did not have much effect on DR5, DcR1, and DcR2. Apoptosis caused by TRAIL seems to need the expression of one or both of its death-domain receptors, DR4 or DR5[39].

The migration of cancer cells plays a crucial role in cancer invasion and metastasis. Cancer cells have a wide range of pathways for migration and invasion. These include tactics for the individual as well as collective cell migration. We used a wound-healing assay to determine cell migration and observed inhibitory effect of PL on cell migration. Liu *et al*. reported that, in the scratch-wound culture model, PL has successfully suppressed migration of human glioma cells (LN229 or U87 MG) but not normal astrocytes. They proposed that the main factor promoting the decrease of migrated cells in the scratched area after PL treatment within the timeframe of their model was cell migration but not cell cycle or cell proliferation[40]. Cancer therapeutics that are designed to target migration and metastasis have not been verified to be effective in lessening of speed tumor progression in clinical trials[41–43]. Yet, the role of PL in cell migration is clear in certain cell lines. Our study was performed on a prostate cancer cell line and we noticed the inhibitory effect of PL on this cell line.

In conclusion, our cell-based studies affirm apoptosis-inducing antiproliferative activities of PL. PL can strengthen apoptosis caused by TRAIL in human cancer cells through the up-regulation of DR4 and pro-apoptotic proteins, as well as down-regulation of anti-apoptotic proteins. Our work provides the basis for clinical trials of patients with prostate cancer.

Conflict of interest statement

We declare that there is no conflict of interest.

Funding

This work was supported by the Turkish Scientific Council (TUBITAK), Grant# 115S942.

Authors' contributions

GK and TS designed the study and made the critical revision of the article with AC and NT. Collecting test data, drafting the article and getting a final approval were done by MA, OM, BYD, AC, and DUC. Data analysis and interpretation was done by FK, OM, and GK. In addition, TS was responsible for the supervision, and GK was responsible for project funding acquisition.

References

[1] Rawla P. Epidemiology of prostate cancer. *World J Oncol* 2019; **10**(2): 63-89.

[2] Taitt HE. Global trends and prostate cancer: A review of incidence, detection, and mortality as influenced by race, ethnicity, and geographic location. *Am J Mens Health* 2018; **12**(6): 1807-1823.

[3] Cuzick J, Thorat MA, Andriole G, Brawley OW, Brown PH, Culig Z, et al. Prevention and early detection of prostate cancer. *Lancet Oncol Oct* 2014; **15**(11): e484-e492.

[4] Tucker D, Anderson M, Miller F, Vaz K, Anderson-Jackson L, McGrowder D. Dietary antioxidants in the chemoprevention of prostate cancer. In: *Antioxidants*. NY, USA: IntechOpen.; 2019. Doi: 10.5772/intechopen.85770

[5] Cao Y, Nishihara R, Wu K, Wang M, Ogino S, Willett WC, et al. Population-wide impact of long-term use of aspirin and the risk for cancer. *JAMA Oncol* 2016; **2**(6): 762-769.

[6] Parnes HL, House MG, Tangrea JA. Prostate cancer prevention: Agent development strategies. *Recent Results Cancer Res* 2014; **202**: 121-131.

[7] Patil T, Bernard B. Complications of androgen deprivation therapy in men with prostate cancer. *Prostate* 2018; **32**(9): 470-474.

[8] Rodrigues T, Reker D, Schneider P, Schneider G. Counting on natural products for drug design. *Nature Chem* 2016; **8**(6): 531.

[9] Yadav V, Prasad S, Sung B, Kannappan R, Aggarwal BB. Targeting inflammatory pathways by triterpenoids for prevention and treatment of cancer. *Toxins* 2010; **2**: 2428-2466.

[10]Poprac P, Jomova K, Simunkova M, Kollar V, Rhodes CJ, Valko M. Targeting free radicals in oxidative stress-related human diseases. *Trends Pharmacol Sci* 2017; **38**(7): 592-607.

