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RESEARCH

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Effect of physicochemical parameters on the L-methioninase activity of *Methylobacterium* sp. and its in vitro anticancer activity in combination with tamoxifen citrate

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Abstract

Background Methionine dependence is a metabolic abnormality observed exclusively in cancer cells. Methionine depletion using methioninase is therefore an attractive strategy for cancer treatment. The current study focuses on the purification of L-Methioninase from a bacterial isolate, *Methylobacterium* sp. JUBTK33, for its anticancer application in conjunction with Tamoxifen in MCF-7, HepG2, and HeLa cancer cell lines.

Results L-methioninase was purified from *Methylobacterium* sp. JUBTK33 using a DEAE-Sephadex G-200 column, resulting in a 6.15-fold purification with a specific activity of 17.89 U/mg. At 40 °C and pH 8.5, the enzymatic biochemical characteristics demonstrated increased enzyme activity. Na+ ions (1 mM) significantly enhanced the enzyme's activity, while Li+, Mn++, Ni++, Fe++, and K+ had little impact. The highest activity was observed at a 225 µM (2.5%) substrate concentration of methionine, with V_{max} and K_m values of 0.48 U/mL/min and 48.23 µM, respectively. The enzyme's potential anticancer effect in combination with TAM was evaluated on HepG2, MCF-7, and HeLa cell lines. It was found to be highly effective on MCF-7 cell lines, with a combination of L-MET-TAM (5 and 10 µg/mL) resulting in 3.72% and 1.0% cell viabilities, and IC_{50} values of 9.701 µg/mL and 5.72 µg/mL, respectively. On the normal HEK-293 cell line, the combination of L-MET-TAM (10 µg/mL) demonstrated approximately an 18% protective effect compared to TAM alone.

Conclusion The combination approach demonstrated remarkable success against cancer cells in vitro, highlighting the need for further investigations to develop it into an effective treatment strategy.

Keywords L-methioninase, Breast cancer, Methionine, Anticancer, Combination therapy

Background

Methionine dependency is a metabolic defect in cancer cells that prevents their growth in media lacking methionine [1]. This unique feature is considered a major hallmark of cancer, known as the Hoffman effect [2]. In methionine-deprived conditions, cancer cells become arrested in the S-G2 phase of the cell cycle and eventually undergo apoptosis [3]. Based on this, a new therapeutic approach has been developed to target cancer cells using

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the enzyme L-methioninase to degrade methionine and hinder their growth and proliferation.

The enzyme L-methioninase is primarily found in bacteria, fungi, and plants but is absent in mammals. Species of bacteria such as *Pseudomonas putida*, *Aeromonas* sp., *Citrobacter freundii*, *Brevibacterium linens*, *Lactococcus lactis*, *Methylobacterium* sp. JUBTK33, and *Clostridium sporogenes* are known to secrete the L-methioninase enzyme [4–6]. However, there are no reports of L-methioninase from any other species of *Methylobacterium* so far. The anticancer potential of L-methioninase, inducing methionine starvation, has been demonstrated in various cancer cell lines, including breast, kidney, colon, lungs, prostate, and others [7]. This therapeutic approach makes cancer cells more sensitive to chemotherapeutic drugs and has been shown to reduce systemic toxicity [8, 9].

Building on this idea, the current study aims to purify the L-methioninase enzyme from *Methylobacterium* sp. JUBTK33 [6], investigate the influence of external parameters on its enzyme activity, and enhance its anticancer potential through a combination approach with tamoxifen citrate.

Materials and methods

L-methioninase producing bacterial strain

Methylobacterium sp. JUBTK33, isolated from the soil of a fenugreek farmland in Sethuvalli, Tamil Nadu, India, and identified as the highest L-methioninase producer among 75 microbial isolates in our previous study report [6], was selected for the current study.

Chemicals

The chemicals utilized in the current investigation were L-methionine, Pyridoxal-5-phosphate, and L-ascorbic acid (sourced from HIMEDIA, India), Coomassie Brilliant Blue, Bovine Serum Albumin (BSA), MEM media, fetal bovine serum, trypsin, MTT dye (all procured from HIMEDIA, India), di-sodium monohydrogen phosphate (Na_2HPO_4), monopotassium dihydrogen phosphate (KH_2PO_4), di-potassium monohydrogen phosphate (K_2HPO_4), sodium chloride (NaCl), magnesium sulfate ($MgSO_4$), calcium chloride ($CaCl_2$), D-Glucose, phenol red, agar, trichloroacetic acid, and Nessler's reagent (also known as potassium tetraiodomercurate (II)), all of which meet analytical grade standards (sourced from Merck, Mumbai, India).

