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Downregulation of Cyclin-D, Wnt3a, and C-Myc in prostate cancer by dose-dependent combination of concentrated marine minerals and curcumin

Syafika Alaydrus[1*], Sriwidodo Sriwidodo[2], Ajeng Diantini[3], Riezki Amalia[3], Wahyuni Wahyuni[4], Ayu Wulandari[5]

[1]Department of Pharmacology and Clinical Pharmacy, STIFA Pelita Mas Palu, Palu, Indonesia.
[2]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Universitas Padjadjaran, Bandung, Indonesia.
[3]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Bandung, Indonesia.
[4]Faculty of Pharmacy, Halu Oleo University, Kendari, Indonesia.
[5]Departments of Technology Pharmacy, STIFA Pelita Mas Palu, Palu, Indonesia.

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ABSTRACT

Prostate cancer presents a significant global health challenge, often exhibiting resistance to chemotherapy drugs and causing severe side effects from conventional treatments. These side effects include toxicity to normal cells and mineral deficiencies, which can lead to complications such as acute diarrhea, electrolyte imbalances, and chemotherapy-induced peripheral neuropathy. Natural compounds like curcumin offer promising synergistic anticancer properties with relatively low toxicity and can reduce co-delivered drug resistance. Concurrently, concentrated marine mineral (CMM) solutions, rich in essential minerals, are being explored as adjunct therapies to mitigate chemotherapy-induced mineral deficiencies and potentially enhance curcumin's efficacy and uptake. This study evaluates the comparative cytotoxic effects of curcumin, CMM, and their combination against DU145 prostate cancer cells and HEK293 normal kidney cells, using cisplatin as a benchmark. Curcumin and CMM demonstrate potent inhibition of DU145 cells, classifying them as highly active while showing reduced cytotoxicity towards HEK293 cells compared to cisplatin. Combining curcumin and CMM enhances cytotoxicity against prostate cancer cells while mitigating toxicity to normal cells. Moreover, the combined treatment effectively downregulates Cyclin-D1, Wnt3a, and C-Myc expression in prostate cancer cells, with optimal effects observed at a 5 ppm curcumin and 5 ppm CMM ratio. These results underscore the potential of curcumin and CMM as a synergistic therapeutic strategy for prostate cancer, offering enhanced efficacy and reduced side effects compared to conventional cisplatin chemotherapy.

INTRODUCTION

Prostate cancer (PCa) is a leading cause of mortality among men, with an approximate lifetime risk of diagnosis reaching 12.5% [1]. The number of new cases is expected to rise from 1.4 million in 2020 to 2.9 million in 2040, accounting for 15% of all cancer cases [2]. A major challenge in PCa management is its tendency to develop resistance to multiple treatments [3–5]. This includes alterations in the androgen receptor (AR) pathway, such as AR amplification, mutations, and the emergence of AR splice variants that remain active even in the absence of androgens [6]. Additionally, cancer cells can produce their androgens or become androgen-independent, activate alternative growth pathways, and evade apoptosis [7,8]. This resistance leads to limited treatment options, increased morbidity and mortality, and a significant decline in patients' quality of life.

Recent studies have highlighted the potential of curcumin in overcoming drug resistance and re-sensitizing cancer cells to chemotherapeutic drugs [9]. Curcumin is reported to increase doxorubicin efficacy in castration-resistant PCa treatment by enhancing apoptosis, inducing endoplasmic

*Corresponding Author
Syafika Alaydrus, Departments of Pharmacology and Clinical Pharmacy, STIFA Pelita Mas Palu, Palu, Indonesia.
E-mail: syafikaalaydrus39 @ gmail.com

reticulum stress, and inhibiting survival pathways [10]. While PCa cells (DU145, LNCaP, and PC-3) are generally resistant to TRAIL, curcumin can make them more susceptible to TRAIL-induced apoptosis [11–13]. When used alongside radiation, curcumin markedly enhances the effectiveness of radiation by inducing apoptosis and increasing clonogenic inhibition. This synergy adjusts the Bax/Bcl2 ratio and triggers the activation of cytochrome c, caspase-9, and caspase-3, demonstrating that curcumin effectively increases the sensitivity of PCa cells to radiation [14]. In addition to being used in combination therapies, curcumin exhibits potent effects when used as a single agent. Studies have shown that curcumin lowers AR expression, prevents AR from binding to the androgen response element on the prostate-specific antigen (PSA) gene, and reduces PSA levels in LNCaP cells [15]. It enhances the expression of microRNA miR-30a-5p and reduces the expression of PCNA clamp-associated factor, showing that curcumin can suppress the malignant biological behaviors of PCa [16]. However, despite the potential benefits of these combination therapies, patients undergoing chemotherapy frequently suffer from mineral deficiencies in magnesium, potassium, sodium, zinc, and iron, which are crucial for maintaining various physiological functions [17]. Significant metabolic damage might still occur if mineral consumption exceeds the recommended daily amount [18]. These deficiencies can lead to complications such as diarrhea, electrolyte imbalances, and chemotherapy-induced peripheral neuropathy (CIPN) [19,20]. The disruption of these minerals can exacerbate the patient's overall health condition, leading to additional treatments and longer recovery times.

