ejpmr, 2025, 12(4), 743-749

SJIF Impact Factor 7.065

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EFFECT OF ZINC PYRITHIONE ON INFLAMMATION-INDUCED EPITHELIAL-MESENCHYMAL TRANSITION IN COLORECTAL CANCER CELL LINES

V. Brahmaiah, G. Deepthi Reddy, B. Y. Kavitha and G. Deepika*

Institute of Genetics and Hospital for Genetic Diseases, Osmania University, Begumpet, Hyderabad, 500016.

***Corresponding Author: Dr. G. Deepika**

Institute of Genetics and Hospital for Genetic Diseases, Osmania University, Begumpet, Hyderabad, 500016.

Article Received on 20/12/2024 **Article Revised on 10/02/2025** **Article Accepted on 21/03/2025**

ABSTRACT

Background: Colorectal cancer is a major global health concern, with metastasis significantly contributing to patient mortality. Inflammation within the tumor microenvironment plays a critical role in promoting CRC progression, notably by inducing the epithelial-mesenchymal transition. EMT is a process where epithelial cells lose their characteristic cell-cell adhesion and gain migratory and invasive properties, facilitating metastasis. Zinc Pyrithione, a coordination complex based on zinc, has demonstrated antimicrobial and anti-proliferative effects. The study revealed that Zinc Pyrithione efficiently reduces epithelial-mesenchymal transition in colorectal cancer cell lines HT-29 and SW480 in the context of inflammation and TGF-β stimulation. **Methods:** Colorectal cancer cell lines SW480 and HT-29 were exposed to inflammatory conditions to induce EMT. Subsequently, the cells were treated with varying concentrations of ZnPT. The expression of epithelial and mesenchymal markers, specifically E-cadherin, N-cadherin, Vimentin, and Snail, was then assessed using Western blot analysis to determine the impact of ZnPT on these key EMT markers. **Results:** ZnPT treatment effectively attenuated inflammation-induced EMT in both SW480 and HT-29 cells. This attenuation was characterized by a reversal of the inflammation-induced changes in EMT marker expression. ZnPT treatment led to an increase in the levels of the epithelial marker E-cadherin, which is typically downregulated during EMT, and a concomitant decrease in the levels of mesenchymal markers N-cadherin, Vimentin, and Snail, which are typically upregulated during EMT. **Conclusion:** ZnPT demonstrates a significant ability to suppress inflammation-induced EMT in CRC cell lines by modulating the expression of key EMT markers. These findings suggest that ZnPT holds promise as a therapeutic agent for preventing CRC metastasis by targeting inflammation-driven EMT processes. Further studies are needed to elucidate the precise mechanisms of action and to evaluate the in vivo efficacy of ZnPT in CRC models. Zinc is an essential trace element, playing important roles in cellular processes. Disruptions in zinc levels can have deleterious effects on cell.

KEYWORD:- Zinc Pyrithione, Colorectal Cancer, Epithelial-Mesenchymal Transition, Metastasis, LPS, TGF-β, Inflammation.

INTRODUCTION

Colorectal cancer ranks as one of the most prevalent malignancies worldwide, posing a significant threat to human health.[1] The progression of CRC is a complex process involving multiple genetic and epigenetic alterations, coupled with intricate interactions within the tumor microenvironment.[2] Metastasis, the spread of cancer cells from the primary tumor to distant sites, remains the leading cause of cancer-related deaths.[3] The EMT is a pivotal process in cancer progression, allowing cancer cells to acquire migratory and invasive properties necessary for metastasis.[4] Inflammatory cytokines, such as TGF-β, TNFα, IL-1, IL-6, and IL-8 activate transcription factors that drive EMT.[4] These transcription factors include Smads, NF-κB, STAT3, Snail, Twist, and Zeb.[4] Understanding the mechanisms that regulate EMT is crucial for developing effective strategies to prevent CRC metastasis. Inflammation has been recognized as a critical player in the development and progression of CRC.[4] Chronic inflammation in the tumor microenvironment can promote tumor initiation, growth, angiogenesis, and metastasis. Inflammatory mediators, such as cytokines and chemokines, can induce EMT in cancer cells, thereby enhancing their invasive potential. Among the various mechanisms driving EMT, the activation of EMT-related transcription factors like Snail, Slug, Twist, and ZEB1/2 plays a crucial role.[5] These transcription factors repress the expression of epithelial markers such as E-cadherin and induce the expression of mesenchymal markers such as N-cadherin and Vimentin. Specifically, the pro-inflammatory cytokine TNFα, which is produced by macrophages and

