Research Square

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Dual TRPM2–PLA_2 Blockade Induces Redox Reprogramming and Selective Apoptosis in Prostate Cancer Cells

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Research Article

Keywords: Prostate cancer, Phospholipase A2, Transient receptor potential melastatin 2 channel, N-(p-amylcinnamoyl) anthranilic acid (ACA)

Posted Date: May 5th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9476315/v1

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Additional Declarations: No competing interests reported.

Abstract

Purpose:
Dysregulated calcium signalling and lipid metabolism contribute to prostate cancer progression. This study aimed to evaluate whether dual inhibition of transient receptor potential melastatin 2 (TRPM2) channels and phospholipase A_2 (PLA_2) by N-(p-amylcinnamoyl) anthranilic acid (ACA) affects proliferation, apoptosis, migration, and redox balance in prostate cancer cells.

Methods:
DU-145, PC-3, and LNCaP cells were treated with increasing concentrations of ACA at different time points. Cell viability was measured using the MTT assay. Apoptosis was analysed by Annexin V-FITC/PI staining and flow cytometry. Colony formation and wound healing assays were used to assess clonogenic capacity and migration. Oxidative status was determined by measuring total oxidant status (TOS), total antioxidant status (TAS), and oxidative stress index (OSI).

Results:
ACA reduced cell viability in a concentration- and time-dependent manner, with PC-3 cells showing the highest sensitivity. A marked increase in apoptosis was observed in androgen receptor-positive LNCaP cells, whereas DU-145 and PC-3 cells showed minimal apoptotic changes. Colony formation was suppressed in LNCaP and PC-3 cells but not in DU-145 cells. Migration was inhibited in all cell lines, with the strongest effect in PC-3 cells. ACA also decreased TOS levels in all groups and reduced OSI values in LNCaP and DU-145 cells.

Conclusion:
ACA exerts selective anticancer effects by modulating proliferation, migration, apoptosis, and redox balance. Its pro-apoptotic activity appears to depend on androgen receptor status, suggesting that dual TRPM2–PLA_2 targeting may represent a context-dependent therapeutic strategy in prostate cancer.

1. Introduction

Prostate cancer represents a major global health burden, with millions of new cases diagnosed annually and a substantial proportion of patients progressing to advanced disease stages [1–3]. Although early-stage prostate cancer is associated with a favourable prognosis, with long-term survival rates approaching 99%, the clinical outcome dramatically worsens once metastasis develops [4–6]. Indeed, metastatic prostate cancer remains a leading cause of cancer-related mortality among men worldwide. In addition, current therapeutic approaches are often accompanied by significant adverse effects, which complicate disease management and highlight the necessity for more effective and targeted treatment strategies [7, 8].

N-(p-amylcinnamoyl) anthranilic acid (ACA) has been identified as a pharmacological agent capable of inhibiting phospholipase A_2 (PLA_2). Notably, accumulating evidence indicates that ACA also acts as an inhibitor of the transient receptor potential melastatin 2 (TRPM2) channel, suggesting a dual-target

mechanism [9, 10]. Previous studies have demonstrated that ACA interferes with intracellular Ca^{2+} influx, thereby modulating key cellular signalling pathways [11, 12]. Calcium ions (Ca^{2+}) function as critical second messengers that regulate a wide range of cellular processes, including gene expression, proliferation, migration, and cell death. Dysregulation of Ca^{2+} homeostasis has been strongly implicated in tumorigenesis, angiogenesis, and metastatic progression. Consequently, targeting aberrant Ca^{2+} signalling has emerged as a promising strategy in cancer therapy [13]. TRPM2, a non-selective cation channel permeable to Ca^{2+} and activated under oxidative stress conditions, has been reported to be expressed in multiple cancer types, including prostate cancer [14]. Inhibition of TRPM2 function or expression has been shown to suppress tumour cell proliferation and viability across various malignancies, such as breast, gastric, pancreatic, prostate, and hematological cancers [15].

In addition to Ca^{2+}-dependent pathways, lipid signalling also plays a crucial role in cancer progression. Eicosanoids derived from cyclooxygenase (COX) and lipoxygenase (LOX) pathways contribute to tumour growth, angiogenesis, and metastasis. Enzymes such as PLA_2 regulate the availability of arachidonic acid, a precursor for these bioactive lipids, thereby influencing tumour biology. Elevated PLA_2 expression has been reported in prostate cancer tissues, and its activity has been associated with tumour growth and progression [16]. However, the role of PLA_2 in cancer appears to be context-dependent, as it may function as either a tumour promoter or suppressor depending on the cancer type [17].