Concentrated marine mineral (CMM) solutions, rich in essential minerals such as magnesium, potassium, and calcium, have been proposed as adjunct therapies to address the mineral deficiencies caused by chemotherapy [21]. These solutions are even suggested to have a role in inhibiting the metastatic potential of breast cancer [22]. Additionally, the complexation between curcumin and certain minerals can improve solubility and enhance cellular uptake [23]. Consequently, combining curcumin and CMM might yield more optimal cancer therapeutic effects while minimizing the side effects of co-administered chemotherapy drugs. The potential synergistic effects of combining curcumin with CMM have not been thoroughly explored, prompting us to investigate how this combination influences cancerous and normal cells compared to standard treatments. This study aims to compare the cytotoxic effects of curcumin and CMM and their combination against prostate cancer cells (DU145) and normal kidney cells (HEK293) relative to cisplatin. This research explored the mechanisms of action of curcumin and CMM, particularly in terms of gene expression changes related to cancer cell proliferation and survival, such as Cyclin-D1, Wnt3a, and C-Myc. Additionally, we aim to identify the optimal ratios of curcumin and CMM that maximize anticancer efficacy while minimizing toxicity to normal cells.

METHODS

Materials

In this study, we used DU145 and HEK-293 cell lines from the Cellular and Molecular Biology Laboratory, Faculty of Pharmacy, Universitas Padjadjaran. We also used WST-8, PBS, fetal bovine serum (Sigma), Dulbecco's modified eagle medium (DMEM) with high glucose (Sigma), penicillin-streptomycin (Sigma), TrypLE trypsin (Gibco), phosphate buffer saline 10X (Lonza), KAPA SYBR FAST One-Step Qrt-PCR Master Mix (2x) Universal (Kapa Biosystem), and SensiFASTTM SYBR® No-ROX One-Step Kit (Meridian Bioscience). Curcumin used in this study was obtained from Punca Loka Nusantara, with a purity of less than 95%. The sea mineral concentrate was sourced from Pamekasan, Madura, with sampling points located approximately 500–750 m from the coastline and at a depth of about 1–1.1 m [24,25].

Suspension preparation

In preparing the combination of sea mineral concentrate and curcumin, CMM and curcumin were weighed according to the amounts specified in Table 1. Curcumin was then dissolved using Span 60, and CMM and distilled water were added. The mixture was stirred and heated until a homogeneous solution was formed, and then distilled water was added to make up to 30 ml.

In vitro cytotoxicity

Cell culture

The DU145 prostate cancer cell line and the HEK-293 normal cell line were grown in DMEM with 10% FBS, 100 IU/ml penicillin, and 10 µg/ml streptomycin, maintained at 37°C in a 5% CO_2 environment. The flasks were incubated until cells reached 80%–90% confluence (~48–72 hours). Cells were harvested by adding 1–2 ml of 0.25% trypsin for 3–5 minutes at 37°C. The trypsinized cells were transferred to conical tubes, and DMEM was added to 10 ml. Cells were centrifuged for 5 minutes at 2,000 rpm. Following removal of the supernatant, the pellet was resuspended in 1 ml of medium, and cell numbers were measured with a hemocytometer.

Table 1. Suspension formula of curcumin and CMM mix.