[11]Raza MH, Siraj S, Arshad A, Waheed U, Aldakheel F, Alduraywish S, et al. ROS-modulated therapeutic approaches in cancer treatment. *J Cancer Res Clin Oncol* 2017; **143**(9): 1789-1809.

[12]Möhler H, Pfirrmann RW, Frei K. Redox-directed cancer therapeutics: Taurolidine and piperlongumine as broadly effective antineoplastic agents (review). *Int J Oncol* 2014; **45**(4): 1329-1336.

[13]Raj L, Ide T, Gurkar AU, Foley M, Schenone M, Li X, et al. Selective killing of cancer cells by a small molecule targeting the stress response to ROS. *Nature* 2011; **475**(7355): 231-234.

[14]Parkinson E, Hergenrother PJ. Runaway ROS as a selective anticancer strategy. *Chem Med Chem* 2011; **6**(11): 1957-1959.

[15]De Miguel D, Lemke J, Anel A, Walczak H, Martinez-Lostao L. Onto better TRAILs for cancer treatment. *Cell Death Differ* 2016; **23**(5): 733-747.

[16]Zhang L, Fang B. Mechanisms of resistance to TRAIL-induced apoptosis in cancer. *Cancer Gene Ther* 2005; **12**(3): 228-237.

[17]Droz JP, Albrand G, Gillessen S, Hughes S, Mottet N, Oudard S, et al. Management of prostate cancer in elderly patients: Recommendations of a task force of the International Society of Geriatric Oncology. *Eur Urol* 2017; **72**(4): 521-531.

[18]Xiong XX, Liu JM, Qiu XY, Pan F, Yu SB, Chen XQ. Piperlongumine induces apoptotic and autophagic death of the primary myeloid leukemia cells from patients *via* activation of ROS-p38/JNK pathways. *Acta Pharmacol Sin* 2015; **36**(3): 362.

[19]Roh JL, Kim EH, Park JY, Kim JW, Kwon M, Lee BH. Piperlongumine selectively kills cancer cells and increases cisplatin antitumor activity in head and neck cancer. *Oncotarget* 2014; **5**(19): 9227-9238.

[20]Jin HO, Lee YH, Park JA, Lee HN, Kim JH, Kim JY, et al. Piperlongumine induces cell death through ROS-mediated CHOP activation and potentiates TRAIL-induced cell death in breast cancer cells. *J Cancer Res Clin Oncol* 2014; **140**(12): 2039-2046.

[21]Gong LH, Chen XX, Wang H, Jiang QW, Pan SS, Qiu JG, et al. Piperlongumine induces apoptosis and synergizes with cisplatin or paclitaxel in human ovarian cancer cells. *Oxid Med Cell Longev* 2014; **2014**: 906804.

[22]Makhov P, Golovine K, Teper E, Kutikov A, Mehrazin R, Corcoran A, et al. Piperlongumine promotes autophagy *via* inhibition of Akt/mTOR signalling and mediates cancer cell death. *Br J Cancer* 2014; **110**(4): 899-907.

[23]Liu JM, Pan F, Li L, Liu QR, Chen Y, Xiong XX, et al. Piperlongumine selectively kills glioblastoma multiforme cells *via* reactive oxygen species accumulation dependent JNK and p38 activation. *Biochem Biophys Res Commun* 2013; **437**(1): 87-93.

[24]Koff J, Ramachandiran S, Bernal-Mizrachi L. A time to kill: Targeting apoptosis in cancer. *Int J Mol Sci* 2015; **16**(2): 2942-2955.

[25]McIlwain DR, Berger T, Mak TW. Caspase functions in cell death and disease. *Cold Spring Harb Perspect Biol* 2013; **5**(4): a008656.

[26]Alvarez-Paggi D, Hannibal L, Castro MA, Oviedo-Rouco S, Demicheli V, Tortora V, et al. Multifunctional cytochrome c: Learning new tricks from an old dog. *Chem Rev* 2017; **117**(21): 13382-13460.