L-methioninase assay and specific activity of the enzyme

To assess L-methioninase activity in the culture supernatants containing the crude enzymes, we employed a modified version of the standard Nesslerization method, following the established protocol with minor changes [10]. The assay procedure involved the incubation of 1 mL of a 1% L-methionine substrate, prepared in a phosphate buffer (50 mmol, pH 7.0), with the addition of 0.1 mL of pyridoxal phosphate (100 µmol) and 1 mL of the crude enzyme extract. These components were thoroughly mixed and allowed to incubate for 60 min at 37 °C. To halt the enzyme-catalyzed reaction, 0.1 mL of trichloroacetic acid (1.5 mol) was added. The ammonia released during the reaction was quantified by introducing 0.5 mL of Nessler's reagent (comprising $HgCl_2$, KI, and NaOH), and the resulting color change was measured with a UV–VIS spectrophotometer (Shimadzu, Japan) at 480 nm against an enzyme blank. The calculated enzyme activity was expressed as µmol/min/mL. The assays were conducted three times, and the average value was taken into consideration. Specific activity was calculated by dividing the L-methioninase activity by the total protein content and expressed as unit activity per milligram (U/mg) of protein [6].

Total protein estimation

Lowry's method was used to determine the total protein content of the extracted enzyme, with bovine serum albumin serving as the standard [11].

Enzyme purification

L-methioninase purification was performed using the acetone precipitation method with a 1:2 ratio of sample to acetone [12]. The precipitate was collected and subsequently subjected to partial purification.

Anion exchange DEAE column chromatography

The acetone-precipitated enzyme was passed through a DEAE cellulose column (Bio-Rad, UK) that had been pre-equilibrated with 0.05 M potassium phosphate buffer at pH 6.5. NaCl (0.12 M) was used as the elution buffer for the enzyme. Fifty 1 mL/min fractions were collected in separate tubes, and enzyme activity was determined using Nessler's method. Active fractions were collected, pooled, and further purified using a Sephadex G-200 column [13].

Sephadex G-200 purification

The active fractions from the DEAE column were passed through a Sephadex G-200 column. The column was equilibrated with 0.05 M potassium phosphate buffer at pH 6.5. The enzyme was eluted at a rate of 1 mL/min with 0.12 M NaCl, and 50 fractions were collected and checked for enzyme activity. Active fractions were pooled and lyophilized for further use [13].

Molecular weight determination and activity staining

The purity of semi-purified (SPMet) and Sephadex G-200 column-purified (SG purified) enzymes was assessed, and their molecular weights were determined using 10% SDS–polyacrylamide gel electrophoresis following standard protocols [14]. The activity of the purified enzyme was confirmed by activity staining on modified starch agar plates supplemented with phenol red (0.07% w/v) as the indicator. The development of pink color will indicate the release of ammonia by the action of L-methioninase on methionine.

Evaluation of physicochemical parameters on SG purified L-methioninase enzyme activity

The effect of temperature on L-methioninase activity

By incubating the reaction mixture in a water bath for 1 h at various temperatures (20, 30, 40, 50, 60, and 70 °C), the effect of temperature on L-methioninase enzyme was determined [13]. The enzyme activity was determined after it was pre-incubated as per previously given methodology.

The effect of pH on L-methioninase activity

To determine the optimum pH for L-methioninase activity, acetate buffer (pH 5–6), phosphate buffer (pH 6.5–7.5), and Tris–HCl buffer (pH 8–9) were used. Enzymatic activity was determined following Nessler's method, after incubating each reaction mixture at 37 °C for 30 min [13].

The effect of metal ions on L-methioninase activity

The effect of various metal ions, including Fe++, Li+, Na+, Ni++, Mn++, and K+, on L-methioninase activity was determined by pre-incubating the purified L-methioninase enzyme with metal ions at concentrations of 1 and 5 mM in 50 mM phosphate buffer at 37 °C for 1 h. After pre-incubation, enzyme activity was assessed following Nessler's method [13].

Kinetics of column-purified L-methioninase enzyme

We utilized a conventional procedure to investigate how varying substrate concentrations (45 mM, 90 mM, 135 mM, 180 mM, 225 mM, and 280 mM) impacted the activity of L-methioninase. From the Lineweaver–Burk plot, we determined the enzymatic kinetic parameters, namely the Michaelis–Menten constant (K_m) and the maximum velocity (V_{max}) [13].