No.	Material	Function	Formula											
			A	B	C	D	E	F	G	H	I	J	K	L
1.	Curcumin (ppm)	Active compound	12.5	12.5	12.5	12.5	25	25	25	25	50	50	50	50
2.	CMM (ppm)	Active compound	0	100	500	1000	0	100	500	1000	0	100	500	1000
3.	Span 60 (% v/v)	Surfactant	1.212											
4.	Aquadest	Solvent	ad 100%											

Cell treatment

The test samples were assessed for their effects on the DU145 prostate cancer cell line and the HEK-293 normal cell line using the WST-8 assay. The cells were grown in DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. Cells were plated in 96-well plates and incubated at 37°C with 5% CO_2 for 24 hours. After this incubation period, the medium was replaced with fresh culture medium, and the samples, in varying concentrations, were introduced, with cisplatin as a positive control and DMSO as a blank. Following another 24 hours incubation, WST-8 reagent was added, and the plates were incubated for 2–4 hours. Absorbance was then measured at 450 nm using a Tecan Infinite spectrophotometer. The cell viability rate was calculated based on Equation 1.

$$Survival\ rate\ (\%) = \frac{Absorbance_{Sample} - Absorbance_{Blanco}}{Absorbance_{Negative\ Control} - Absorbance_{Blanco}} \times 100\%$$

Quantitative real-time PCR (qRT-PCR) analysis

***RNA* Isolation**

Cell isolation using GeneZolTM follows the protocol below. Cells that have undergone seeding and treatment were subsequently detached and harvested by adding 750 µl of Ribozol to the surface of the 6-well plate. Pipetting was performed to resuspend the Ribozol and ensure the cells were uniformly detached from the plate surface. Cells harvested with Ribozol were then transferred to a 1.5 ml microtube for storage at −80°C or for immediate isolation.

The next step was phase separation, where 100 µl of chloroform was added to the cell-Ribozol mixture and vortexed for 30 minutes until homogeneous. The lysate was then centrifuged at 12,000–16,000 g for 15 minutes. After centrifugation, three distinct phases were observed: an aqueous phase (transparent), containing RNA; an interphase (white), containing DNA; and an organic phase (pink), consisting of Ribozol, chloroform, organic molecules like lipids, carbohydrates, and proteins separated after cell lysis. The aqueous phase containing RNA was transferred to a new sterile 1.5 ml microtube, while the remaining microtube was added with 100 µl of nuclease free water (NFW) and centrifuged again at the same speed and duration. The newly formed aqueous phase was combined with the previous one in the same microtube.

Next, RNA precipitation was performed by adding isopropanol in a volume equal to the volume of the aqueous phase obtained. The microtube was vortexed and homogenized by inverting several times, then incubated for 10 minutes. After incubation, the microtube was centrifuged at 12,000–16,000 g for 10 minutes. The RNA pellet was formed at the bottom and sides of the tube. The pellet was washed with 500 µl of ethanol, vortexed, and centrifuged at 12,000–16,000 g for 5 minutes. This RNA washing process was repeated 2–3 times. After the final wash, the supernatant was carefully removed without disturbing the pellet, and the pellet was air-dried for 5–10 minutes. The pellet should not be over-dried to maintain its solubility.

The final step was RNA resuspension or pellet dissolution using 20–50 µl of NFW, depending on the pellet size obtained. If a large pellet was visible, a larger volume of NFW was used for RNA dissolution, while a smaller pellet required less NFW. After adding NFW, the microtube was vortexed for 15 seconds until homogeneous and then incubated at 55°C–60°C for 10–15 minutes to ensure the RNA was fully dissolved in NFW. The isolated RNA sample was then stored at −70°C until used.

Gene expression analysis

The reverse transcription reaction and quantitative analysis were carried out using two kits, namely KAPA SYBR FAST One-Step Qrt-PCR Master Mix (2x) Universal and SensiFASTTM SYBR® No-ROX One-Step Kit with a volume of 20 µl for each reaction. The primer sequences used (Macrogen) can be seen in Table 2.

A final volume of 20 µl KAPA Biosystem (without RNA template) was prepared using PCR-grade water. KAPA SYBR FAST qPCR Master Mix (2X) was added to reach a 1X concentration, accounting for 10 µl. Optional 10 mM dUTP was added at a final concentration of 0.2 mM, contributing 0.4 µl. The forward and reverse primers, each at a concentration of 200 µM, were added in volumes of 0.4 µl. Additionally, 50X KAPA RT Mix was incorporated at a 1X concentration, totaling 0.4 µl. Template RNA (1 pg) was added, contributing 2 µl to the mixture.

The Meridian Bioscience kit (without template RNA) was made by mixing the SensiFASTTM SYBr® No-ROX One-Step Mix at a final concentration of 1X in 10 µl total volume, forward and reverse primers at 400 µM each (0.8 µl each), reverse transcriptase (0.2 µl), RiboSafe RNAse Inhibitor (0.4

Table 2. Primer PCR used in the gen expression analysis.