CD4-positive T lymphocytes, plays a crucial role in EMT, as it upregulates NF-κB signaling pathways, leading to overexpression of transcription factors Snail and Slug. The Wnt signaling pathway is tightly regulated in normal colon stem cells and is one of the most common pathways that is dysregulated in colon cancer, not only in familial cases of CRC but also in the majority of sporadic CRC cases.[5] Wnt signaling induces EMT by inhibiting glycogen synthase kinase-3 to stabilize β-catenin. When β-catenin is active, it translocates to the nucleus to directly activate ZEB1 and SNAI1.[2] The EMT-inducing transcription factors are upregulated directly or indirectly by TGF-β–Smad signaling in the early stages of carcinoma.[6] Lipopolysaccharide is a potent endotoxin derived from the outer membrane of Gram-negative bacteria and is known to trigger a strong inflammatory response in host cells.[7] LPS is also regarded as a crucial factor in postoperative recurrence of CRC and can increase the formation of liver and lung metastasis of CRC.[7] In CRC, increased levels of LPS can induce or contribute to carcinogenesis, tumor progression, invasion, and metastasis.[8] LPS activates the TLR4 inflammatory signaling pathway, playing a crucial role in human cancer invasion and metastasis.[8] The intricate interplay between the gut microbiota and CRC pathogenesis is increasingly recognized, with the microbial composition significantly influencing the trajectory of tumor development and its subsequent progression.[9] When the gut barrier is damaged, it triggers the diffusion of small molecules and bacteria, such as LPS, into the host systemic circulation and causes the expansion of inflammation.[8] LPS induces EMT by binding to the TLR4 receptor, activating downstream signaling pathways, such as NF-κB and MAPK, leading to the upregulation of EMT-related transcription factors and the downregulation of epithelial markers.[7] Zinc Pyrithione, a coordination complex of zinc, has garnered attention for its diverse biological activities, including antimicrobial, antifungal, and anti-proliferative properties. ZnPT has been shown to inhibit the growth of various cancer cells, including CRC cells, through mechanisms involving cell cycle arrest, apoptosis induction, and inhibition of angiogenesis. Interestingly, CXCL8, MMP7, tissue inhibitor of metalloproteinase 1, and components of the epithelial-to-mesenchymal transition were found to be highly expressed in CRC tumors.[10] However, the effect of ZnPT on EMT in CRC cells, particularly in the context of inflammation, remains largely unexplored. In this study, we investigated the effect of ZnPT on LPS-induced EMT in CRC cell lines SW480 and HT-29. We hypothesized that ZnPT could attenuate LPS-induced EMT by modulating the expression of key EMT markers. Our findings demonstrate that ZnPT effectively inhibits LPS-induced EMT in CRC cells by upregulating E-cadherin expression and downregulating N-cadherin, Vimentin, and Snail expression. These results suggest that ZnPT has the potential to be developed as a therapeutic agent for preventing CRC metastasis by targeting inflammation-driven EMT processes.[11][7]

MATERIALS AND METHODS

Cell Culture: Human colorectal cancer cell lines HT-29 and SW480 were obtained from a certified cell repository. Cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin. Cells were maintained at 37°C in a humidified atmosphere with 5% CO_2. Cells were routinely passaged to maintain exponential growth and were used for experiments between passages 5 and 20.

Reagents: Zinc Pyrithione was purchased from Sigma-Aldrich. Stock solutions of ZnPT were prepared in DMSO and stored at -20°C. Lipopolysaccharide from *E. coli* serotype O111:B4 was obtained from Sigma-Aldrich. Transforming Growth Factor-beta was purchased from R&D Systems. Antibodies against E-cadherin, N-cadherin, Vimentin, Snail, and β-actin were sourced from Cell Signaling Technology or equivalent suppliers. All other chemicals and reagents were of analytical grade.

EMT Induction: To induce EMT, cells were seeded at a density of 1×10^6 cells/well in 6-well plates and allowed to adhere overnight. Cells were then pre-treated with LPS (1 µg/mL) or TGF-β (10 ng/mL) for 2 hour. Control cells received no pretreatment.

ZnPT Treatment: After EMT induction, cells were treated with ZnPT at three concentrations (IC12.5, IC25, and IC50) for 24 hours in the same medium. The IC50 values for ZnPT in HT-29 and SW480 cells were previously determined using an MTT assay. Control cells received the vehicle at a concentration equivalent to that used in the ZnPT treatment groups (less than 0.1% v/v).

Protein Extraction and Western blot analysis: Following the 24-hour treatment, cells were washed twice with ice-cold PBS and lysed using RIPA buffer supplemented with protease and phosphatase inhibitors. Cell lysates were incubated on ice for 30 minutes, followed by centrifugation at 12,000 x g for 15 minutes at 4°C. Protein concentration was determined using the Bradford assay. Equal amounts of protein (20-30 µg) were separated by SDS-PAGE on 10% polyacrylamide gels and transferred to PVDF membranes. Membranes were blocked with 5% non-fat milk in TBST (Tris-buffered saline with 0.1% Tween-20) for 1 hour at room temperature and then incubated with primary antibodies overnight at 4°C. The following primary antibodies were used: anti-E-cadherin (1:1000), anti-N-cadherin (1:1000), anti-Vimentin (1:1000), anti-Snail (1:1000), and anti-β-actin (1:5000). After washing three times with TBST, membranes were incubated with HRP-conjugated secondary antibodies (1:5000) for 1 hour at room temperature. Blots were developed using enhanced chemiluminescence reagent and visualized using a ChemiDoc imaging system.[12] Band intensities were quantified using ImageJ software, and protein expression was normalized to β-actin.

