Iranian Journal of Microbiology

Volume 18 Number 2 (April 2026) 217-225
DOI: http://doi.org/10.18502/ijm.v18i2.21298

ORIGINAL ARTICLE

The effects of *Salmonella enterica* cell extract on the gene expression of bleomycin hydrolase, puromycin-sensitive aminopeptidase, and thimet oligopeptidase in human colorectal adenocarcinoma cells

Rozalin Mardani[1], Majid Borhaniasl[1], Mohammad Amin Ghatee[1,2], Seyed Abdolmajid Khosravani[1], Kasra Sharifi[3], Khodakaram Jahanbin[4], Asghar Sharifi[1*]

[1]*Cellular and Molecular Research Center, Yasuj University of Medical Sciences, Yasuj, Iran*

[2]*Professor Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*

[3]*Student Research Committee, Medical College, Islamic Azad University, Kazeroon Branch, Kazeroon, Iran*

[4]*Department of Pathology, Legal Medicine Research Center, Legal Medicine Organization, Yasuj, Iran*

Received: October 2024, **Accepted:** February 2026

ABSTRACT

Background and Objectives: *Salmonella enterica* survival depends on evading CD8+ T-cell recognition, a process governed by the MHC Class I antigen presentation pathway. The cytosolic aminopeptidases—bleomycin hydrolase (*BH*), puromycin-sensitive aminopeptidase (*PSA*), and thimet oligopeptidase (*TOP*)—are critical "final trimmers" that generate the precise peptide epitopes required for MHC Class I loading. This study investigated whether *S. enterica* cell extract modulates the transcriptional expression of these key enzymes in HT-29 cells, potentially revealing a mechanism of immune evasion mediated by bacterial structural components.

Materials and Methods: Human colorectal adenocarcinoma HT-29 cells were treated with varying concentrations of *S. enterica* extract. The gene expression levels of *BH*, *PSA*, and *TOP* were quantified using qRT-PCR at multiple time points (6, 12, 24, 48, and 72 hours) post-treatment. Data were statistically analyzed to evaluate significant modulations relative to untreated controls.

Results: The transcriptional response to *S. enterica* extract was highly selective, with *TOP* demonstrating the most robust and rapid induction. Significant upregulation of *TOP* occurred as early as 6 hours post-treatment and reached its maximum induction at 48 hours. Conversely, *BH* levels remained largely indistinguishable from untreated controls. *PSA* expression showed no statistically significant alterations relative to the control group throughout the study. These findings suggest that *S. enterica* components preferentially target the "terminator" peptidase *TOP* to potentially disrupt the MHC Class I antigen presentation pathway.

Conclusion: *S. enterica* cell extract significantly and selectively alters the expression of *TOP*, a critical cytosolic peptidase involved in the final stages of antigen processing. This targeted modulation likely serves as a mechanism for immune evasion by facilitating the destruction of immunogenic epitopes, thereby rendering infected cells less visible to the adaptive immune system.

Keywords: Aminopeptidases; HT-29 cells; *Salmonella enterica*; Cysteine endopeptidases; Metalloendopeptidases

[*]Corresponding author: Asghar Sharifi, Ph.D, Cellular and Molecular Research Center, Yasuj University of Medical Sciences, Yasuj, Iran.
Tel: +98-9171048255 Fax: +98-7433232019 Email: asghar.sharifi@yums.ac.ir

Copyright © 2026 The Authors. Published by Tehran University of Medical Sciences.
This work is licensed under a Creative Commons Attribution-Non Commercial 4.0 International license (https://creativecommons.org/licenses/by-nc/4.0/). Noncommercial uses of the work are permitted, provided the original work is properly cited.

INTRODUCTION

Salmonella enterica is a prominent enteric pathogen capable of causing serious gastrointestinal infections. As a rod-shaped, Gram-negative bacterium that thrives in both aerobic and anaerobic environments (1), it accounts for the bulk of gastrointestinal tract infections, imposing a significant burden on global public health and the economy (2). This bacterium is a significant health concern worldwide, often spreading through contaminated food and water, especially from sources like poultry, eggs, and unwashed produce (1). Infections can range from self-limiting gastroenteritis to severe systemic illnesses like typhoid fever, particularly affecting vulnerable groups such as children, the elderly, and immunocompromised individuals. Each year, millions of people suffer from non-typhoidal *Salmonella* infections, resulting in significant morbidity and deaths (3).

A key feature of *Salmonella* is its ability to invade and survive inside host cells, thereby avoiding detection by the immune system (4). It utilizes a sophisticated toolkit of virulence factors to survive intracellularly and manipulate host processes to evade immune responses (4). One way it achieves this is by affecting the antigen presentation pathway, which is crucial for the immune system to detect and destroy infected cells (5). Antigen presentation involves Major Histocompatibility Complex (MHC) molecules, especially MHC class I, which displays intracellular peptides to cytotoxic T lymphocytes (CTLs) (6). These peptides originate from proteins broken down by the proteasome and are further trimmed by cytosolic aminopeptidases, including bleomycin hydrolase (*BH*), puromycin-sensitive aminopeptidase (*PSA*), and thimet oligopeptidase (*TOP*) (7). The disruption of these enzymes can hinder antigen processing, thereby facilitating the evasion of immune surveillance by pathogens like *Salmonella* (7, 8).