Based on these observations, the present study aimed to investigate the biological effects of ACA, a dual TRPM2 and PLA_2 inhibitor, in prostate cancer cell lines (DU-145, PC-3, and LNCaP), with a particular focus on proliferation, apoptosis, migration, and redox regulation.

2. Materials and Methods

2.1 Cell sources and experimental materials

Three human prostate cancer cell lines—DU-145, PC-3, and LNCaP—were included in the experimental design. All cell lines were procured from ATCC (USA). Cells were propagated in RPMI-1640 medium enriched with L-glutamine (NutriCulture, UK), supplemented with 10% heat-inactivated fetal bovine serum (Biosera, France) and 1% penicillin–streptomycin solution (Gibco, USA).

Reagents utilized during experimental procedures comprised trypsin-EDTA (0.25%, Gibco, USA), TriPure reagent for simultaneous isolation of RNA, DNA, and proteins (Roche, USA), phosphate-buffered saline (Grisp, Portugal), MTT (Calbiochem, Germany), and cDNA synthesis kit (iScript™, Bio-Rad, USA).

2.2 Culture conditions

Cells were maintained under controlled environmental conditions (37°C, 5% CO_2, humidified atmosphere). Culture media were routinely renewed at 48-hour intervals. Subculturing procedures were performed when cells approached optimal density, ensuring maintenance of exponential growth conditions.

2.3 Determination of cell viability (MTT assay)

To evaluate the cytotoxic profile of ACA, cell viability was quantified using a colorimetric MTT-based approach. The non-malignant MRC-5 fibroblast cell line was incorporated as a reference control.

Cells (DU-145, PC-3, LNCaP, and MRC-5) were distributed into 96-well plates at a density of 1×10^4 cells/well and allowed to attach overnight. Subsequently, cells were exposed to graded concentrations of ACA (16–192 µM) or a reference compound (5–200 µM) across three incubation periods (24, 48, and 72 hours).

At the end of treatment, MTT solution was introduced into each well, and plates were incubated until visible formazan crystal formation occurred. Crystals were then solubilized using DMSO, and optical density values were recorded at 570 nm. Dose–response relationships and IC_{50} values were computed using GraphPad Prism 9.

2.4 Clonogenic survival analysis

The long-term proliferative potential of cells was assessed via colony formation assay. Cells were initially seeded at low density (1,000 cells per well in 6-well plates) to allow discrete colony growth. Following attachment, cells were exposed to ACA at concentrations corresponding to IC_{50} values.

During the 10-day incubation period, media containing ACA were replenished every 48 hours. At the endpoint, colonies were fixed, stained with crystal violet, and quantified using ImageJ software.

2.5 Assessment of migratory capacity

Cell motility was investigated using an in vitro wound closure model. Cells were seeded into 6-well plates and cultured until near-confluent monolayers (~ 85%) were obtained. A linear mechanical disruption was introduced using a sterile pipette tip.

Migration into the wound area was monitored at predetermined time points (0, 16, and 24 hours). Images were captured and analyzed digitally, and the extent of wound closure was quantified using ImageJ. All experiments were conducted in triplicate.

2.6 Evaluation of oxidative status

To determine redox alterations, extracellular levels of total oxidants (TOS) and total antioxidants (TAS) were measured following 72-hour ACA exposure. Commercial assay kits (Rel Assay Diagnostics, Turkey) were used in accordance with manufacturer protocols.

Absorbance readings were obtained at 530 nm (TOS) and 660 nm (TAS). The oxidative stress index (OSI) was derived by calculating the ratio of TOS to TAS and multiplying by 100, as described previously [18, 19].

2.7 Statistical Analysis

Data were statistically analyzed using GraphPad Prism version 9. All results are presented as mean ± standard deviation (SD). Nonlinear regression analysis of dose-response curves was used to calculate IC_{50} values. One-way analysis of variance (ANOVA) was used as the statistical method. A significance level of < 0.05 was accepted.

3. Results

3.1 ACA reduces viability of prostate cancer cells

Exposure to ACA resulted in a measurable decline in cell viability across prostate cancer cell lines. This reduction became more evident with increasing concentrations and longer exposure durations.

Among the tested models, PC-3 cells exhibited the greatest sensitivity to ACA, as indicated by the lowest IC_{50} value (49.15 µM at 48 h). DU-145 and LNCaP cells displayed comparatively higher IC_{50} values (75.51 µM and 78.59 µM at 48 h, respectively). Viability assessments performed at 24, 48, and 72 hours confirmed a progressive reduction in metabolic activity (Fig. 1).