Statistical analysis: Data are presented as mean ± standard deviation from at least three independent experiments. Statistical significance between groups was determined by one-way ANOVA followed by Tukey's post hoc test. A p-value < 0.05 was considered statistically significant. Statistical analyses were performed using GraphPad Prism software.

RESULTS

To investigate the therapeutic potential of zinc pyrithione (ZnPT) in modulating inflammation- and growth factor-induced epithelial-mesenchymal transition (EMT), a series of in vitro experiments were conducted using two human colorectal cancer cell lines, SW480 and HT-29. EMT induction was stimulated using lipopolysaccharide (LPS) and transforming growth factor-beta (TGF-β) (a positive control for EMT), and subsequent treatments with various concentrations of ZnPT were evaluated for their ability to reverse EMT characteristics. EMT status was assessed by examining the protein expression levels of E-cadherin (epithelial marker), and N-cadherin, Vimentin, and Snail (mesenchymal markers) through Western blot analysis, followed by densitometric quantification.

LPS-Induced EMT in SW480 and HT-29 Cells

Upon treatment with LPS (1 µg/mL for 24 hours), both cell lines underwent a marked transition toward a mesenchymal phenotype. The western blot results (Figure 1&2) and its quantification shows that, in SW480 cells, E-cadherin expression dramatically decreased to 0.46, while the expression levels of N-cadherin, Vimentin, and Snail increased significantly to 1.37, 2.02, and 2.94 respectively. A similar pattern was observed in HT-29 cells, where E-cadherin declined to 0.69, while N-cadherin, Vimentin, and Snail levels rose to 1.65, 1.4, and 1.93, respectively **(**Table 1&2). These findings clearly demonstrate that LPS effectively induces EMT through inflammatory signaling in both colorectal cancer cell lines.

ZnPT Attenuates LPS-Induced EMT in a Dose-Dependent Manner

ZnPT treatment following LPS stimulation resulted in a concentration-dependent reversal of EMT in both cell lines. The western blot results (Figure 1&2) and its quantification shows that, at the **IC12.5 dose**, E-cadherin expression in SW480 cells improved modestly to 0.77, while mesenchymal markers decreased to 0.44 (N-cadherin), 0.67 (Vimentin), and 1.9 (Snail). In HT-29 cells, E-cadherin increased to 0.69, and N-cadherin, Vimentin, and Snail decreased to 1.65, 1.4, and 1.93 respectively. When treated with **IC25 ZnPT**, a stronger effect was observed. In SW480, E-cadherin expression rose to 0.96, while mesenchymal markers decreased further (N-cadherin: 0.28; Vimentin: 0.34; Snail: 0.68). Correspondingly, HT-29 cells showed E-cadherin at 1.06, with mesenchymal markers reducing to 0.75 (N-cadherin), 0.36 (Vimentin), and 0.53 (Snail). At the highest concentration, **IC50**, ZnPT exhibited maximal suppression of EMT features. In SW480 cells, E-cadherin increased significantly to 1.4, and N-cadherin, Vimentin, and Snail levels declined to 0.12, 0.47, and 0.47 respectively. Similarly, in HT-29 cells, E-cadherin reached 1.5, and mesenchymal markers were substantially downregulated to 0.9 (N-cadherin), 0.11 (Vimentin), and 0.12 (Snail) (Table 1&2). These results confirm that ZnPT effectively reverses LPS-induced EMT in both cell lines in a dose-dependent manner, restoring epithelial characteristics and suppressing mesenchymal transformation.

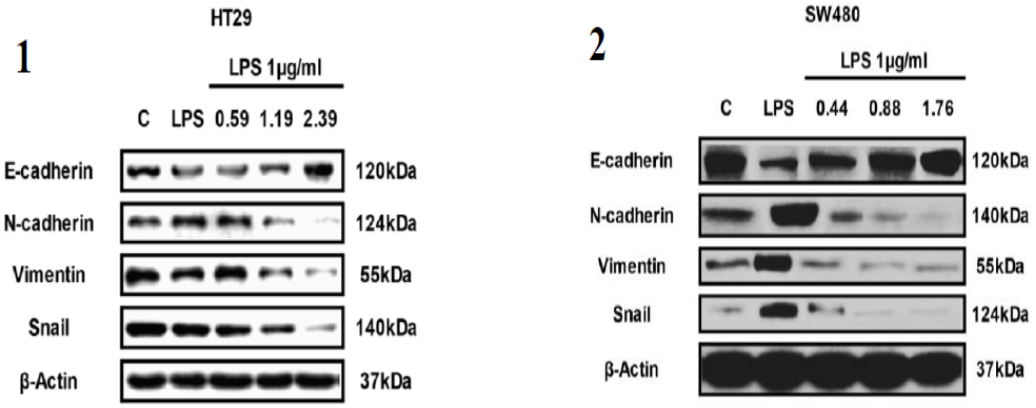

Figure 1 & 2: Western blot analysis showing the effect of ZnPT on LPS-induced EMT in HT-29 and SW480 cells. The figure illustrates the changes in E-cadherin, N-cadherin, Vimentin, and Snail expression. β-actin was used as a loading control.