The rise of multidrug-resistant (MDR) strains of *Salmonella* has exacerbated the global health impact of this pathogen (9). Widespread overuse of antibiotics in medicine and agriculture has led to the emergence of strains resistant to multiple common antibiotics, further complicating treatment and increasing healthcare costs and mortality rates (10, 11). Consequently, a comprehensive understanding of the molecular interactions between *Salmonella* and its host is crucial for developing novel therapeutic interventions (12).

Recent research has focused on how *Salmonella* modulates host immune responses (4, 12), yet less is known about its impact on cytosolic aminopeptidases that are essential for antigen processing (13). While the role of the proteasome in antigen processing is well documented, the downstream cytosolic aminopeptidases serve as the final gatekeepers that define the peptide repertoire available for MHC Class I presentation. Dysregulation of these enzymes can lead to the destruction of immunogenic epitopes or the generation of "decoy" peptides, rendering infected cells invisible to cytotoxic T lymphocytes. We hypothesized that *Salmonella* may exploit this pathway to evade immune surveillance. Furthermore, this study utilizes a whole-cell bacterial extract rather than live infection. This experimental design allows us to isolate the effects of Pathogen-Associated Molecular Patterns (PAMPs)—such as LPS and flagellin—on host gene expression, independent of the confounding variables associated with active bacterial invasion, replication, and bacteria-induced cytotoxicity.

MATERIALS AND METHODS

Cell line and culture conditions. The human colorectal adenocarcinoma cell line HT-29 was utilized in this study. Cells were purchased from the Pasteur Institute of Iran and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin. Cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2. For all experiments, cells were seeded into 6-well plates at a density of 1×10^6 cells per well and allowed to adhere for 24 hours prior to treatment (14).

Preparation of *Salmonella enterica* cell extract. The *Salmonella enterica* strain used in this study was ATCC 13076. Bacteria were cultured overnight in Luria-Bertani (LB) broth at 37°C with constant shaking. Bacterial cells were harvested by centrifugation at 5,000 × g for 10 minutes, washed twice with sterile phosphate-buffered saline (PBS), and resuspended in PBS. The bacterial suspension was then sonicated on ice for five cycles (30 seconds on/30 seconds off) using a probe-tip sonicator. The resulting suspension was centrifuged at 10,000 × g for 20 minutes at 4°C to remove insoluble cell debris (15). The supernatant,

containing the soluble bacterial extract, was sterilized using a 0.22 µm filter and stored at -20°C until required.

Treatment of HT-29 cells with *Salmonella* extract. HT-29 cells were treated with *S. enterica* cell extract at various concentrations. The use of cell extract was intended to evaluate the host cellular response to soluble bacterial PAMPs and structural components in the absence of active bacterial replication and type III secretion system-mediated cytotoxicity. The stock extract was diluted in normal saline to achieve final experimental concentrations of 25%, 50%, and 75% (v/v). Untreated cells served as a negative control. All treatments were performed in triplicate to ensure statistical reproducibility (16).

RNA extraction and cDNA synthesis. Total RNA was extracted from HT-29 cells following treatment using the TRIzol reagent (Sina-Gen, Tehran, Iran) according to the manufacturer's instructions. The concentration and purity of the isolated RNA were quantified using a NanoDrop spectrophotometer (Thermo Scientific) by assessing the 260/280 nm ratio. Complementary DNA (cDNA) was synthesized from 1–2 µg of total RNA using the SinaClon First Strand cDNA Synthesis Kit following the manufacturer's protocol (17).

Quantitative real-time PCR (qRT-PCR). The expression levels of *BH*, *PSA*, and *TOP* were quantified using quantitative real-time PCR (qRT-PCR). Primers specific for each target gene and the housekeeping gene GAPDH were designed using Primer3 software or sourced from previous literature (18-21). The full primer sequences, amplicon sizes, and amplification efficiencies are detailed in Table 1 and Fig. 1. The qRT-PCR reactions were carried out using SYBR Green PCR Master Mix in a Bio-Rad CFX96 real-time PCR detection system under the following conditions:

Reaction setup. Each reaction was performed in a final volume of 10 µL, containing 5 µL of 2× SYBR Green Master Mix, 1 µL of cDNA template, 0.5 µL of each forward and reverse primer, and 3 µL of nuclease-free water.

Cycling conditions. The thermal cycling program consisted of an initial denaturation at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds. A melting curve analysis was performed immediately following amplification to confirm product specificity. Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method, adjusted for primer efficiency where appropriate, with GAPDH serving as the internal control (22).