3.2 Apoptotic response is restricted to LNCaP cells

Flow cytometric analysis using Annexin V-FITC/PI staining revealed a pronounced apoptotic response following ACA treatment in LNCaP cells. The proportion of apoptotic cells increased dramatically compared to untreated controls (from ~ 6% to ~ 84%).

In contrast, DU-145 and PC-3 cells did not demonstrate a comparable increase in apoptotic fractions, suggesting that ACA-induced cytotoxicity in these cells is not primarily mediated through apoptosis (Fig. 2).

3.3 Clonogenic capacity is differentially affected

The ability of prostate cancer cells to form colonies was variably influenced by ACA exposure. A substantial reduction in colony number was observed in LNCaP and PC-3 cells, indicating impaired proliferative capacity.

DU-145 cells, however, showed limited responsiveness, with colony formation remaining largely unaffected under similar treatment conditions (Fig. 3).

3.4 Migration is markedly suppressed by ACA

Untreated cells demonstrated efficient wound closure over time, particularly in PC-3 and DU-145 groups, reflecting high baseline motility.

ACA exposure significantly attenuated this migratory behavior in all cell lines. The inhibitory effect was most pronounced in PC-3 cells, where wound closure was markedly delayed. Reduced migration was

also evident in LNCaP and DU-145 cells (Fig. 4).

3.5 ACA alters oxidative balance

Baseline antioxidant capacity differed among cell lines, with PC-3 cells exhibiting the highest TAS levels. Upon ACA treatment, TAS levels decreased in PC-3 cells, increased in DU-145 cells, and remained relatively unchanged in LNCaP cells.

TOS measurements revealed elevated basal oxidant levels in DU-145 and LNCaP cells compared to PC-3 cells. ACA treatment led to a consistent reduction in TOS across all groups, with the most substantial decrease observed in LNCaP cells.

Analysis of OSI demonstrated minimal change in PC-3 cells, whereas a marked reduction was detected in both LNCaP and DU-145 cells, indicating a shift toward a less oxidative intracellular environment.

4. Discussion

The present study demonstrates that dual inhibition of TRPM2 channels and PLA_2 activity results in a multifaceted antitumour response in prostate cancer cells. Our findings indicate that ACA exerts its effects not only by reducing cellular proliferation but also by impairing migratory capacity, altering redox homeostasis, and selectively inducing apoptosis in androgen-responsive cells. These observations highlight the therapeutic potential of simultaneously targeting calcium signalling and lipid-mediated pathways in prostate cancer.

TRPM2 is a unique member of the TRP channel family, characterized by its sensitivity to oxidative stress and its role in intracellular Ca^{2+} regulation. This channel has been implicated in multiple cellular processes, including survival, proliferation, and stress responses [20–22]. In cancer biology, TRPM2 has gained attention due to its contribution to the maintenance of mitochondrial integrity, cellular bioenergetics, and DNA repair mechanisms, all of which support tumour cell survival [15]. Consistent with previous reports, inhibition of TRPM2 has been associated with reduced tumour growth and increased susceptibility to cell death across different cancer types [22].

Elevated TRPM2 expression has been reported in prostate cancer cells compared to normal prostate tissue, suggesting a role in tumour progression [23]. Genetic or pharmacological suppression of TRPM2 has been shown to disrupt hypoxia-related signalling pathways, increase mitochondrial reactive oxygen species (ROS) production, and ultimately promote cell death [24]. These findings support the concept that TRPM2 functions as a pro-survival factor in cancer cells. Interestingly, although antioxidant strategies have traditionally been considered beneficial, emerging evidence suggests that excessive suppression of ROS may paradoxically enhance tumour progression, whereas controlled elevation of oxidative stress can induce cancer cell death [25–31]. Cancer cells rely on a finely tuned redox balance, and disruption of this balance—either by excessive ROS accumulation or depletion—can compromise

cellular homeostasis [32, 33]. In this context, the observed reduction in TOS levels in our study may interfere with pro-survival redox signalling, thereby contributing to the anticancer activity of ACA.

A particularly striking finding of this study is the pronounced apoptotic response observed in LNCaP cells following ACA treatment. Unlike DU-145 and PC-3 cells, which are androgen-independent, LNCaP cells retain androgen receptor (AR) signalling and exhibit hormone sensitivity. AR signalling has been closely linked to both Ca^{2+} homeostasis and oxidative stress regulation [34, 35]. Therefore, disruption of TRPM2-mediated Ca^{2+} influx and redox balance by ACA may preferentially compromise AR-dependent survival pathways, leading to enhanced apoptosis in these cells. This selective sensitivity suggests that ACA may have greater therapeutic relevance in androgen-sensitive prostate cancer.