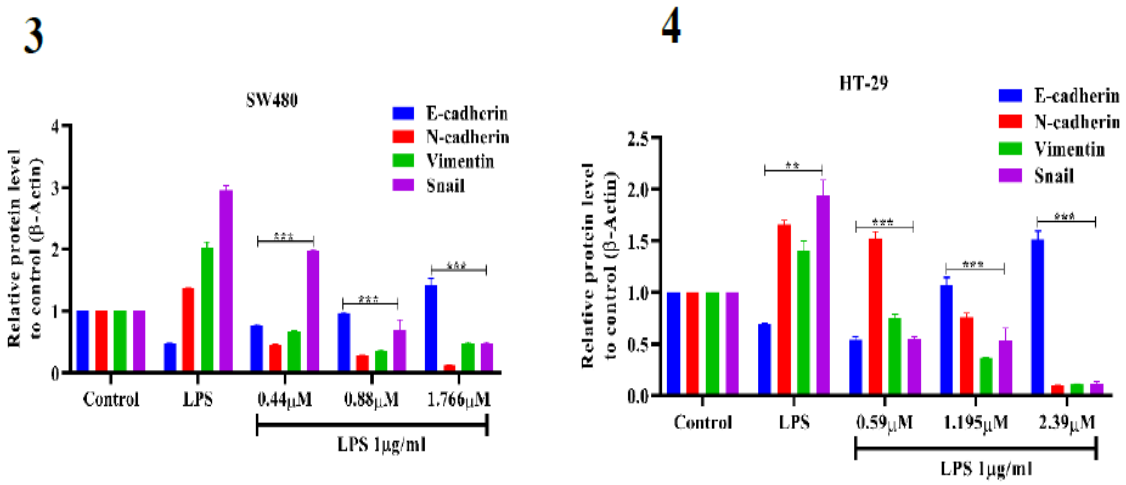

Figure 3&4: Quantitative analysis of EMT marker protein expression in HT-29 and SW480 colorectal cancer cell lines following LPS stimulation and zinc pyrithione (ZnPT) treatment. Bar graphs represent the relative expression levels of E-cadherin, N-cadherin, Vimentin, and Snail proteins normalized to β-Actin. Cells were treated with LPS (1 μg/mL) to induce epithelial-mesenchymal transition (EMT) and subsequently co-treated with increasing concentrations of ZnPT. In both cell lines, LPS exposure significantly downregulated E-cadherin and upregulated mesenchymal markers (N-cadherin, Vimentin, and Snail), indicating induction of EMT. ZnPT treatment restored E-cadherin expression and dose-dependently suppressed mesenchymal marker expression in both cell lines, suggesting its role in reversing EMT. Data represent the mean ± standard deviation (S.D.) from three independent experiments.

Table 1: Relative protein expression levels of EMT markers in HT-29 cells treated with LPS and varying concentrations of zinc pyrithione (ZnPT), normalized to β-Actin. Values represent the mean ± standard deviation (S.D.) from densitometric analysis of Western blot bands using ImageJ software. E-cadherin levels decreased with LPS exposure but increased progressively with ZnPT treatment. Conversely, LPS-induced expression of N-cadherin, Vimentin, and Snail decreased in a dose-dependent manner upon ZnPT treatment, indicating reversal of EMT.

Name of the protein	(HT29) Mean of Relative protein level to control (β-Actin) ± S.D.				
	Control	LPS	0.59μM	1.19μM	2.39μM
E-cadherin	1 ± 0	0.692 ± 0.005	0.538 ± 0.30	1.067 ± 0.075	1.514 ± 0.082
N-cadherin	1 ± 0	1.658 ± 0.04	1.527 ± 0.060	0.756 ± 0.041	0.096 ± 0.005
Vimentin	1 ± 0	1.4 ± 0.1	0.748 ± 0.035	0.361 ± 0.005	0.113 ± 0.002
Snail	1 ± 0	1.93 ± 0.152	0.55 ± 0.013	0.534 ± 0.113	0.121 ± 0.012

Table 2: Relative protein expression levels of EMT markers in SW480 cells treated with LPS and varying concentrations of zinc pyrithione (ZnPT), normalized to β-Actin. Values represent the mean ± standard deviation (S.D.) from densitometric analysis of Western blot bands using ImageJ software. E-cadherin levels decreased with LPS exposure but increased progressively with ZnPT treatment. Conversely, LPS-induced expression of N-cadherin, Vimentin, and Snail decreased in a dose-dependent manner upon ZnPT treatment, indicating reversal of EMT.