Cytotoxicity assay and safety assessment (Toxicity study)

The cytotoxicity of crude, semi-purified (SPMet), and Sephadex G (SG) purified L-methioninase (L-MET) alone and in combination with tamoxifen citrate (TAM) was tested on HepG2 (Liver cancer), MCF-7 (Breast cancer), and HeLa cells (Cervical cancer), with Doxorubicin (DOX) serving as the positive control (standard), using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay as per the standard methodology [15]. The cancer cells were incubated for 24 h, 48 h, and 96 h with different doses (10, 50, 100, 200, 400 µg/mL) of the enzyme in combination with 5 µg/mL and 10 µg/mL of TAM. After the treatment duration cytotoxicity was assessed by treating with MTT dye for 3 h, and later taking the optical density at 540 nm in an ELISA reader (Lisa Plus, Aspan, India). The safety and toxicity of the enzyme were assessed on Healthy human embryonic kidney (HEK-293) cell lines through the MTT assay.

Statistical analysis

In this present study, every experiment was conducted in triplicate, and the outcomes were expressed as Mean ± standard error. The experimental data were analyzed using one-way/two-way ANOVA within the Graph Pad Prism version 6 software. To assess the mean differences, the Dunnett's multiple range test, also known as the Dunnett's multiple comparison test, was employed. Statistical significance was considered at a p value less than 0.05.

Results

Purification of L-methioninase from *Methylobacterium* sp. JUBTK33 and determination of its molecular weight

The extracellular L-methioninase from *Methylobacterium* sp. JUBTK33 was purified using cold acetone precipitation, ion exchange, and Sephadex G-200 gel filtration chromatography. With a 4.3-fold purification, the acetone-precipitated enzyme demonstrated enzyme activity of 1.5 ± 0.91 U/mL/min and a specific activity of 12.60 U/mg (Table 1). Each purification step resulted in an increased specific activity of the enzyme. The acetone-precipitated sample was dialyzed to concentrate the protein, which was then further purified using a DEAE cellulose ion exchange column. The specific activity was increased to 14.75 U/mg with a 5.07-fold purification. Furthermore, through a Sephadex G-200 column purification step, the specific activity was increased to 17.89 U/mg (6.15-fold purification). The purity and homogeneity of the L-MET were confirmed through SDS-PAGE (Fig. 1A). A single band with an approximate molecular weight of 82 kDa was observed. The L-methioninase activity of SPMet's was confirmed by performing an activity staining assay on agar plates (Fig. 1B).

Table 1 Purification of Methioninase from *Methylobacterium* sp. JUBTK33

Purification	Enzyme activity (U/mL/min)	Total protein content (U/mL)	Specific activity (U/mg)	Purification fold
Crude sample	1.26 ± 0.12	700	2.91	1
SPMet's—1 mg/mL	1.5 ± 0.91	119	12.60	4.30
DEAE column purified	0.9 ± 0.11	61	14.75	5.07
Sephadex G-200 column purified	0.34 ± 0.14	19	17.89	6.15