Statistical analysis. All experiments were repeated in triplicate, and data are presented as the mean ± standard error (SEM). Statistical analysis was performed using SPSS version 25.0 and GraphPad Prism version 8.0.1. The ΔCt values were subjected to one-way ANOVA followed by Tukey's post hoc test (23), while the non-parametric Kruskal-Wallis test was used to compare results across different experimental days. P-values less than 0.05 were considered statistically significant (24).

Ethical approval. This study was reviewed and approved by the Ethics Committee of Yasuj University of Medical Sciences (Ethics code: IR.YUMS.REC.1402.031).

RESULTS

In this study, we evaluated the effect of *Salmonella* extract on the transcriptional expression of *BH*, *PSA*, and *TOP* across various time points. The results are summarized in Figs. 1-3, which depict the normalized fold changes in gene expression relative to the control group across a range of extract concentrations.

Differential expression of bleomycin hydrolase: 6 to 24 hours post-treatment. Quantitative analysis revealed that *BH* expression remained largely unaffected by *Salmonella* extract treatment during the early time points. At 6, 12, and 24 hours, no statistically significant differences were observed between any of the treated groups and the untreated control (Figs. 1A-C). While minor fluctuations were observed between specific treatment concentrations—such as a slight increase in the 100% group compared to the 25% group ($P < 0.05$)—these changes remained within a narrow range (approx. ± 5%) of the baseline, suggesting minimal biological modulation during the first 24 hours.

Table 1. Primer sequences, amplicon characteristics, and real-time PCR efficiency parameters for target and reference genes used in this study. Note: R^2 values and Efficiency (%) were derived from standard curves generated using serial dilutions of cDNA template.

Gene Symbol	Primer Sequence (5' → 3')	Amplicon Size (bp)	Slope	Y-Intercept	R^2	Efficiency (%)
BLMH (*BH*)	F: CCTCATCTCTAACAGCTCAGGA	181	-3.165	33.878	0.988	106.99
	R: CCCTCCCTCCACTCTACTCT					
NPEPPS (*PSA*)	F: TACCAGGCATTCGTGACCTT	178	-3.495	33.649	0.991	93.25
	R: ATCCCCAGGTTACAGCTCAG					
THOP1 (*TOP*)	F: CTCTGATGTTTTCTCCCGCG	171	-3.403	33.693	0.992	96.74
	R: AAATGGAGGTGAGGACGAGG					
GAPDH	F: TGGGCTACACTGAGCACCAG	134	-3.522	32.884	0.994	92.29
	R: CAGCGTCAAAGGTGGAGGAG					