Name of the protein	(SW480) Mean of Relative protein level to control (β-Actin) ± S.D.				
	Control	LPS	0.44μM	0.88μM	1.76μM
E-cadherin	1 ± 0	0.465 ± 0.021	0.77 ± 0.017	0.960 ± 0.014	1.413 ± 0.120
N-cadherin	1 ± 0	1.371 ± 0.010	0.449 ± 0.008	0.286 ± 0.003	0.127 ± 0.001
Vimentin	1 ± 0	2.022 ± 0.095	0.675 ± 0.010	0.348 ± 0.018	0.473 ± 0.021
Snail	1 ± 0	2.947 ± 0.089	1.970 ± 0.021	0.687 ± 0.164	0.474 ± 0.020

TGF-β (A positive control for EMT induction)-Induced EMT and Its Reversal by ZnPT

To further validate ZnPT's anti-EMT effect in a non-inflammatory context, EMT was induced using TGF-β a positive control (1 μg/mL for 24 hours). The western blot results (Figure 5&6) and its quantification shows that the following TGF-β treatment, SW480 cells showed a sharp reduction in E-cadherin expression to 0.82, with a simultaneous increase in N-cadherin (0.1.68), Vimentin (2.46), and Snail (2.11). HT-29 cells followed a similar trend, with E-cadherin declining to 0.28, while mesenchymal markers were elevated to 5.95 (N-cadherin), 4.69 (Vimentin), and 3.68 (Snail), indicating strong induction of EMT. Treatment with ZnPT at IC12.5 led to partial restoration of epithelial features. In SW480, E-cadherin rose to 1.11, and mesenchymal markers decreased to 0.88 (N-cadherin), 1.63 (Vimentin), and 1.96 (Snail). HT-29 cells showed similar

improvements, with E-cadherin at 0.84 and reductions in N-cadherin (3.78), Vimentin (3.0), and Snail (0.60). At the IC25 dose, a more pronounced reversal of EMT was noted. In SW480 cells, E-cadherin increased to 1.85, and N-cadherin, Vimentin, and Snail were reduced to 0.93, 1.24, and 1.35, respectively. HT-29 cells demonstrated similar trends, with E-cadherin at 2.13, and mesenchymal markers decreasing to 1.0, 1.73, and 0.65, respectively. With IC50 ZnPT treatment, maximal epithelial recovery and mesenchymal suppression were observed in both cell lines. SW480 cells exhibited E-cadherin expression at 2.34 and significant downregulation of N-cadherin (0.56), Vimentin (0.55), and Snail (0.67). HT-29 cells reflected consistent effects, with E-cadherin at 3.46 and N-cadherin, Vimentin, and Snail reduced to 0.55, 0.32, and 0.29, respectively (Table 3&4).

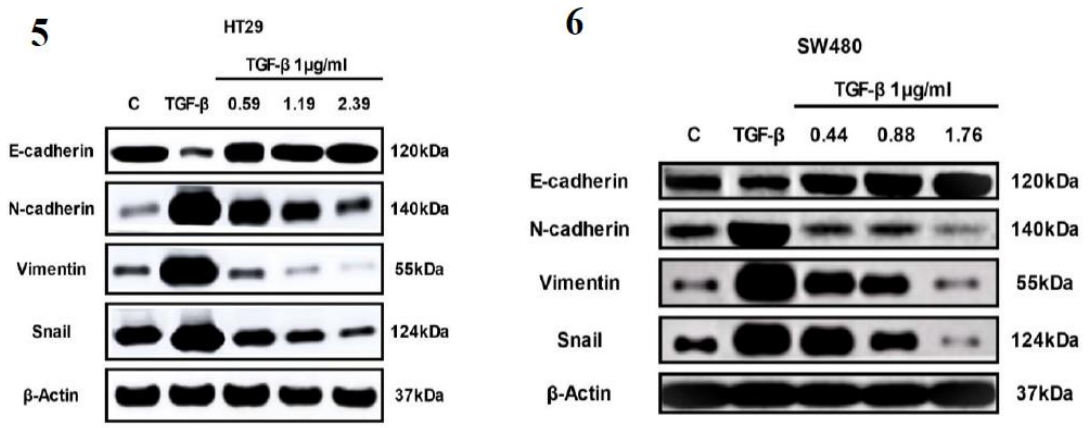

Figure 5&6: Western blot analysis showing the effect of ZnPT on a positive control, TGF-β-induced EMT in HT-29 and SW480 cells. The figure illustrates the changes in E-cadherin, N-cadherin, Vimentin, and Snail expression. β-actin was used as a loading control.