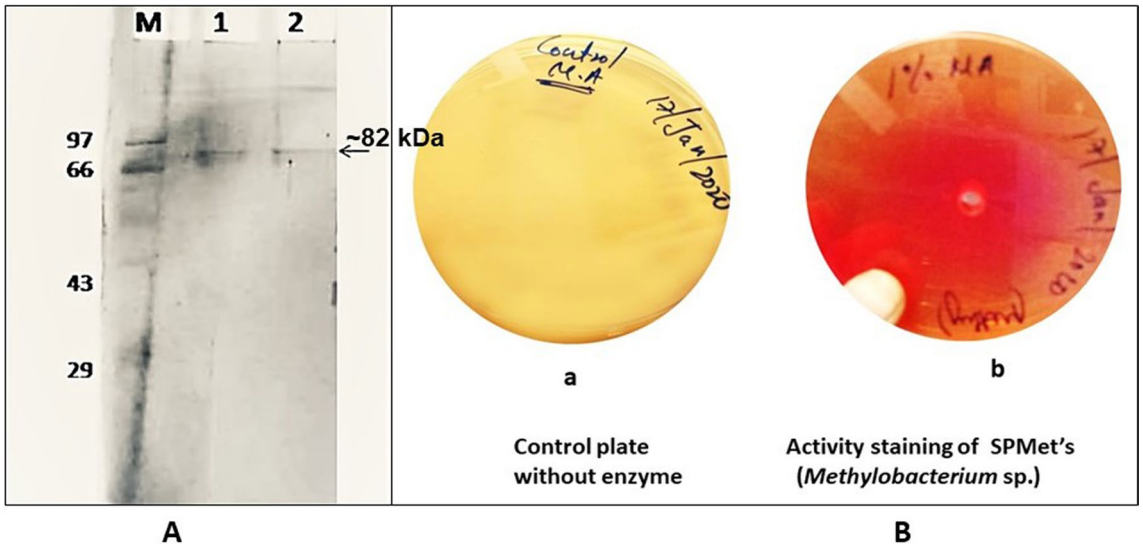

Fig. 1 **A** Molecular weight and purity of L-methioninase by SDS-PAGE. Lane M—Mol. wt. marker, Lane 1—SPMet's, and Lane 2—L-MET. **B** Plate assay of activity staining of SPMet's from *Methylobacterium* sp. JUBTK33, where (a) control plate without the enzyme, and (b) plate inoculated with SPMet's

Effect of physio-chemical parameters on the activity of L-MET enzyme

Effect of temperature of L-methioninase activity

The study investigated the effects of temperature on L-methioninase enzyme activity across a range from 20 to 70 °C. At 40 °C, there was a slight increase in enzyme activity to 0.35 ± 0.026 U/mL/min, while at 37 °C, it measured 0.33 ± 0.036 U/mL/min. Enzyme activity increased as the temperature approached 40 °C, but further increases to 50, 60, and 70 °C significantly inhibited enzyme activity ($p < 0.001$). This suggests that the optimum temperature for L-methioninase enzyme activity from *Methylobacterium* sp. JUBTK33 lies in the range of 35–40 °C (Fig. 2a).

Effect of pH on L-methioninase activity

We analyzed the influence of pH levels ranging from 5 to 9 on enzyme activity. At pH 7 (control), the L-methioninase enzyme from *Methylobacterium* sp. exhibited an activity of 0.33 ± 0.017 U/mL/min (in phosphate buffer). Acidic pH of 5 and 5.5 has significantly reduced ($p < 0.05$) the enzyme activity, but it increased with increasing pH; at pH 8.5 (using Tris–HCl buffer), enzyme activity measured 0.38 ± 0.012 U/mL/min. Further increases in pH resulted in a decrease in L-methioninase activity. While pH did not have a significant impact on enzyme activity, it did show a significant negative effect at pH lower than 6 (Fig. 2b).

Effect of metal ions on enzyme activity

At pH 7 and 37 °C, we investigated the effect of various metal ions, including Fe++, Li+, Na+, Ni++, Mn++, and K+, on L-methioninase activity at concentrations of 1 mM and 5 mM. Figure 2c illustrates that Na+ ions at a concentration of 1 mM significantly ($p < 0.05$) increased the enzyme activity to 0.38 ± 0.021 U/mL/min compared to the control (0.34 ± 0.016 U/mL/min). Enzyme activity remained unaltered when the concentration was increased to 5 mM. The metal ions Li+, Mn++, Ni++, and Fe++ (at both 1 mM and 5 mM concentrations) had no significant impact on L-methioninase activity,