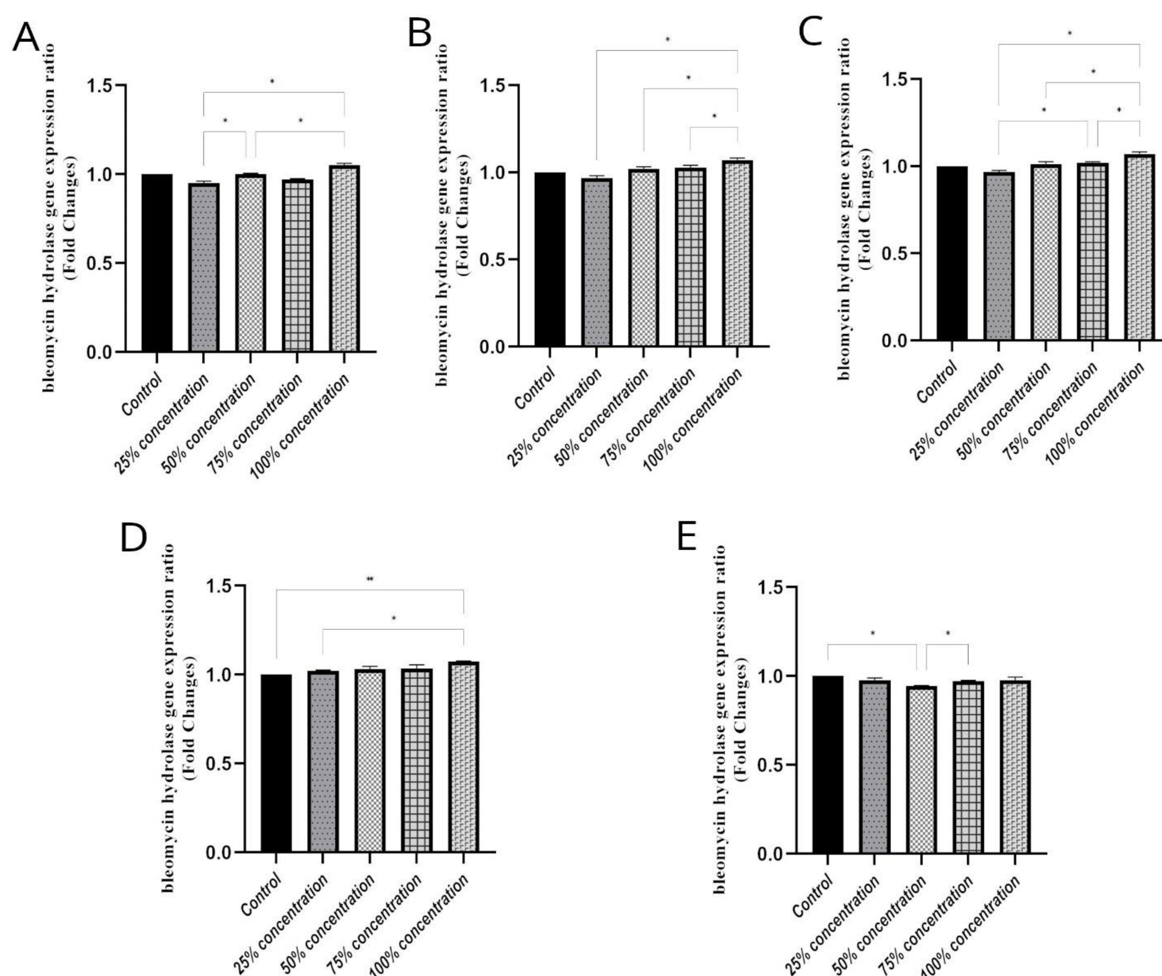

Fig. 1. The bar graphs illustrate the normalized fold changes in bleomycin hydrolase gene expression following treatment with varying concentrations of *Salmonella* extract. Each graph (A-E) represents different time points: 6 hours (A), 12 hours (B), 24 hours (C), 48 hours (D), and 72 hours (E). The x-axis shows the concentrations of *Salmonella* extract (25%, 50%, 75%, 100%), and the y-axis represents the normalized fold change in gene expression. Asterisks indicate statistically significant differences compared to the control group, with * p-value ≤ 0.05, ** p-value ≤ 0.01, *** p-value ≤ 0.001, and **** p-value ≤ 0.0001.