Gen	Primer	Annealing temperature (°C)	References
C-Myc	F:5'-CGCGGATCCCTGGATTTTTTTCGGGTAGTG-3'	58	Jiang *et al*. [26]
	R:5'-CCGCTCGAGCGCACAAGAGTTCCGTAGCT-3'		
β-actin	F:5'-CAAGAGATGGCCACGGCTGCT-3'	60	Takeuchi *et al.* [27]
	R:5'-TCCTTCTGCATCCTGTCGGCA-3'		
Cyclin D1	F:5'-CCGCCTCACACGCTFCCTCTC-3'	60	Jiang *et al.*
	R:5'-TCCTCCTCGCGGCCTTGGGG-3'		

µl), and PCR-grade water added to reach a final volume of 18 µl. Template RNA, ranging from 1 pg to 1 µg, was added at 2 µl.

Thermal cycling

The reaction mixture, totaling 18 µl, was aliquoted into PCR tubes. Subsequently, 2 µl of template RNA (ranging from 1 pg to 1 µg RNA per 20 µl) was added to each tube. For the KAPA Biosystem protocol, reverse transcription was conducted at 42°C for 5 minutes to synthesize cDNA. This was followed by enzyme activation at 95°C for 3 minutes to prepare the enzymes for PCR. Denaturation was performed at 95°C for 1–3 seconds to separate the DNA strands. Annealing, extension, and data collection were carried out, with annealing occurring at 60°C for ≥ 20 seconds. On the other hand, using the Meridian Bioscience protocol, reverse transcription was conducted at 45°C for 10 minutes. Enzyme activation followed at 95°C for 2 minutes to ensure optimal enzyme performance. Denaturation was then performed at 95°C for 5 seconds to denature the DNA. Annealing occurred at 60°C for 10 seconds, followed by extension at 72°C for 5 seconds to elongate the DNA strands.

Statistical analysis

The data are expressed as mean ± standard error of the mean. Statistical analysis was performed using GraphPad Prism software version 9.0.0. One-way analysis of variance followed by Tukey's post hoc test was conducted to assess statistical significance. These analyses evaluated the differences in % survival rate and gene expression values across the groups tested. A *p*-value of < 0.05 was considered statistically significant.

RESULTS

***In vitro* cytotoxicity of individual material**

The results in Table 3 indicated that curcumin and CMM exhibited better inhibitory activity than cisplatin against DU145 prostate cancer cells, categorizing it as highly active. Compared to their effects on DU145 cells, higher IC_{50} values of curcumin and CMM against HEK293 cells indicate reduced cytotoxic effects, suggesting that these test samples exert less influence on normal cells than the reference control. Cisplatin demonstrated the lowest IC_{50} value on HEK293 cells, lower than that observed on DU145 cells, highlighting its significant cytotoxic impact on normal kidney cells. Conversely, CMMs exhibited the highest IC_{50} value at 49.36 ppm, indicating minimal impact on HEK293 normal cells.

***In vitro* toxicity of mixed curcumin and concentrated marine mineral**

In the IC_{50} test, it was observed that curcumin exhibited stronger effects individually compared to CMM. However, in combination form, the suspension of curcumin and CMM resulted in higher cytotoxicity on prostate cancer cells across all mixture ratios compared to the single curcumin group at all concentrations (12.5, 25, and 50 ppm). This indicates that combining curcumin and

Table 3. Cytotoxicity of curcumin, CMM, and cisplatin against DU145 and HEK293 cells.

Compound	IC_{50} (ppm)	Cytotoxicity class[a]
Against DU145		
Curcumin	2.60	Highly active
Concentrated marine mineral	8.64	Highly active
Cisplatin	16. 79	Active
Against HEK293		
Curcumin	8.15	Highly active
Concentrated marine mineral	49.36	Active
Cisplatin	2.00	Highly active

Note: [a]The cytotoxic activity against cancer cells was categorized as follows: IC_{50} values ≤ 10 mg/l were considered highly active, IC_{50} values ≤ 10—100 mg/l were considered active, IC_{50} values of 100—500 mg/l were considered moderately active, and IC_{50} values > 500 mg/l were considered less active [28].