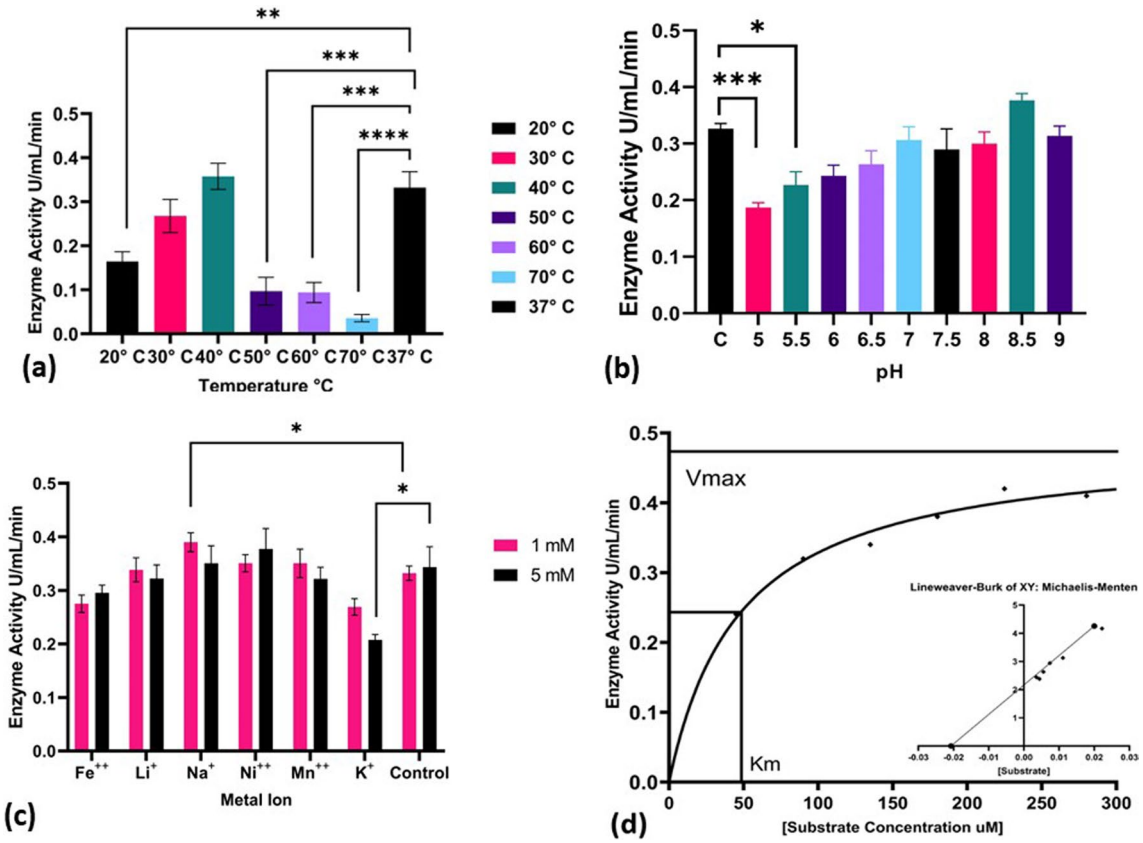

Fig. 2 The effect of physicochemical parameters on L-methioninase activity is represented graphically. **a** Temperature; **b** pH; **c** metal ions, and **d** substrate concentration (MM and LB plot). Data were expressed as Mean ± SE with $n = 3$; two-way ANOVA using Dunnett's multiple comparisons test was performed. Data showed significance with ****$p < 0.0001$; ***$p < 0.001$; **$p < 0.01$; and *$p < 0.05$

whereas K+ at 1 mM had no significant effect but K + at 5 mM significantly suppressed enzyme activity ($p < 0.05$).

Effect of substrate concentration on enzyme activity and determination of its V_{max} and K_m

The effect of substrate concentration on enzyme activity and the determination of its V_{max} and K_m values were studied for L-methioninase from *Methylobacterium* sp. JUBTK33. This analysis revealed a hyperbolic relationship between the rate of reaction and the substrate concentration. The saturation point was observed at 225 µM (2.5% of methionine), resulting in an enzyme activity of 0.42 ± 0.017 U/mL/min. Using Michaelis–Menten (MM) and Lineweaver–Burk (LB) plots, we determined the V_{max} and K_m values to be 0.48 U/mL/min and 48.23 µM, respectively (see Fig. 2d). The obtained K_m value demonstrates good specificity for the L-methioninase enzyme.

In vitro anticancer activity of L-MET in combination with tamoxifen citrate on cancer cell lines

The study aimed to evaluate the anticancer potential of L-methioninase from *Methylobacterium* sp. on human hepatocarcinoma (HepG2), human breast carcinoma (MCF-7), and human cervical carcinoma (HeLa) cell lines. Figure 3 illustrates the anticancer activity of the enzyme in combination with Tamoxifen.

At 96 h, the anticancer activity of L-MET was most effective at a concentration of 400 µg/mL. Monotherapies on liver (HepG2) cancer cells resulted in cell viabilities of 19.93% for Methioninase (400 µg/mL), 26.56% for TAM (10 µg/mL), and 21.98% for DOX (10 µg/mL). In contrast, the combination of L-MET-TAM (5 µg/mL) and L-MET-TAM (10 µg/mL) resulted in viabilities of 18.49% and 3.39%, respectively, with IC_{50} values of 28.56 µg/mL and 10.69 µg/mL, respectively (Fig. 3a). The decrease in cell viabilities with the combination of L-MET-TAM (10 µg/mL) was significantly more than that with the monotherapy of L-MET or DOX ($p < 0.05$).