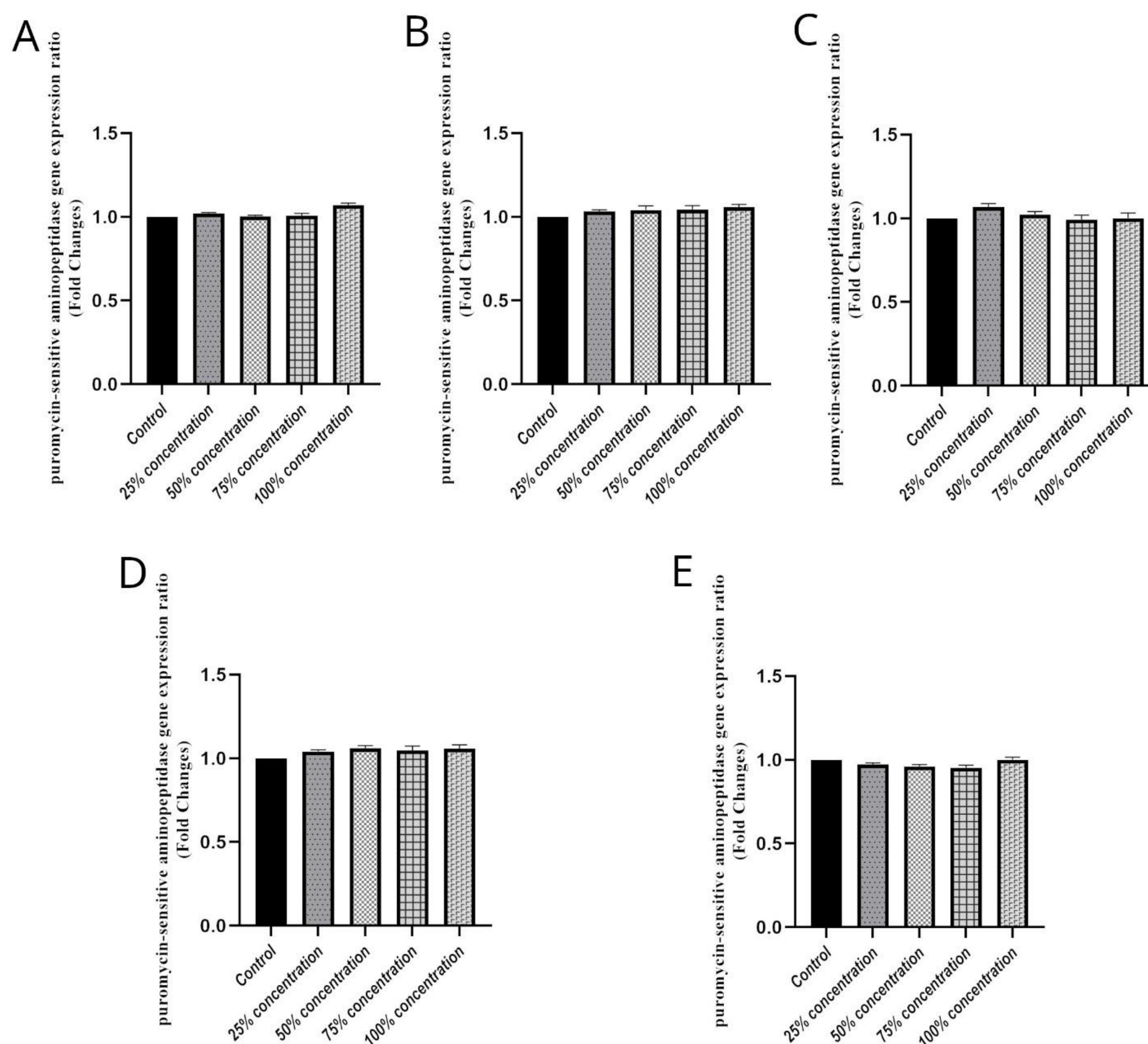

Fig. 2. The bar graphs illustrate the normalized fold changes in puromycin-sensitive aminopeptidase gene expression following treatment with varying concentrations of *Salmonella* extract. Each graph (A-E) represents different time points: 6 hours (A), 12 hours (B), 24 hours (C), 48 hours (D), and 72 hours (E). The x-axis shows the concentrations of *Salmonella* extract (25%, 50%, 75%, 100%), and the y-axis represents the normalized fold change in gene expression. Asterisks indicate statistically significant differences compared to the control group, with * p-value ≤ 0.05, ** p-value ≤ 0.01, *** p-value ≤ 0.001, and **** p-value ≤ 0.0001.

48 hours post-treatment. significant induction of *BH* gene expression was observed at 48 hours, specifically in the group treated with 100% extract concentration compared to the control ($P < 0.05$; Fig. 1D). This group also showed a significant increase relative to the 25% concentration group ($P < 0.05$), while lower concentrations (25%-75%) did not deviate significantly from the control.

72 hours post-treatment. By 72 hours, *BH* expression levels began to stabilize. The only significant variation relative to the control was observed at the 50% concentration ($P < 0.05$; Fig. 1E). Other groups, including the 100% concentration, no longer showed significant differences compared to the untreated control, suggesting a transient nature of the *BH* response.

Differential expression of puromycin-sensitive aminopeptidase. Overall, the expression of *PSA* remained relatively stable throughout the study. At 6-, 12-, 24-, and 48-hours post-treatment, no statistically significant differences in *PSA* gene expression were observed between any of the treated groups and the control, nor among the treated groups themselves