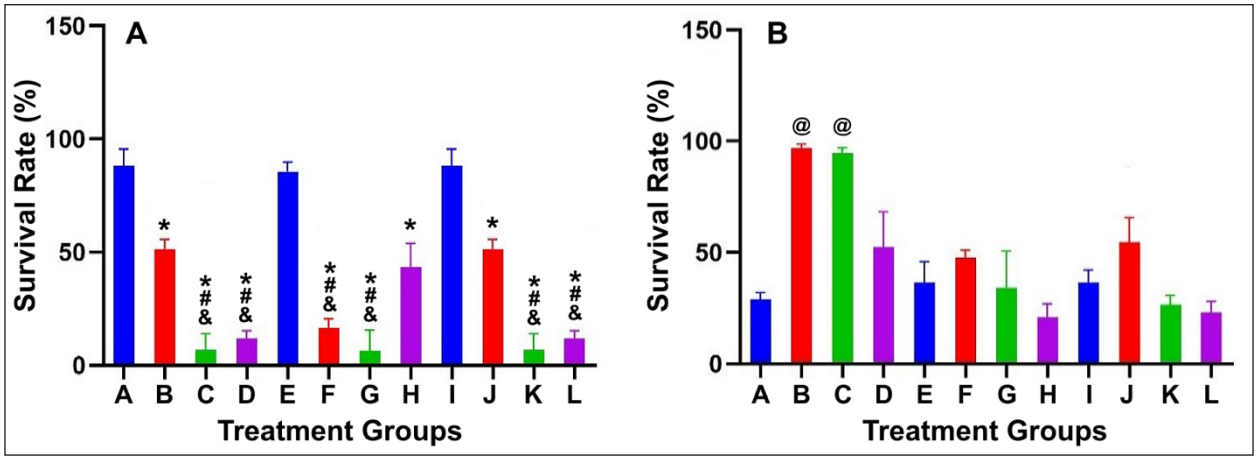

Figure 1. Cell survival rate (%) of (A) DU145 and (B) HEK293 treated with curcumin and CMM suspension. CMM stands for a CMM.

Note: (*, $p < 0.01$) shows a significant difference compared to curcumin alone group (A, E, I); (#, $p < 0.01$) compared to group B and J; (&, $p < 0.01$) compared to group H; and (@, $p < 0.01$) compared to other groups.

CMM therapy is superior to curcumin alone. Increasing the ratio of curcumin in the mixture did not show better toxic effects. However, increasing the concentration of CMM in the mixture ratio demonstrated more potent toxic effects in several formulations across different ratios of curcumin concentrations (Fig. 1A).

In HEK293 cells, toxic effects were observed in all single curcumin groups at various concentrations. However, cell survival rates significantly increased in the mixture of 12.5 ppm curcumin and 100–500 ppm CMM. This pattern was also observed in groups B, F, and J (Fig. 1B). This indicates that adding CMM can reduce curcumin's toxic effects on normal cells. Adding more CMM (>500 ppm) did not improve normal cell safety. Therefore, using CMM in combinations needs to ensure appropriate mixture ratios.

Cytotoxic selectivity index

The selectivity of anticancer drugs can be measured by calculating the IC_{50} of the compound/mixture on normal cells divided by its IC_{50} value on cancer cells, with a selectivity index >10 indicating high selectivity [29]. Based on Table 4, it can be seen that the selectivity of the suspension of curcumin and CMM mixture significantly increased compared to its single treatments, both at 100 and 500 ppm CMM. The high selectivity of the combined test substances indicates the potential of marine mineral concentrate and curcumin combination as safe chemopreventive agents.

Downregulation of Cyclin-D, Wnt3a, and C-Myc

Based on IC_{50} observations and cell survival rates, the optimal dose for killing prostate cancer cells while maintaining normal cell viability was 12.5 ppm curcumin and 100 ppm CMM. However, the dose used at this stage was reduced to ensure more apparent gene expression observations so that the cells do not die and continue expressing their genes. Therefore, the combination dose set was 2.5–5 ppm for curcumin and 5–12.5 ppm for CMM.

In Figure 2, it can be seen that all test samples and positive controls show the ability to reduce the expression of Cyclin-D, Wnt3a, and C-Myc to near-normal values (healthy cells). For Cyclin-D and C-Myc (Fig. 2A and C), although there was a significant decrease, the cisplatin treatment group remains significantly higher compared to the normal control group, with the cisplatin group also substantially higher than all other test groups in terms of C-Myc gene expression. Only the single curcumin group (2.5 and 5 ppm), the single CMM group at 5 ppm, and the combination of 5 ppm curcumin with both doses of CMM (5 and 12.5 ppm) experienced a decrease in the expression of all tested genes. It did not show significant differences from the normal control values. Based on this finding, effective mixture dosage is CMM 5 ppm + curcumin 5 ppm.