For cervical (HeLa) cancer cells, a cell viability of 17.43% was observed with L-MET at 400 µg/mL, while the combination of L-MET-TAM (5 µg/mL) and L-MET-TAM (10 µg/mL) resulted in 10.16% and 4.16% viabilities, respectively, with IC_{50} values of 8.25 µg/mL and 9.29 µg/mL, respectively (Fig. 3b). These viability percentages

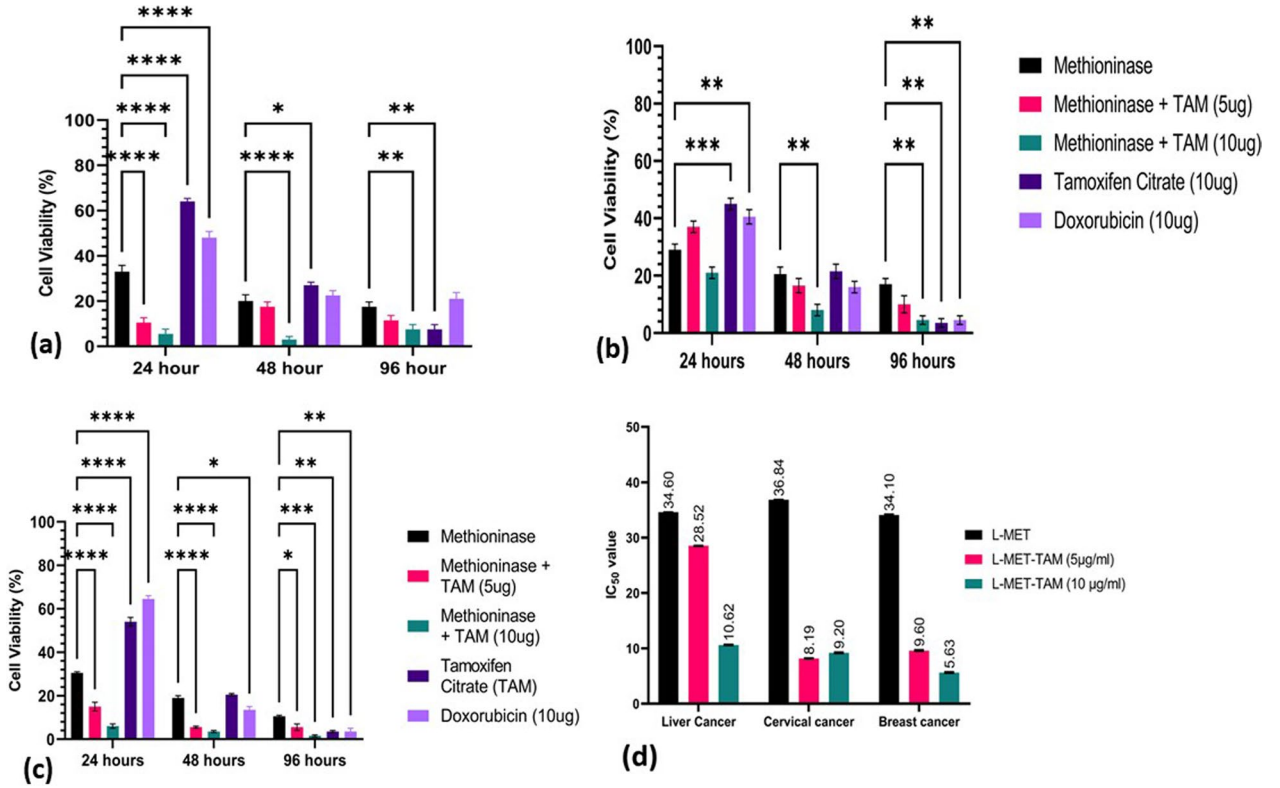

Fig. 3 Graphical representation of cytotoxicity of L-MET in combination with TAM (5 and 10 µg/mL) on **a** liver cancer; **b** cervical cancer; **c** breast cancer; and **d** IC_{50} values. Data were expressed as Mean ± SE with $n = 3$; two-way ANOVA using Dunnett's multiple comparisons test was performed. Data showed significance with ****$p < 0.0001$; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$

were found to be significantly ($p < 0.01$) lesser than that with the monotherapy of L-MET and DOX.

The highest anticancer potential was observed in the breast cancer cell line (MCF-7). Monotherapies on MCF-7 cells resulted in cell viabilities of 8.99% for L-MET (400 µg/mL), 3.92% for TAM (10 µg/mL), and 2.86% for DOX (10 µg/mL). In contrast, the combination of L-MET-TAM (5 µg/mL and 10 µg/mL) resulted in 3.72% and 1.0% cell viabilities, with IC_{50} values of 9.701 µg/mL and 5.72 µg/mL, respectively (Fig. 3c). These percentage cell viabilities were significantly ($p < 0.01$) lower than that with the monotherapy of L-MET and DOX. L-methioninase alone demonstrated anticancer effects with IC_{50} values of 34.62 µg/mL, 36.88 µg/mL, and 34.18 µg/mL on liver, cervical, and breast cancer cell lines, respectively (Fig. 3d), which got greatly reduced by combining with TAM.