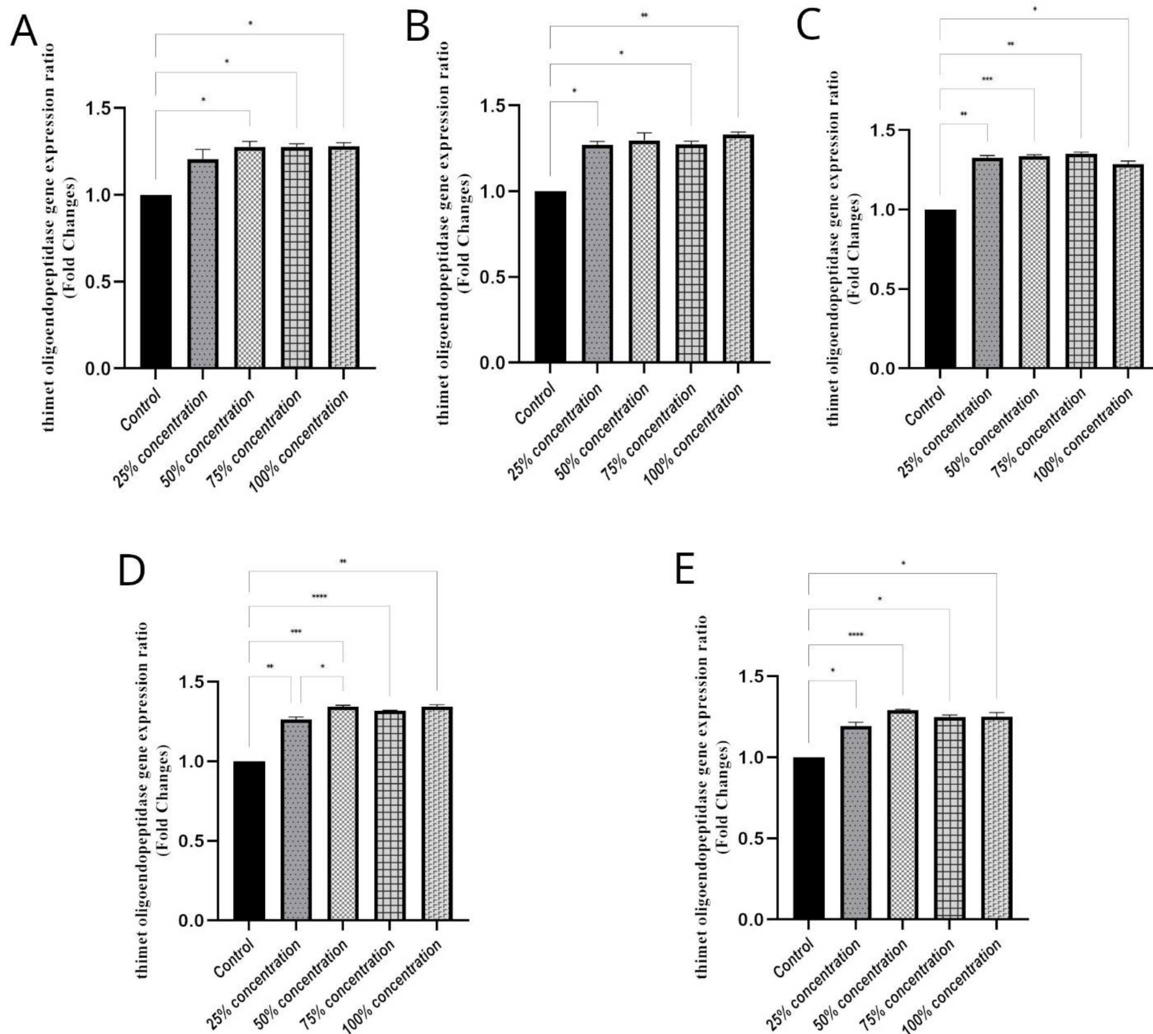

Fig. 3. The bar graphs illustrate the normalized fold changes in thimet oligopeptidase gene expression following treatment with varying concentrations of *Salmonella* extract. Each graph (A-E) represents different time points: 6 hours (A), 12 hours (B), 24 hours (C), 48 hours (D), and 72 hours (E). The x-axis shows the concentrations of *Salmonella* extract (25%, 50%, 75%, 100%), and the y-axis represents the normalized fold change in gene expression. Asterisks indicate statistically significant differences compared to the control group, with * p-value ≤ 0.05, ** p-value ≤ 0.01, *** p-value ≤ 0.001, and **** p-value ≤ 0.0001.

(Figs. 2A-D). While minor trends were noted—such as a non-significant increase at the 100% concentration at 6 hours (Fig. 2A) and a slight upward trend across all concentrations at 48 hours (Fig. 2D)—these did not reach statistical significance. By 72 hours (Fig. 2E), a non-significant decreasing trend in expression was observed for most concentrations, with the exception of the 100% group, which maintained a slight increase relative to the control.

Differential expression of thimet oligopeptidase: 6 hours post-treatment. The *TOP* gene demonstrated a rapid response to treatment, with an increase in expression observed in all treated cells. This upregulation was significant for cells treated with 50%, 75%, and 100% concentrations compared to the control group ($P < 0.05$) (Fig. 3A).

12 hours post-treatment. At 12 hours, the expression profile for *TOP* remained elevated. A significant increase was observed in cells treated with 25% and 75% concentrations compared to the control group ($P \leq 0.05$), while cells treated with the 100% concentration showed a higher degree of significance ($P \leq 0.01$) (Fig. 3B).

24 hours post-treatment. By 24 hours, all treated groups exhibited a significant increase in *TOP* expression compared to the control. The most pronounced increase was observed in cells treated with the 50% concentration ($P \leq 0.01$), while the 100% concentration group also maintained significantly higher expression ($P \leq 0.05$) (Fig. 3C).

48 hours post-treatment. The most substantial induction of *TOP* expression was recorded at 48 hours. All treated groups showed significant and notable increases relative to the control. Specifically, cells treated with 50% and 75% concentrations showed a highly significant increase ($P \leq 0.001$), while the 25% and 100% concentrations also demonstrated strong induction ($P \leq 0.01$). Furthermore, the 50% concentration group showed a significant difference when compared to the 25% concentration group ($P \leq 0.05$) (Fig. 3D).

72 hours post-treatment. The significant upregulation of *TOP* persisted after 72 hours in all treated groups ($P \leq 0.05$). The 50% concentration group continued to show the most notable increase relative to other groups ($P \leq 0.001$). Additionally, cells treated with the 100% concentration displayed a significant increase ($P \leq 0.01$), and the 50% group remained significantly higher than the 25% concentration group ($P \leq 0.05$) (Fig. 3E).