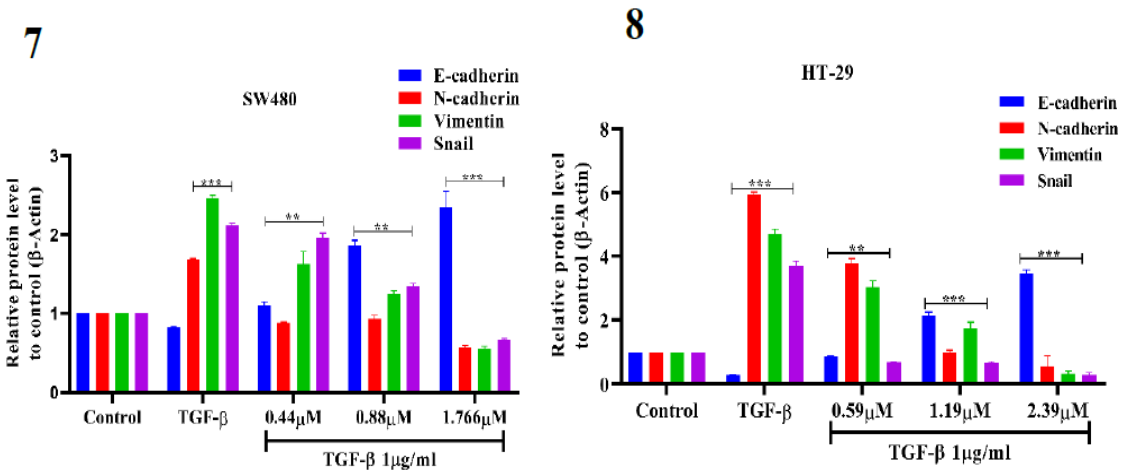

Figure 7&8: Quantitative analysis of EMT marker protein expression in HT-29 and SW480 colorectal cancer cell lines following a EMT positive control, TGF-β stimulation and zinc pyrithione (ZnPT) treatment. Bar graphs represent the relative expression levels of E-cadherin, N-cadherin, Vimentin, and Snail proteins normalized to β-Actin. Cells were treated with TGF-β (1 µg/mL) to induce epithelial-mesenchymal transition (EMT) and subsequently co-treated with increasing concentrations of ZnPT. In both cell lines, TGF-β exposure significantly downregulated E-cadherin and upregulated mesenchymal markers (N-cadherin, Vimentin, and Snail), indicating induction of EMT. ZnPT treatment restored E-cadherin expression and dose-dependently suppressed mesenchymal marker expression in both cell lines, suggesting its role in reversing EMT. Data represent the mean ± standard deviation (S.D.) from three independent experiments.

Table 3: Relative protein expression levels of EMT markers in HT-29 cells treated with TGF-β and varying concentrations of zinc pyrithione (ZnPT), normalized to β-Actin. Values represent the mean ± standard deviation (S.D.) from densitometric analysis of Western blot bands using ImageJ software. E-cadherin levels decreased with LPS exposure but increased progressively with ZnPT treatment. Conversely, TGF-β -induced expression of N-cadherin, Vimentin, and Snail decreased in a dose-dependent manner upon ZnPT treatment, indicating reversal of EMT.

Name of the protein	(SW480) Mean of Relative protein level to control (β-Actin) ± S.D.				
	Control	TGF-β	0.44µM	0.88µM	1.76µM
E-cadherin	1 ± 0	0.825 ± 0.004	1.112 ± 0.033	1.858 ± 0.070	2.344 ± 0.206
N-cadherin	1 ± 0	1.68 ± 0.012	0.883 ± 0.006	0.937 ± 0.037	0.567 ± 0.025
Vimentin	1 ± 0	2.463 ± 0.033	1.631 ± 0.154	1.245 ± 0.043	0.552 ± 0.032
Snail	1 ± 0	2.119 ± 0.026	1.966 ± 0.051	1.357 ± 0.020	0.670 ± 0.012

Table 4: Relative protein expression levels of EMT markers in SW480 cells treated with TGF-β and varying concentrations of zinc pyrithione (ZnPT), normalized to β-Actin. Values represent the mean ± standard deviation (S.D.) from densitometric analysis of Western blot bands using ImageJ software. E-cadherin levels decreased with LPS exposure but increased progressively with ZnPT treatment. Conversely, TGF-β -induced expression of N-cadherin, Vimentin, and Snail decreased in a dose-dependent manner upon ZnPT treatment, indicating reversal of EMT.