In addition to calcium signalling, lipid-mediated pathways also play a critical role in tumour biology. PLA_2 enzymes regulate the release of arachidonic acid, which serves as a precursor for eicosanoids involved in inflammation, proliferation, and metastasis. Increased PLA_2 activity has been documented in various cancer types, including prostate cancer, and has been associated with tumour growth and progression [36–38]. Inhibition of PLA_2 has been shown to suppress proliferation and reduce tumour development in multiple experimental models [39]. In the present study, the observed decrease in colony formation and migration supports the notion that ACA interferes not only with Ca^{2+}-dependent mechanisms but also with inflammatory lipid signalling pathways. This dual-target effect may underlie the broader anticancer activity of ACA compared to single-pathway inhibitors.

PC-3 cells, which represent a highly aggressive and metastatic phenotype, exhibited a significant reduction in migratory capacity following ACA treatment. This finding is particularly relevant, as metastasis remains the primary cause of mortality in prostate cancer. The dependence of metastatic processes on Ca^{2+}-regulated cytoskeletal dynamics and eicosanoid signalling has been well established [40, 41]. Therefore, inhibition of these pathways by ACA may contribute to the suppression of metastatic potential.

Interestingly, despite the marked reduction in viability observed in PC-3 cells, no significant increase in apoptosis was detected. This discrepancy suggests that ACA may induce alternative forms of cell death, such as necrosis, autophagy, or ferroptosis, in androgen-independent cell lines. It is well recognized that cancer cells can activate distinct cell death pathways depending on their genetic and metabolic context [42]. The relatively high antioxidant capacity of PC-3 cells may also contribute to their resistance to apoptosis, enabling them to better adapt to redox perturbations [43, 32].

Taken together, these findings support the hypothesis that TRPM2 and PLA_2 pathways are functionally interconnected and cooperatively contribute to tumour progression through regulation of Ca^{2+} signalling, redox balance, and lipid mediator production. Targeting both pathways simultaneously appears to generate a more comprehensive antitumour response by disrupting multiple survival mechanisms within cancer cells. This approach may be particularly advantageous in advanced prostate cancer, where resistance to single-target therapies is common.

Despite these promising findings, several limitations should be acknowledged. The study was conducted exclusively under in vitro conditions, and therefore, the results require validation in in vivo models. Furthermore, direct assessment of TRPM2 and PLA_2 activity, as well as detailed analysis of Ca^{2+} flux, mitochondrial function, and downstream signalling pathways such as NF-κB and MAPK, would provide additional mechanistic insight.

In conclusion, ACA demonstrates potent anticancer effects in prostate cancer cells by inhibiting proliferation and migration, modulating oxidative balance, and inducing apoptosis in androgen-sensitive cells. Dual targeting of TRPM2 and PLA_2 represents a promising therapeutic strategy that warrants further investigation.

Declarations

Conflicts of interest

All authors declare no conflicts of interest.

Funding

No sources of funding were received.

Author Contribution

S.S.O. and M.Ç. conceived the idea. M.Ç., R.Y. and S.S.O. performed MTT, flow cytometry, migration assay and colony formation tests. M.K. and İ.D. performed TAS, TOS and OSI analyses. M.Ç. and S.S.O. took the lead in writing the article. All authors discussed the results and contributed to the final manuscript.

Data Availability

ll data supporting the findings of this study are available within the paper and its Supplementary Information.