Table 4. Cytotoxic selectivity index of curcumin, CMM, and their combination.

Material	Selectivity index
Cisplatin	0.77
Curcumin	3.13
CMM	5.71
Suspension of 12.5 curcumin + 100 ppm CMM	237.45
Suspension of 12.5 curcumin + 500 ppm CMM	234.01

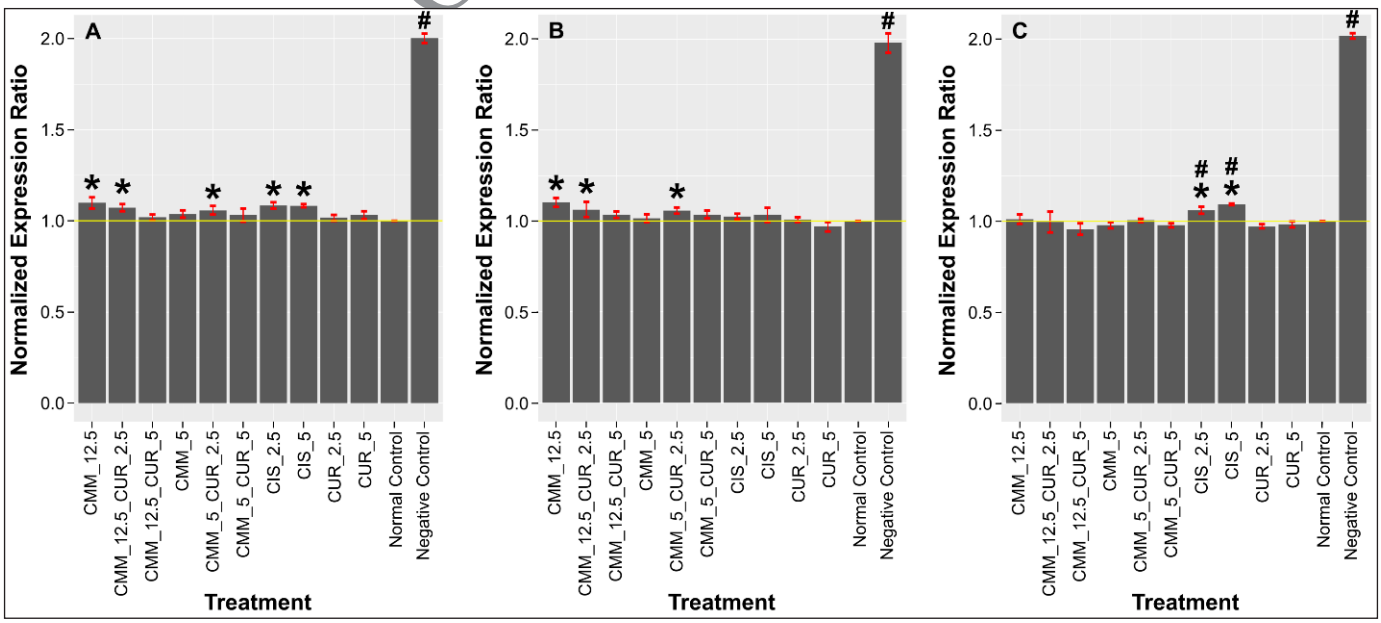

Figure 2. Impact of curcumin, concentrated deepsea mineral (CMM), and their combination on (A) Cyclin-D1, (B) Wnt3a, and (C) C-Myc level in normalized value.

Note: (*, $p < 0.05$) indicates a significant difference compared to the normal control group. (#, $p < 0.05$) indicates a significant difference from other groups. The yellow line is the normalized expression ratio of normal cells.

DISCUSSION

Cancer therapy generally can cause mineral deficiencies in the body, leading to side effects associated with these deficiencies, such as diarrhea and other electrolyte disorders [30,31]. The minerals commonly deficient include magnesium, potassium, sodium, zinc, iron, and others [30,32]. Therefore, some studies have been conducted incorporating a single type of mineral in chemotherapy to prevent related side effects. A diet rich in magnesium and calcium during chemotherapy has been reported to reduce the potential for CIPN [19].