Name of the protein	(HT29) Mean of Relative protein level to control (β-Actin) ± S.D.				
	Control	TGF-β	0.59µM	1.19µM	2.39µM
E-cadherin	1 ± 0	0.282 ± 0.007	0.840 ± 0.038	2.138 ± 0.104	3.468 ± 0.105
N-cadherin	1 ± 0	5.952 ± 0.057	3.780 ± 0.14	1.0 ± 0.048	0.551 ± 0.322
Vimentin	1 ± 0	4.692 ± 0.159	3.029 ± 0.209	1.735 ±0.200	0.323 ± 0.086
Snail	1 ± 0	3.687 ± 0.163	0.675 ± 0.017	0.655 ± 0.033	0.291 ± 0.063

DISCUSSION

This study provides compelling evidence for the ability of Zinc Pyrithione to reverse epithelial-mesenchymal transition induced by lipopolysaccharide and transforming growth factor-β in colorectal cancer cells. Our key findings demonstrate that ZnPT effectively restores epithelial characteristics and suppresses mesenchymal features in both HT-29 and SW480 cell line. These results strongly suggest that ZnPT holds promise as a therapeutic agent for preventing CRC metastasis by targeting inflammation-driven EMT processes. The induction of EMT by LPS and TGF-β is a well-established phenomenon in CRC.[7] LPS, acting through the TLR4/NF-κB pathway, initiates a cascade of events that lead to the downregulation of E-cadherin and the upregulation of mesenchymal markers. In CRC cells, TLR4 activation by LPS can promote cancer invasion and metastasis.[7] Similarly, TGF-β, a potent inducer of EMT, promotes Smad-mediated transcriptional reprogramming, resulting in a loss of epithelial cell adhesion and an increased capacity for migration and invasion. Our results corroborate these established findings, as evidenced by the decreased expression of E-cadherin and increased expression of N-cadherin, Vimentin, and Snail in LPS/TGF-β-treated cells. These EMT-related protein expression changes are indicative of a shift from an epithelial to a mesenchymal phenotype.[14] The capacity of ZnPT to reverse these EMT-associated changes is particularly noteworthy. ZnPT treatment led to a dose-dependent restoration of E-cadherin expression and a concurrent suppression of mesenchymal markers. This suggests that ZnPT interferes with the signaling pathways that drive EMT in the context of inflammation and TGF-β stimulation. It has been shown that zinc supplementation can counteract TGF-β-induced EMT by modulating Smad signaling and restoring E-cadherin expression. Considering that ZnPT is a zinc coordination complex, a comparable mechanism may be in effect. Several studies suggest that ZnPT can disrupt key cellular functions, including mitochondrial membrane integrity and protein synthesis, which could influence the expression of EMT markers. The implications of these findings for CRC therapy are far-reaching. EMT is a critical process in cancer metastasis, enabling tumor cells to detach from the primary tumor, invade surrounding tissues, and colonize distant sites. By reversing EMT, ZnPT may inhibit these processes and prevent the spread of CRC cells. Furthermore, EMT has been linked to drug resistance in cancer.[13],[15] Reversing EMT may, therefore, enhance the sensitivity of CRC cells to chemotherapy and improve treatment outcomes. Several studies align with our findings, underscoring the pro-EMT effects of LPS and TGF-β in CRC models. For instance, Peng et al. demonstrated that LPS facilitates EMT through the TLR4/NF-κB pathway, leading to the downregulation of E-cadherin and the upregulation of N-cadherin and Vimentin in CRC cells.[16],[17] Other studies have shown that compounds like curcumin can counteract LPS-induced EMT in colorectal cancer cells by modulating similar marker expressions.[18] Furthermore, it has been reported that small compounds can efficiently reverse EMT by targeting transcriptional regulators like Snail, a strategy mirrored in our findings where ZnPT dramatically downregulated Snail expression. While our study provides strong evidence for the anti-EMT effects of ZnPT, further research is needed to fully elucidate the underlying mechanisms of action. Future studies could investigate the specific signaling

pathways targeted by ZnPT, as well as the effects of ZnPT on cell migration, invasion, and metastasis *in vivo*. It would also be valuable to explore the potential of ZnPT in combination with other chemotherapeutic agents for the treatment of CRC. Further studies should consider investigating the effects of Zinc on the expression of ZEB1, a critical transcription factor for EMT induction in CRCs.[19] Examination of ZnPT's impact on cell migration and invasion, perhaps through wound-healing assays or transwell migration assays, would provide a more comprehensive understanding of its anti-metastatic potential.

CONCLUSION

our study demonstrates that ZnPT effectively reverses LPS/TGF-β-induced EMT in CRC cells. These findings suggest that ZnPT has potential as a therapeutic agent for preventing CRC metastasis by targeting inflammation-driven EMT processes. Further research is warranted to fully elucidate the mechanisms of action and to evaluate the clinical potential of ZnPT in the treatment of CRC.