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Figures

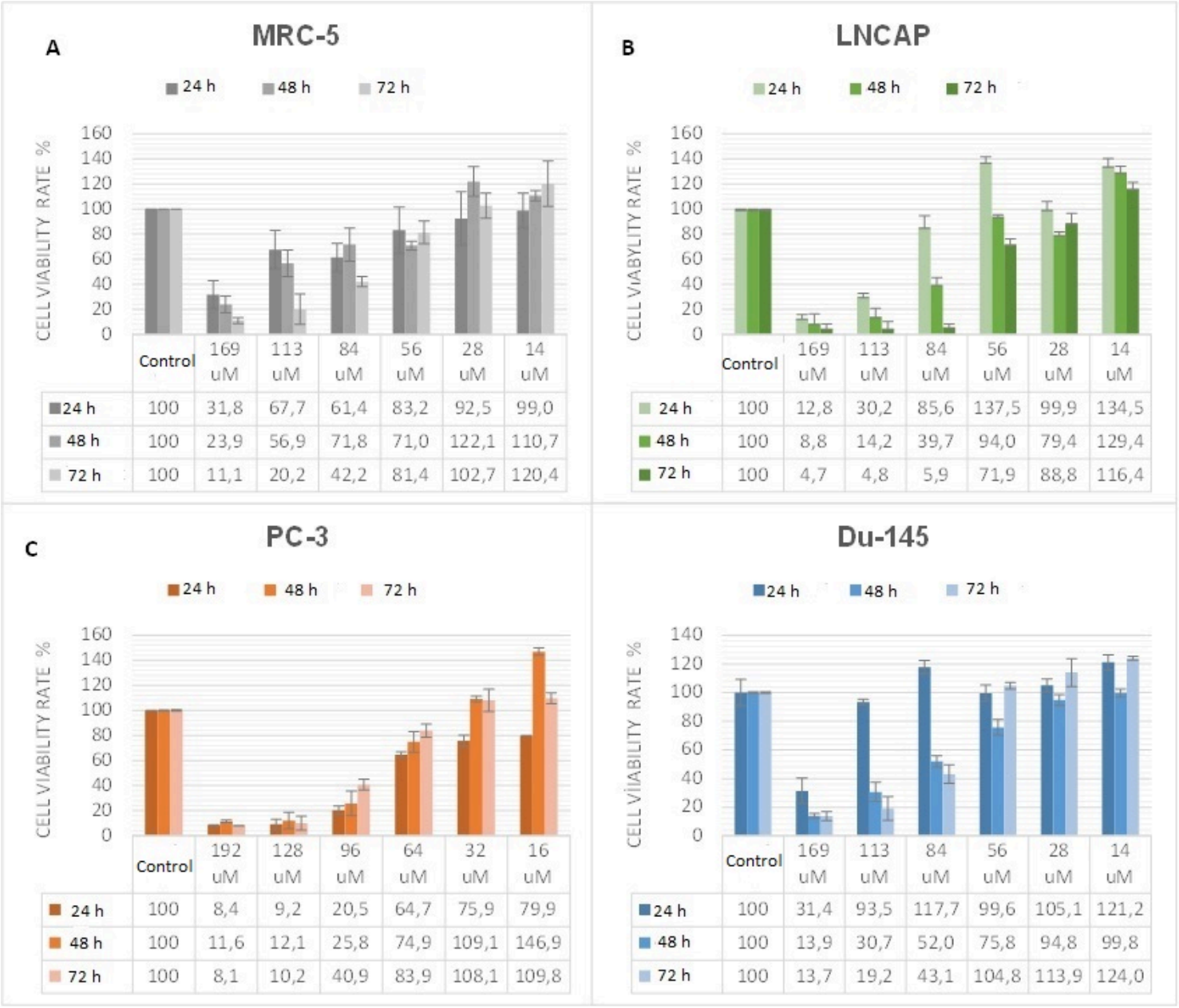


Figure 1

Effect of ACA on cell viability in prostate cancer cell lines and control cells. MRC-5 (non-cancerous), LNCaP, PC-3, and DU-145 cells were exposed to increasing concentrations of ACA for different time intervals. Cell viability was assessed using the MTT assay. Results represent the mean of three independent experiments. IC_{50} values indicate the concentration required to reduce cell viability by 50%.