Additionally, L-methioninase from *Methylobacterium* sp. was found to be more effective than that from *Trichoderma harzianum* at inhibiting cancer growth [6]. The combination approach had the lowest IC_{50} value compared to monotherapies, with IC_{50} values of 14.12 µg/mL and 20.07 µg/mL on HepG2 and MCF-7, respectively. The combination of L-methioninase and tamoxifen citrate on the HeLa cell line represents the first-ever report with the lowest IC_{50} value and promising anticancer potential, to the best of our knowledge.

In vitro protective effect of L-MET in combination with tamoxifen citrate on healthy HEK-293 cell lines

We measured the protective effect of L-methioninase in combination with tamoxifen citrate based on their

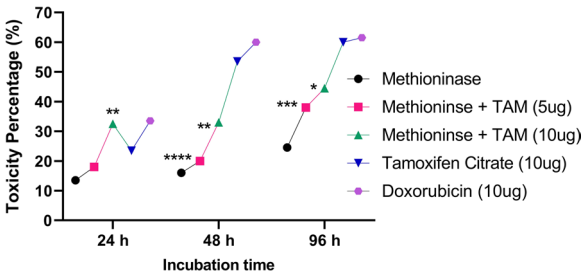

Fig. 4 Graphical representation of toxic effect of the treatment groups on healthy human kidney embryonic cell line (HEK-293). Two-way ANOVA was performed using Dunnett's multiple comparisons test. Data showed significance with ****$p < 0.0001$; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$

toxicity effects on healthy cell lines. Figure 4 illustrates the toxicity percentages of the treatment groups after various incubation times (24, 48, and 96 h). After 96 h of incubation, the monotherapies of L-MET (400 µg/mL), the positive standard TAM (10 µg/mL), and the standard DOX (10 µg/mL) resulted in toxicities of 24%, 59%, and 60%, respectively. In contrast, the combination of L-MET (400 µg/mL) and TAM (5 µg/mL and 10 µg/mL) resulted in toxicities of 37.8% and 44.79%, respectively, which are significantly ($p < 0.05$) lesser than that with the monotherapy of TAM at 10 µg/mL. Compared to the positive standard TAM alone, the combination effect (L-MET-TAM 10 µg/mL) successfully demonstrated an 18% protective effect on healthy cells.

This combination approach convincingly showed that by combining TAM with L-methioninase, the toxic effects caused by the chemotherapeutic drug TAM could be significantly reduced. The protective effect observed with the L-MET-TAM combination also suggests promising anticancer potential, indicating that this therapeutic approach has the potential for further development into an effective treatment strategy.

Discussion

The therapeutic potential of L-methioninase in reducing plasma methionine has been extensively explored. L-methioninase, isolated from bacteria, has been evaluated for its anticancer effects on various cancer cell lines. In our study, L-methioninase from *Methylobacterium* sp. JUBTK33 demonstrated promising anticancer potential against MCF-7, HepG2, and HeLa cancer cell lines. Furthermore, this study represents the first investigation into the combination of L-methioninase with Tamoxifen.

The purified enzyme from *Methylobacterium* sp. was observed to have a molecular weight of 82 kDa. It is worth noting that L-methioninase from different sources exhibits varying molecular weights. For instance, El Awady et al. [16] reported the molecular weight of purified L-methioninase from *Streptomyces variabilis* 3MA2016 as 45 KDa, and Javia et al. [17] found a molecular weight of 46 kDa for the enzyme from *Alcaligenes aquatilis*, while a molecular weight of 48 kDa was reported earlier for the enzyme from *Trichoderma harzianum* [18]. The purified L-methioninase from *Streptomyces* sp. DMMMH4 revealed a slightly higher molecular mass of 47 kDa [7]. On the other hand, L-methioninase from *Brevibacterium linens* BL2, an enzyme reliant on pyridoxal phosphate, possesses a native molecular weight of around 170 kDa, composed of four identical subunits, each weighing approximately 43 kDa [19]. Recent research on *Pseudomonas* sp.'s MGL indicated a molecular mass exceeding 40 kDa when observed through SDS-PAGE, while native PAGE indicated a total molecular mass of approximately 140–150 kDa [20]. In the same study, it was demonstrated that the MGL produced by *Pseudomonas* sp. consists of four identical subunits in terms of both molecular mass and charge, indicating a homotetrameric configuration. Hence, the molecular weight of 82 kDa for the L-methioninase from *Methylobacterium* sp. in our current study could be attributed to a homodimeric configuration, although further studies are needed to confirm this.