DISCUSSION

The findings of this study demonstrate a selective and time-dependent modulation of cytosolic peptidases in HT-29 cells following exposure to *Salmonella enterica* cell extract. While we investigated three enzymes integral to the MHC Class I antigen presentation pathway, our results reveal a clear hierarchy of response: *TOP* serves as the primary transcriptional target, whereas *BH* and *PSA* remained largely stable relative to untreated controls. The MHC Class I pathway is the cornerstone of host defense against intracellular pathogens, responsible for displaying intracellular peptides to CD8+ T cells (25, 26). This process relies on a precise sequence of proteolytic steps where the proteasome performs initial cleavage, followed by cytosolic aminopeptidases that act as "final trimmers" to generate epitopes of 8–10 amino acids in length (27, 28). Our data indicates that *TOP* demonstrated a rapid and sustained upregulation, significant as early as 6 hours and peaking at 48 hours across multiple concentrations. In the context of immunology, *TOP* is often described as a "terminator" of MHC Class I ligands (29). While some trimming is necessary, excessive *TOP* activity can degrade potential 8–10 mer epitopes into fragments too short (3-7 amino acids) for MHC loading (30, 31). The robust induction of *TOP* by *S. enterica* components suggests a targeted mechanism of epitope destruction. By shifting the enzymatic balance toward over-trimming, the pathogen may reduce the density of immunogenic peptides on the cell surface, effectively rendering infected cells "invisible" to cytotoxic T lymphocytes (CTLs) (29, 32).

A critical observation in this study is the relative lack of modulation for *PSA* and *BH*. *PSA* expression showed no significant alterations throughout the 72-hour period. Similarly, *BH* expression remained statistically indistinguishable from the control for the first 24 hours. Significant upregulation for *BH* was only observed at 48 hours at the 100% extract concentration and sporadically at 72 hours. This suggests that *S. enterica* does not induce a generalized, non-specific increase in all cytosolic peptidases. Instead, the bacterium appears to exert a preferential influence on *TOP*. This selectivity is scientifically significant; it implies that *Salmonella* PAMPs specifically trigger signaling pathways that favor *TOP*-mediated peptide degradation over the broader trimming activities of *PSA* or *BH*. This may allow the pathogen to tailor its immune evasion strategy based on the specific enzymatic vulnerabilities of the host cell.

While these transcriptional profiles provide insight into host-pathogen interactions, several constraints must be acknowledged. A primary limitation is the use of the HT-29 adenocarcinoma cell line. As a neoplastic model, these cells possess metabolic and enzymatic baselines that differ from healthy physiological tissue, which may influence absolute expression levels. Future suggestions include validating these findings in primary human intestinal organoids or healthy epithelial tissues. Additionally, we utilized a crude whole-cell extract to isolate the effects of structural PAMPs, such as LPS and flagellin. This approach effectively models the host response to bacterial components but lacks the dynamic variables of a live infection, such as Type III Secretion System (T3SS) effectors. We suggest that subsequent research employ live infection models and fractional purification of the extract to identify the specific

bacterial proteins driving *TOP* upregulation. Finally, as this study focused on mRNA expression, we recommend future protein-level validation (e.g., Western blotting) and functional CTL activation assays to confirm the impact on actual antigen presentation.

CONCLUSION

In summary, *Salmonella enterica* cell extract significantly and selectively upregulates the expression of *TOP* in HT-29 cells. The minimal impact on *BH* and *PSA* suggests that *S. enterica* specifically targets the "terminator" phase of the peptide-trimming machinery. This alteration likely serves as a strategic mechanism for immune evasion by facilitating the destruction of immunogenic epitopes before they can be presented to the adaptive immune system. These findings highlight *TOP* as a critical subject for future research.

ACKNOWLEDGEMENTS

We would like to express our gratitude to Dr. Asghar Sharifi for his invaluable guidance throughout this research. We also extend our thanks to the members of the microbiology faculty at Yasuj University for their continued support and encouragement.

Yasuj University of Medical Sciences has financially supported this study. This article is the result of a thesis by Rozalin Mardani to obtain a Doctor of Medicine (M.D.) degree (Grant number: 4000229). There is no conflict of interest in this study.