REFERENCES

1. G. Collin et al., "Intestinal Epithelial Cells Adapt to Chronic Inflammation through Partial Genetic Reprogramming," Cancers, 2023; 15, 3: 973. doi: 10.3390/cancers15030973.
2. M. A. Sorolla et al., "Microenvironmental Reactive Oxygen Species in Colorectal Cancer: Involved Processes and Therapeutic Opportunities," Cancers, Multidisciplinary Digital Publishing Institute, 2021; 5037, 09: 13-20. doi: 10.3390/cancers13205037.
3. E. Kang, J. Seo, H. Yoon, and S. Cho, "The Post-Translational Regulation of Epithelial–Mesenchymal Transition-Inducing Transcription Factors in Cancer Metastasis," International Journal of Molecular Sciences, Multidisciplinary Digital Publishing Institute, 2021; 3591, 30: 22-7. doi: 10.3390/ijms22073591.
4. I. Chattopadhyay, R. Ambati, and R. Gundamaraju, "Review Article Exploring the Crosstalk between Inflammation and Epithelial- Mesenchymal Transition in Cancer." Jun, 2021; 15.
5. I. Briede, D. Balodis, J. Gardovskis, and I. Strumfa, "Stemness, Inflammation and Epithelial–Mesenchymal Transition in Colorectal Carcinoma: The Intricate Network." Nov, 2021; 29.
6. M. Saitoh, "Transcriptional regulation of EMT transcription factors in cancer," Seminars in Cancer Biology, vol. 97. Elsevier BV, 2023; 21: 05. doi: 10.1016/j.semcancer.2023.10.001.
7. M. Zhang and K. Liu, "Mechanisms of lipopolysaccharides-driven progression and metastasis of colorectal cancer," Authorea (Authorea), 2024, doi: 10.22541/au.172956260.07831670/v1.
8. Q. Li et al., "Gut Barrier Dysfunction and Bacterial Lipopolysaccharides in Colorectal Cancer," Journal of Gastrointestinal Surgery, Springer Science+Business Media, 2023; 1466, 27: 27-7. doi: 10.1007/s11605-023-05654-4.
9. S. Fan, L. Zhou, W. Zhang, D. Wang, and D. Tang, "Role of imbalanced gut microbiota in promoting CRC metastasis: from theory to clinical application," Cell Communication and Signaling, 2024; 22: 1. doi: 10.1186/s12964-024-01615-9.
10. T. Huang, N. Mokgautsi, Y. Huang, A. T. H. Wu, and H.-S. Huang, "Comprehensive Omics Analysis of a Novel Small-Molecule Inhibitor of Chemoresistant Oncogenic Signatures in Colorectal Cancer Cell with Antitumor Effects," Cells, 2021; 10, 8: 1970. doi: 10.3390/cells10081970.
11. A. Iyer, M. R. Shaik, J. Raufman, and G. Xie, "The Roles of Zinc Finger Proteins in Colorectal Cancer," International Journal of Molecular Sciences, Multidisciplinary Digital Publishing Institute, 2023; 10249, 16: 24- 12. doi: 10.3390/ijms241210249.
12. P. Pedrosa et al., "Targeting Cancer Resistance via Multifunctional Gold Nanoparticles," International Journal of Molecular Sciences, 2019; 20, 21: 5510. doi: 10.3390/ijms20215510.
13. Banyard J, Bielenberg DR. The role of EMT and MET in cancer dissemination. Connect Tissue Res, 2015; 56(5): 403-13. doi: 10.3109/03008207.2015.1060970. Epub 2015 Aug 20. PMID: 26291767; PMCID: PMC4780319.
14. Y. Chen et al., "An epithelial-to-mesenchymal transition-inducing potential of granulocyte macrophage colony- stimulating factor in colon cancer." Jun, 2017; 19.
15. Huang, Y., Hong, W. & Wei, X. The molecular mechanisms and therapeutic strategies of EMT in tumor progression and metastasis. *J Hematol Oncol*, 2022; 15: 129. https://doi.org/10.1186/s13045-022-01347-8
16. C. Buhrmann, M. Yazdi, B. Popper, A. B. Kunnumakkara, B. Aggarwal, and M. Shakibaei, "Induction of the Epithelial-to-Mesenchymal Transition of Human Colorectal Cancer by Human TNF-β (Lymphotoxin) and its Reversal by Resveratrol," Nutrients, 2019; 11, 3: 704. doi: 10.3390/nu11030704.
17. H. Li, Y. Li, D. Liu, and J. Liu, "LPS promotes epithelial–mesenchymal transition and activation of TLR4/JNK signaling," Tumor Biology, 2014; 35, 10: 10429. doi: 10.1007/s13277-014-2347-5.
18. H. Wang, X. Cai, and L. Ma, " Curcumin Modifies Epithelial–Mesenchymal Transition in Colorectal Cancer Through Regulation of miR-200c/EPM5 ," Cancer Management and Research, 2020; 9405. doi: 10.2147/cmar.s260129.
19. E. Zeindl-Eberhart et al., "Epithelial-Mesenchymal Transition Induces Endoplasmic-Reticulum-Stress Response in Human Colorectal Tumor Cells," PLoS ONE, 2014; 9: 1. doi: 10.1371/journal.pone.0087386.