[27]Tait SWG, Green DR. Mitochondria and cell death: Outer membrane permeabilization and beyond. *Nat Rev Mol Cell Biol* 2010; **11**: 621-632.

[28]Xiong S, Mu T, Wang G, Jiang X. Mitochondria-mediated apoptosis in mammals. *Protein Cell* 2014; **5**(10): 737-749.

[29]An WW, Wang MW, Tashiro S, Onodera S, Ikejima T. Norcantharidin induces human melanoma A375-S2 cell apoptosis through mitochondrial and caspase pathways. *J Korean Med Sci* 2004; **19**(4): 560-566.

[30]Cairns RA, Harris IS, Mak TW. Regulation of cancer cell metabolism. *Nat Rev Cancer* 2011; **11**(2011): 85-95.

[31]Fosslien E. Biochemistry of cyclooxygenase (COX)-2 inhibitors and molecular pathology of COX-2 in neoplasia. *Crit Rev Clin Lab Sci* 2000; **37**: 431-502.

[32]Pruthi RS, Derksen JE, Moore D, Carson CC, Grigson G, Watkins C, et al. Phase II trial of celecoxib in prostate-specific antigen recurrent prostate cancer after definitive radiation therapy or radical prostatectomy. *Clin Cancer Res* 2006; **12**(7 Pt 1): 2172-2177.

[33]Bieniek J, Childress C, Swatski MD, Yang W. COX-2 inhibitors arrest prostate cancer cell cycle progression by down-regulation of kinetochore/centromere proteins. *Prostate* 2014; **74**(10): 999-1011.

[34]Reed JC. Apoptosis-regulating proteins as targets for drug discovery. *Trends Mol Med* 2001; **7**: 314-319.

[35]Janeklang S1, Nakaew A, Vaeteewoottacharn K, Seubwai W, Boonsiri P, Kismali G, et al. *In vitro* and *in vivo* antitumor activity of tiliacorinine in human cholangiocarcinoma. *Asian Pac J Cancer Prev* 2014; **15**(17): 7473-7478.

[36]Kismali G, Emen FM, Yesilkaynak T, Meral O, Demirkiran D, Sel T, et al. The cell death pathway induced by metal halide complexes of pyridine and derivative ligands in hepatocellular carcinoma cells - necrosis or apoptosis? *Eur Rev Med Pharmacol Sci* 2012; **16**(8): 1001-1012.

[37]Hu XZ, Xu Y, Hu DC, Hui Y, Yang FX. Apoptosis induction on human hepatoma cells Hep G2 of decabrominated diphenyl ether (PBDE-209). *Toxicol Lett* 2007; **171**(1–2): 19-28.

[38]Grub S, Persohn E, Trommer WE, Wolf A. Mechanisms of cyclosporine A-induced apoptosis in rat hepatocyte primary cultures. *Toxicol Appl Pharmacol* 2000; **163**: 209-220.

[39]Griffith TS, Lynch DH. TRAIL: A molecule with multiple receptors and control mechanisms. *Curr Opin Immunol* 1998; **5**: 559-563.

[40]Liu QR, Liu JM, Chen Y, Xie XQ, Xiong XX, Qiu XY, et al. Piperlongumine inhibits migration of glioblastoma cells *via* activation of ROS-dependent p38 and JNK signaling pathways. *Oxid Med Cell Longev* 2014; **2014**: 653732.

[41]Bravo-Cordero JJ, Hodgson L, Condeelis J. Directed cell invasion and migration during metastasis. *Curr Opin Cell Biol* 2012; **2**: 277-283.

[42]Meral O, Alpay M, Kismali G, Kosova F, Cakir DU, Pekcan M, et al. Capsaicin inhibits cell proliferation by cytochrome c release in gastric cancer cells. *Tumour Biol* 2014; **35**(7): 6485-6492.

[43]Friedl P, Wolf K. Tumour-cell invasion and migration: Diversity and escape mechanisms. *Nat Rev Cancer* 2003; **3**(5): 362-374.