REFERENCES

1. Oludairo OO, Kwaga JK, Kabir J, Abdu PA, Gitanjali A, Perrets A, et al. A review on *Salmonella* characteristics, taxonomy, nomenclature with special reference to non-Typhoidal and Typhoidal salmonellosis. *Zagazig Vet J* 2022; 50: 161-176.
2. Peery AF, Dellon ES, Lund J, Crockett SD, McGowan CE, Bulsiewicz WJ, et al. Burden of gastrointestinal disease in the United States: 2012 update. *Gastroenterology* 2012; 143: 1179-1187.e3.
3. Bhunia AK (2018). Foodborne Microbial Pathogens: Mechanisms and Pathogenesis. 2nd ed. Springer. New York, NY.
4. Hurley D, McCusker MP, Fanning S, Martins M. Salmonella–host interactions–modulation of the host innate immune system. *Front Immunol* 2014; 5: 481.
5. Bueno SM, González PA, Carreño LJ, Tobar JA, Mora GC, Pereda CJ, et al. The capacity of Salmonella to survive inside dendritic cells and prevent antigen presentation to T cells is host specific. *Immunology* 2008; 124: 522-533.
6. Marshall JS, Warrington R, Watson W, Kim HL. An introduction to immunology and immunopathology. *Allergy Asthma Clin Immunol* 2018; 14(Suppl 2): 49.
7. Kloetzel PM, Ossendorp F. Proteasome and peptidase function in MHC-class-I-mediated antigen presentation. *Curr Opin Immunol* 2004; 16: 76-81.
8. Dougan G, John V, Palmer S, Mastroeni P. Immunity to salmonellosis. *Immunol Rev* 2011; 240: 196-210.
9. Parisi A, Crump JA, Glass K, Howden BP, Furuya-Kanamori L, Vilkins S, et al. Health outcomes from multidrug-resistant *Salmonella* infections in high-income countries: a systematic review and meta-analysis. *Foodborne Pathog Dis* 2018; 15: 428-436.
10. Doyle ME. Multidrug-resistant pathogens in the food supply. *Foodborne Pathog Dis* 2015; 12: 261-279.
11. Barie PS. Multidrug-resistant organisms and antibiotic management. *Surg Clin North Am* 2012; 92: 345-391.
12. Bernal-Bayard J, Ramos-Morales F. Molecular mechanisms used by Salmonella to evade the immune system. *Curr Issues Mol Biol* 2018; 25: 133-168.
13. Pishesha N, Harmand TJ, Ploegh HL. A guide to antigen processing and presentation. *Nat Rev Immunol* 2022; 22: 751-764.
14. Freshney RI (2016). Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications. 7th ed. Wiley-Blackwell. Hoboken, NJ.
15. Grabski AC. Advances in preparation of biological extracts for protein purification. *Methods enzymol* 2009; 463: 285-303.
16. Schuerer-Maly CC, Eckmann L, Kagnoff MF, Falco MT, Maly FE. Colonic epithelial cell lines as a source of interleukin-8: stimulation by inflammatory cytokines and bacterial lipopolysaccharide. *Immunology* 1994; 81: 85-91.
17. Chomczynski P, Sacchi N. The single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction: twenty-something years on. *Nat Protoc* 2006; 1: 581-585.
18. Simões PSR, Visniauskas B, Perosa SR, Yacubian EMT, Centeno R, Canzian M, et al. Expression and activity of thimet oligopeptidase (TOP) are modified in the hippocampus of subjects with temporal lobe epilepsy (TLE). *Epilepsia* 2014; 55: 754-762.
19. Perez I, Blanco L, Sanz B, Errarte P, Ariz U, Beitia M, et al. Altered Activity and Expression of Cytosolic Peptidases in Colorectal Cancer. *Int J Med Sci* 2015; 12:

458-467.

20. Thome J, Gewirtz J, Sakai N, Zachariou V, Retz-Junginger P, Retz W, et al. Polymorphisms of the human apolipoprotein E promoter and bleomycin hydrolase gene: risk factors for Alzheimer's dementia? *Neurosci Lett* 1999; 274: 37-40.
21. Koressaar T, Remm M. Enhancements and modifications of primer design program Primer3. *Bioinformatics* 2007; 23: 1289-1291.
22. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 2001; 25: 402-408.
23. Zar JH (2010). Biostatistical Analysis. 5th ed. Pearson. Upper Saddle River, NJ.
24. Kruskal WH, Wallis WA. Use of ranks in one-criterion variance analysis. *JASA* 1952; 47: 583-621.
25. Blanchard N, Shastri N. Cross-presentation of peptides from intracellular pathogens by MHC class I molecules. *Ann N Y Acad of Sci* 2010; 1183: 237-250.
26. Antoniou AN, Powis SJ. Pathogen evasion strategies for the major histocompatibility complex class I assembly pathway. *Immunology* 2008; 124: 1-12.
27. Mamrosh JL, Sherman DJ, Cohen JR, Johnston JA, Joubert MK, Li J, et al. Quantitative measurement of the requirement of diverse protein degradation pathways in MHC class I peptide presentation. *Sci Adv* 2023; 9(25): eade7890.
28. Rock KL, York IA, Goldberg AL. Post-proteasomal antigen processing for major histocompatibility complex class I presentation. *Nat Immunol* 2004; 5: 670-677.
29. York IA, Mo AX, Lemerise K, Zeng W, Shen Y, Abraham CR, et al. The cytosolic endopeptidase, thimet oligopeptidase, destroys antigenic peptides and limits the extent of MHC class I antigen presentation. *Immunity* 2003; 18: 429-440.
30. Saric T, Graef CI, Goldberg AL. Pathway for degradation of peptides generated by proteasomes: a key role for thimet oligopeptidase and other metallopeptidases. *J Biol Chem* 2004; 279: 46723-46732.
31. Ferro ES, Gewehr MC, Navon A. Thimet oligopeptidase biochemical and biological significances: past, present, and future directions. *Biomolecules* 2020; 10: 1229.
32. Silva CL, Portaro FC, Bonato VL, de Camargo AC, Ferro ES. Thimet oligopeptidase (EC 3.4. 24.15), a novel protein on the route of MHC class I antigen presentation. Biochem Biophys Res Commun 1999; 255: 591-595.