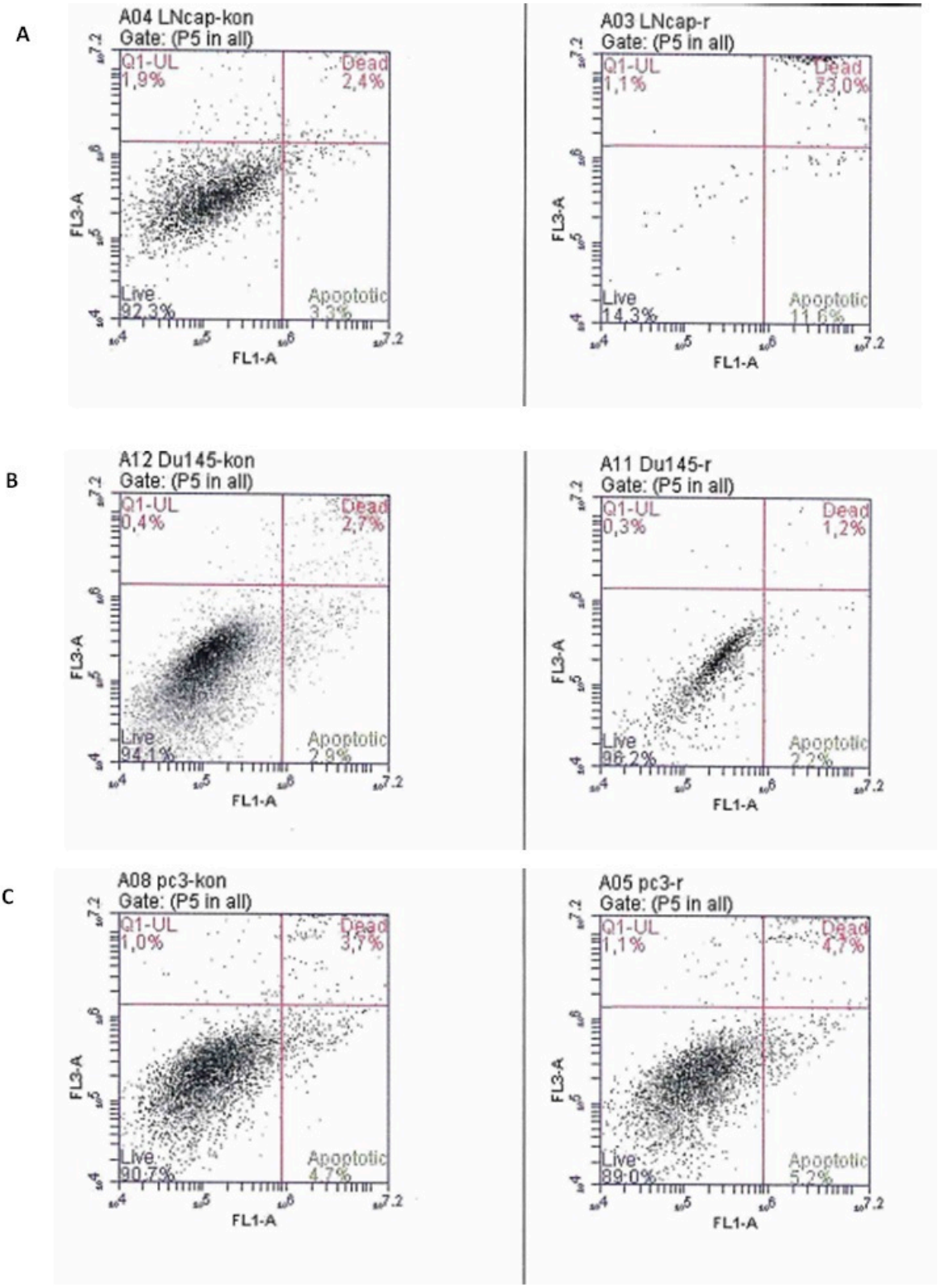


Figure 2

Induction of apoptosis by ACA in prostate cancer cell lines. LNCaP, DU-145, and PC-3 cells were treated with ACA for 24 and 48 hours. Following treatment, cells were collected, washed with cold PBS, and stained using Annexin V-FITC and propidium iodide. Apoptotic cell populations were quantified by flow cytometry analysis.