CMM contains abundant minerals, with as much as 10.88% pure magnesium that can be isolated [24]. CMM can be obtained through appropriate concentration procedures in a safe, consumable, sterilizable, and non-toxic form [25]. Besides addressing various mineral deficiencies occurring during chemotherapy, CMM also contains several anticancer minerals, such as boron [33,34] and manganese [35,36], which can work synergistically with chemotherapy for both the prevention and treatment of cancer. With these advantages, chemotherapy combined with CMM administration will produce more optimal effects with fewer side effects.

Against prostate cancer (DU145), CMM alone was able to inhibit cell growth by 50% (IC_{50}) with a concentration of only 8.64 ppm (Table 3), categorizing it as a very active anticancer agent, similar to curcumin (IC_{50} = 2.6 ppm). These results suggest that both CMM and curcumin possess significant cytotoxic effects against prostate cancer cells, making them promising candidates for further development as therapeutic agents. The low IC_{50} values indicate that these compounds are effective at relatively small concentrations, which is crucial in reducing potential side effects in clinical settings [37]. Moreover, the high potency of CMM, while slightly less than curcumin, still places it in the very active category, showcasing its potential as a powerful natural anticancer compound. Both CMM and curcumin are much more robust than the positive control, cisplatin (IC_{50} = 16.79 ppm), a widely used chemotherapy drug. Cisplatin's higher IC_{50} in DU145 cells suggests that higher drug concentrations are required to achieve the same cytotoxic effect as CMM or curcumin. This is a critical finding, implying that CMM and curcumin could provide anticancer effects at lower doses, potentially reducing the toxic side effects typically associated with higher dosages of conventional chemotherapy like cisplatin. The ability of natural compounds like CMM and curcumin to outperform traditional chemotherapeutic agents, in this context, highlights the potential for developing less harmful but effective treatments for prostate cancer.

In contrast, in normal kidney cells (HEK293), cisplatin alone demonstrated a strong toxic effect, showing the smallest IC_{50} value compared to curcumin and CMM. This suggests that cisplatin exerts significant cytotoxicity on cancer and healthy, non-cancerous cells. The strong toxicity profile of cisplatin in normal cells correlates with known clinical side effects, such as nephrotoxicity, where kidney damage occurs due to cisplatin treatment [38]. The toxicity in kidney cells from cisplatin therapy causes excessive excretion of minerals, leading to deficiencies and long-term renal complications [39].

On the other hand, CMM and curcumin exhibited much higher IC_{50} values in HEK293 cells, with CMM having the highest IC_{50}, indicating that it is less toxic to normal cells than cisplatin. The lower toxicity of CMM in non-cancerous cells implies a better therapeutic index, meaning that CMM can target cancer cells more selectively while sparing normal cells. This selective cytotoxicity is biologically relevant, as it highlights the potential of CMM as a safer therapeutic option with fewer off-target effects, particularly in minimizing damage to vital organs such as the kidneys. The high tolerance of normal cells to CMM further underscores its promise as an anticancer agent with reduced systemic toxicity, a critical aspect in developing effective yet safe cancer treatments.

In combination with CMM, the cytotoxicity of curcumin significantly increased against prostate cancer cells, while toxicity to normal cells decreased. This indicates that CMM can enhance the toxic selectivity of curcumin, as shown in Table 4. Increasing the amount of curcumin in the mixture did not show an increase in toxicity, but increasing the amount of CMM (up to ≤ 500 ppm) increased its toxicity. This suggests that CMM can improve the chemotherapy efficiency of curcumin. Several mechanisms might coincide, including the complexation between curcumin and minerals in CMM. It has been reported that curcumin complexes with several minerals can increase its selectivity toward mouse tumor cells [40,41]. Gallium in CMM that can form complexes with curcumin and curcuminoids has also been reported to significantly increase cell uptake [42].

Curcumin, CMM, and cisplatin alone were shown to significantly reduce the expression of Cyclin-D1, Wnt3a, and C-Myc in prostate cancer cells. This downregulation is particularly notable, as these genes play vital roles in the proliferation and survival of cancer cells [43] , making them critical targets in cancer therapy. The observed reduction in gene expression can even reach levels comparable to those in normal cells when prostate cancer cells are treated with a combination of curcumin and CMM. Notably, a 5 ppm curcumin and 5 ppm CMM (1:1) produced the most optimal suppression effect for all the genes tested, underscoring the potential synergistic effect between these two compounds in targeting multiple oncogenic pathways.