External factors have been observed to affect L-methioninase activity. Considering the results obtained in our study, where a temperature range of 35–40 °C was optimal for L-methioninase from *Methylobacterium* sp., others have reported 45 °C as optimum for *Streptomyces* sp. and 25 °C as optimum for *Brevibacterium linens* [21, 22]. However, L-methioninase from *Trichoderma harzianum*, *Aspergillus* sp., and *P. putida* has their optimal temperature at 35 °C [6, 18]. Additionally, optimal temperature for *Candida tropicalis* L-methioninase has been reported as 45–55 °C, 70 °C for *Streptomyces* sp. DMMMH 4, and 65 °C for *Streptomyces* sp. DMMMH 60 [13, 21]. These findings suggest that the optimal temperature for L-methioninase activity may be species or source-specific.

The activity of L-methioninase from *Methylobacterium* sp. in the current study has a pH optimum between 6 and 8.5, which is consistent with what has been reported for *Brevibacterium linens*, *Aspergillus ustus*, and *Aspergillus flavipes* [6, 19]. Regarding impact of metal ions, as seen in our current study with 1 mM Na+ having a positive effect on L-methioninase activity, similar findings have been reported for *Brevibacterium linens* CNRZ 918, where Na+ was shown to increase enzyme activity [22]. In contrast, Mn++, Ni++, Fe++, and K+ have been found to inhibit *Streptomyces* sp. DMMMH4 and *Trichoderma harzianum* [7, 18], which is contrary to our findings. We observed no significant changes in the enzyme activity of L-methioninase from *Methylobacterium* sp. in response to these metal ions.

When enzyme kinetics were evaluated, the K_m value of 90 mM reported in *Clostridium sporogenes* indicated lower specificity toward methionine, as compared to the K_m value (48.23 µM) obtained for L-methioninase from *Methylobacterium* sp. JUBTK33 [23]. The lower K_m value observed for L-methioninase in our current study provides strong evidence for its high anticancer potential and specificity toward cancer.

To enhance the anticancer potential of L-methioninase, researchers have implemented combination approaches with various chemotherapeutic drugs, such as 5-fluorouracil (5-FU), vincristine, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), and cisplatin [24]. While fewer studies on treating breast cancer using

L-methioninase in combination with chemotherapeutic drugs (such as 5-fluorouracil (5-FU), cisplatin, gemcitabine, and paclitaxel) are available [25], notably, TAM, being a first-line chemotherapeutic drug, has not been studied in vitro in combination with L-methioninase.

Comparing the results with monotherapy using L-methioninase from *Streptomyces* sp. DMMMH4, which resulted in 6% and 5% cell viability on breast and liver cancer cell lines, respectively [7], the newly discovered combination therapeutic approach using TAM along with L-MET from *Methylobacterium* sp. has proven to enhance the anticancer potential against breast cancer (1% cell viability). This approach also demonstrated an 18% protective effect on healthy cells in an in vitro model.

Before this approach can be developed into a new chemotherapeutic treatment strategy, further investigation in an in vivo model is needed.

Conclusion:

The study results lead to the conclusion that the L-methioninase enzyme from the soil-isolated bacterium, *Methylobacterium* sp. JUBTK33, has demonstrated significant enzyme activity and cytotoxicity against cancer cells. When combined with TAM (Tamoxifen), this enzyme exhibited promising anticancer potential, particularly against the MCF-7 cell line, and successfully mitigated the toxic effects of tamoxifen citrate on healthy HEK-293 cell lines. The combination of L-methioninase from *Methylobacterium* sp. with TAM holds great promise as an anticancer therapy. Further research in this direction could pave the way for the development of a new and effective therapeutic strategy for breast cancer.

Abbreviations

SPMet's	Semi-purified L-methioninase
L-MET	Sephadex G purified L-methioninase
TAM	Tamoxifen citrate
DOX	Doxorubicin
HEK-293	Human embryonic kidney cell line
DEAE	Diethylaminoethyl
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide

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Author contributions

KD and VKN contributed to the study conception and design. Material preparation, data collection, and analysis were performed by KD. The first draft of the manuscript was written by KD. The review and comments on the previous version of the manuscript were done by VKN. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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