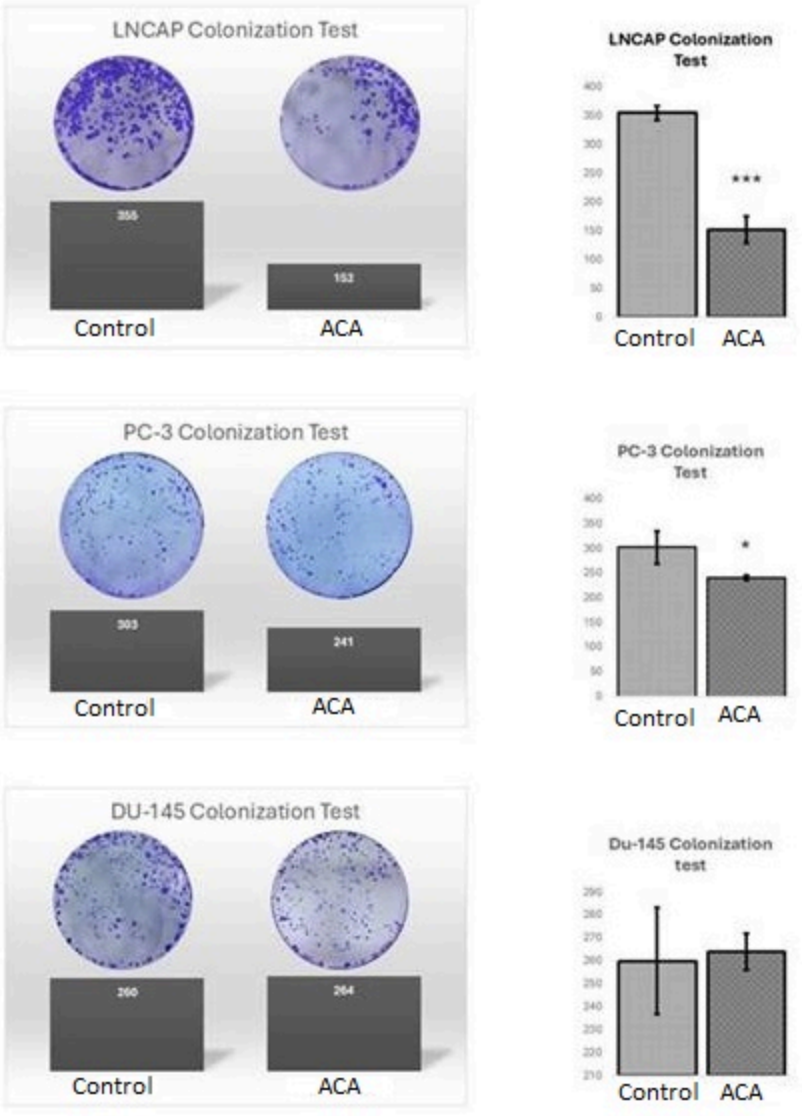


Figure 3

Impact of ACA on clonogenic survival of prostate cancer cells. LNCaP, PC-3, and DU-145 cells were cultured under treatment conditions, and colony formation was evaluated after staining with crystal violet. Data are presented as mean ± SD (n = 3). Statistical significance is indicated as *P < 0.05 and ***P < 0.001.