Cyclin-D1, Wnt3a, and C-Myc are critical regulators involved in the proliferation and survival of cancer cells, particularly in prostate cancer. Cyclin-D1 plays a crucial role in the transition from the G1 to the S phase of the cell cycle, and its overexpression is linked to uncontrolled cell proliferation in various cancers [44] . The downregulation of Cyclin-D1 by natural compounds like curcumin and CMM suggests that these agents may induce cell cycle arrest, limiting cancer cell growth while avoiding the side effects of traditional chemotherapeutics. Similarly, Wnt3a, a pivotal component of the Wnt/β-catenin pathway, is frequently dysregulated in prostate cancer, leading to enhanced tumor growth and metastasis [45] . The reduction of Wnt3a expression by curcumin and CMM highlights their potential to disrupt Wnt signaling, thereby inhibiting tumorigenesis. Moreover, the downregulation of C-Myc, a critical oncogene associated with

aggressive prostate cancer, further underscores the anticancer potential of curcumin and CMM. By targeting C-Myc, these compounds may decrease cancer cells' proliferative and metabolic activity, offering a multi-faceted approach to suppress tumor progression [46].

The study highlights the importance of combination therapy, as the 5 ppm curcumin and 5 ppm CMM ratio produced the most robust suppression of all three genes tested. This synergistic effect likely arises from the ability of curcumin and CMM to target multiple pathways simultaneously, which may be more effective than higher doses of either compound used alone. For instance, CMM at higher doses (12.5 ppm) alone did not significantly inhibit the expression of Cyclin-D1 and Wnt3a ($p > 0.05$), underscoring the dose-dependent nature of CMM's therapeutic effects. However, when combined with 5 ppm curcumin, the downregulation of these genes became significant, highlighting the advantage of combination therapy in achieving more potent anticancer effects with lower doses. This dose dependency and the synergistic relationship between curcumin and CMM are biologically significant because they point to a potential therapeutic strategy that maximizes efficacy while minimizing toxicity. Lower effective doses can reduce the risk of adverse effects commonly seen with higher concentrations of chemotherapy agents like cisplatin. Additionally, targeting multiple oncogenic pathways—Cyclin-D1, Wnt3a, and C-Myc—through combination therapy provides a multi-faceted attack on cancer cells, reducing the likelihood of resistance developing and improving the overall treatment outcome.

Furthermore, the fact that these natural agents can achieve significant gene suppression at relatively low doses highlights their potential as low-toxicity alternatives to traditional chemotherapies. Their ability to selectively target cancer cells while exhibiting reduced cytotoxicity in normal cells (as evidenced by their higher IC_{50} values in normal cells) further underscores the promise of curcumin and CMM as components of prostate cancer therapy. Future studies could explore their role in combination with other therapies to enhance treatment efficacy while minimizing side effects, potentially leading to more holistic and targeted cancer treatment regimens. Although these are preliminary results, an *in vivo* test and clinical studies are necessary to validate our findings.

CONCLUSION

In conclusion, this study demonstrates the potential of combining curcumin and CMM as an effective treatment strategy for prostate cancer. Both compounds show significant cytotoxic effects against DU145 prostate cancer cells, with curcumin exhibiting similar potency to cisplatin but less toxic to normal cells. Combining curcumin and CMM enhances their anticancer effects while reducing harm to healthy cells, suggesting improved therapeutic selectivity compared to cisplatin. Additionally, the synergistic downregulation of key cancer-related genes (Cyclin-D1, Wnt3a, and C-Myc) further supports the efficacy of this combination in targeting multiple pathways involved in prostate cancer progression.

Future studies should focus on optimizing the curcumin-CMM combination, exploring different ratios to maximize the therapeutic benefits while minimizing side effects. *In vivo* animal model validation is also necessary to confirm these findings in a more complex biological setting. Additionally, further investigation into the molecular mechanisms behind the gene suppression observed in this study will provide deeper insights into how curcumin and CMM work together to combat cancer, which could lead to the development of more targeted therapies.

Integrating natural compounds like curcumin with mineral-rich supplements such as CMM represents a promising new direction for prostate cancer treatment. These findings lay the groundwork for future research, potentially leading to safer, more effective therapeutic options that reduce toxicity while enhancing treatment outcomes for prostate cancer patients.

LIST OF ABBREVIATIONS

AR, Androgen receptor; CIPN, Chemotherapy-induced peripheral neuropathy; CMM, Concentrated marine mineral; DMEM, Dulbecco's modified eagle medium; NFW, Nuclease free water; Pca, Prostate cancer; PSA, Prostate-specific antigen

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

PUBLISHER'S NOTE

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USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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