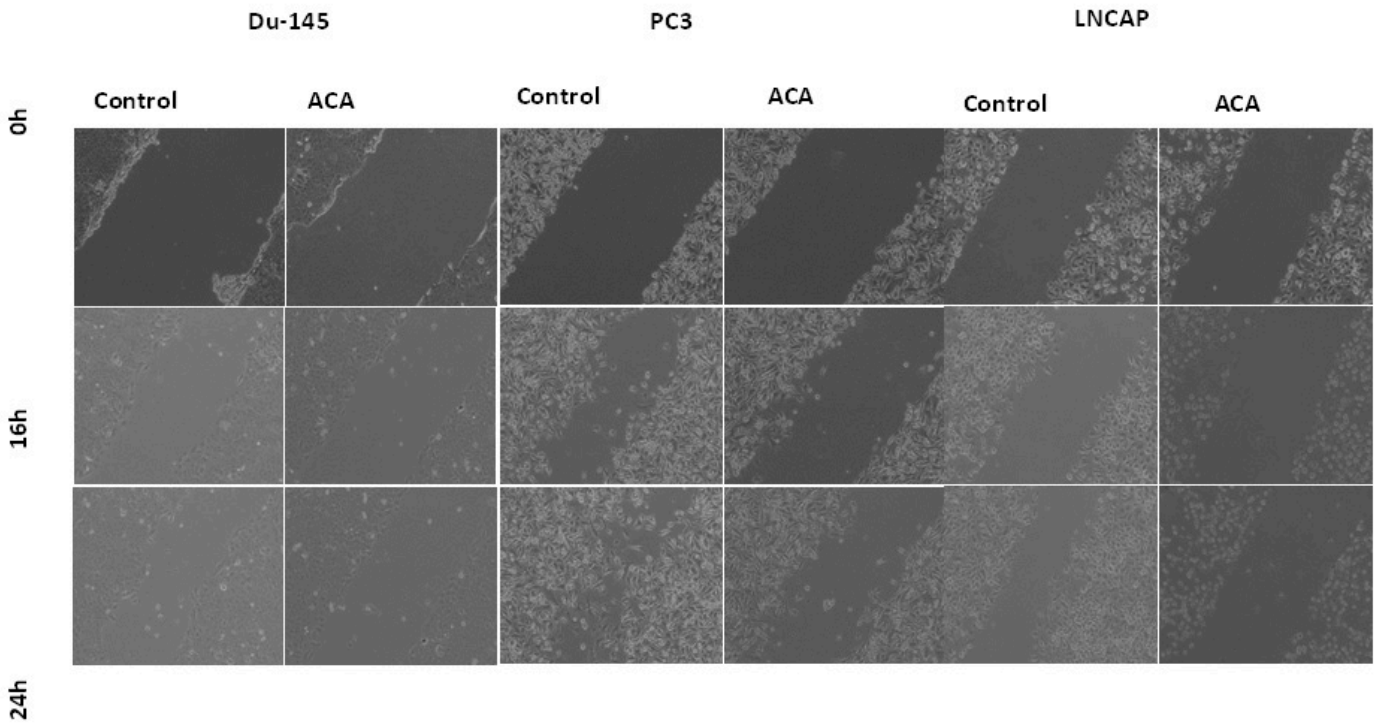


Figure 4

Effect of ACA on cell migration assessed by wound healing assay. Confluent monolayers of DU-145, PC-3, and LNCaP cells were scratched to create a wound area, and migration into the gap was monitored at 0, 16, and 24 hours. Representative images and quantitative analysis demonstrate the extent of wound closure under control and treatment conditions.

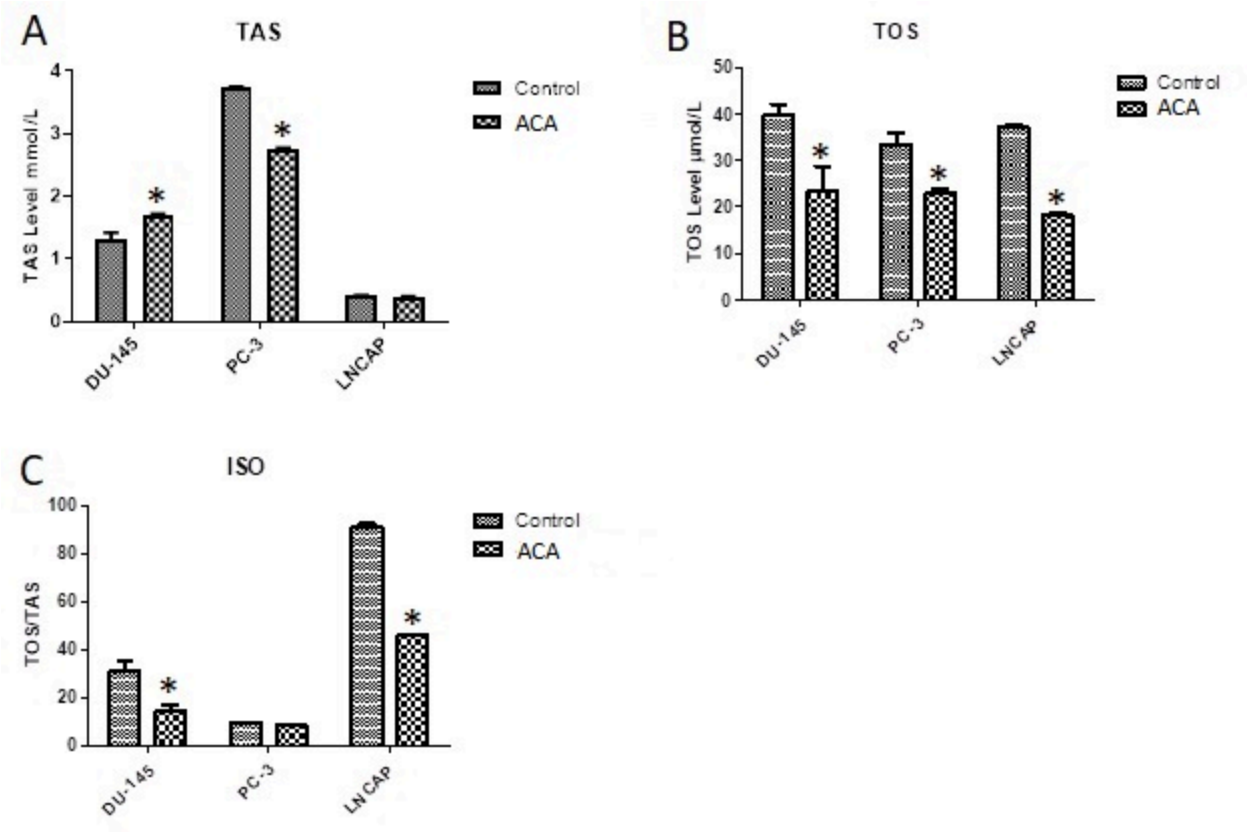


Figure 5

Effects of ACA on oxidative status parameters in prostate cancer cells. Total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) were measured in DU-145, PC-3, and LNCaP cells following treatment. TAS values are expressed in mmol/L, TOS values in µmol/L, and OSI represents the TOS/TAS ratio. Data are shown as mean ± SEM (n = 3), with statistical significance indicated at *P < 0.05.

Supplementary Files

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