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In vitro Anti-HIV-1 Activity of the Recombinant HIV-1 TAT Protein Along With Tenofovir Drug

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Abstract: ***Background***: HIV-1 TAT protein is essential for the regulation of viral genome transcription. The first exon of TAT protein has a fundamental role in the stimulation of the extrinsic and intrinsic apoptosis pathways, but its anti-HIV activity is not clear yet.

Methods: In the current study, we firstly cloned the first exon of the TAT coding sequence in the pET-24a expression vector and then protein expression was done in the *Rosetta* expression host. Next, the expressed TAT protein was purified by Ni-NTA column under native conditions. After that, the protein yield was determined by Bradford kit and NanoDrop spectrophotometry. Finally, the cytotoxicity effect and anti-Scr-HIV-1 activity of the recombinant TAT protein alone and along with Tenofovir drug were assessed by MTT and ELISA, respectively.

Results: The recombinant TAT protein was successfully generated in *E. coli,* as confirmed by 13.5% SDS-PAGE and western blotting. The protein yield was ~150-200 µg/ml. In addition, the recombinant TAT protein at a certain dose with low toxicity could suppress Scr-HIV replication in the infected HeLa cells (~30%) that was comparable with a low toxic dose of Tenofovir drug (~40%). It was interesting that the recombinant TAT protein could enhance anti-HIV potency of Tenofovir drug up to 66%.

Conclusion: Generally, a combination of TAT protein and Tenofovir drug could significantly inhibit HIV-1 replication. It will be required to determine their mechanism of action in the next studies.

Keywords: HIV-1, TAT protein, prokaryotic expression system, highly active antiretroviral therapy, tenofovir drug, anti-HIV activity.

1. INTRODUCTION

Human immunodeficiency virus (HIV) is a progressive infection which affected CD4^{+} immune cells and caused immunodeficiency. As a matter of fact, when the blood CD4 count is theoretically dropped below 200 cells/µL, an acquired immunodeficiency syndrome (AIDS) occurs. With the manifestation of highly active antiretroviral therapy (HAART) and early diagnosis, the life span of HIV patients was extensively increased [1-4]. It was observed that a novel therapeutic HIV vaccine consisting of replication-defective HIV along with viral suppression under antiretroviral therapy (ART) could reduce immune activation/chronic inflammation and latent infection [5]. The RNA genome of HIV consists of at least nine genes, such as *gag*, *pol*, *env*, *tat, rev*, *nef*, *vif*, *vpr*, and *vpu* [6-9]. Some functions of these proteins are mentioned as follows. The Gag proteins of HIV-1 are the main players in virus particle assembly, release and maturation [10]. The HIV-1 Pol gene encodes the enzymes for replication, including protease, reverse transcriptase, RNase H and integrase [11]. Moreover, HIV-1 enters cells through binding between viral envelope glycoprotein (Env) and cellular receptors to initiate virus and cell fusion [12-14]. The HIV-1 Tat protein is a key activator of HIV-1 transcription and is involved in the pathogenesis of HIV-related complications [15-19]. However, HIV-1 replication is significantly de-

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pendent on controlled processing of its RNA, and the activities of Tat and Rev regulatory factors [20, 21]. The HIV-1 Vpu protein contributes to HIV-1-induced CD4 receptor down-regulation and enhances the release of progeny virions from infected cells, as well [22-24]. HIV-1 infectivity factor protein *Vif* promotes the proteasomal degradation of an innate immune factor (A3G), allowing productive viral replication [25-27]. In addition, the Vpr protein was associated with replication efficiency and cytopathogenicity of the virus. HIV-1 Vpr modifies host cell energy metabolism, oxidative status, and proteasome function [28-30]. Finally, the Nef protein plays a major role in virus replication *in vivo* and in the onset of AID. This protein is a primary determinant of viral pathogenesis [31, 32]. Many proteins encoded by the HIV genome possess pro- and/or anti-apoptotic qualities [33]. TAT protein (Transactivator of transcription) is required for successful transcription of the full-length HIV mRNA. This protein consists of two exons; the first exon comprising amino acids 1-72 has the transcriptional functions, and the second exon containing amino acids 73-101 or 73-86 possesses the integrin-binding domains [6, 34, 35]. The functional domains of the first exon include the acidic/proline-rich, the cysteine-rich/ZnF, the core, and the basic and the glutamine-rich domains. The structure of the TAT protein gives it the properties of both transcription promotion and membrane transduction [34]. HIV TAT protein can be secreted from HIV-infected cells and although it has no signal sequence, it can be taken up by non-infected cells [36]. According to the previous studies, the first exon of HIV TAT protein has a dual function on immune cells. TAT protein can cause loss of B cells but activate dendritic cells (DCs) and $CD8^+$ T cells, thus promote earlier and last longer cellular immune response in HIV-infected or -uninfected people [37]. On the other hand, the N-terminal of TAT protein induced apoptosis in different HIV-infected cell lines by a variety of mechanisms, thus it could prevent infection diffusion to uninfected neighbor cells. For example, TAT could up-regulate Fas ligand and increase caspase 8, trigger Bax/mitochondrial death pathway, suppress microtubule dynamics by the degradation of microtubule-associated protein 2, affect mitotic spindle formation and chromosome assembly in mitosis, and induce FOXO3 in infected T-cells [38, 39]. In contrast, the first exon of TAT had anti-apoptotic effects on HIV-infected cells, as well. For instance, TAT protein could make HIV-infected cells resistant to death by the down-regulation of caspase 10 expression and up-regulation of FLIP isoforms. Therefore, these anti-apoptotic effects had a critical impact on HIV pathogenesis. In fact, the previous reports represented that *in vitro* pro- or anti-apoptotic effects of TAT on HIV-infected cells depend upon the type of cell line, endogenous expression vectors, exogenous injection, the dose of the injected TAT and oxygen level [39, 40]. Therefore, for understanding the impact of TAT protein in HIV therapy, our study was focused on the anti-HIV effects of the recombinant TAT protein in HeLa cells which were infected by the first generation of single-cycle replicable (Scr) virions that can replicate only for one cycle and also are safe [41]. Moreover, it was used in combination therapy with the commercially available anti-HIV drug, Tenofovir.

2. METHODS

2.1. Materials

The pET24a (+) expression vector was obtained from Novagen (provided by the Gene bank at Pasteur Institute of Iran). The plasmid extraction mini-kit was purchased from FAVORGEN Company (Taiwan). Isopropyl-D-thiogalacto-pyranoside (IPTG), Ty2x medium, LB medium and Kanamycin were bought from Sigma (Germany). The RPMI-1640 medium, DMEM medium, phosphate-buffered saline (PBS), 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT), 3, 3'-diaminobenzidine (DAB), and penicillin/ streptomycin 100X (PS) were purchased from Gibco (USA). The peroxidase conjugated-anti-His antibody and Ni-NTA agarose column were obtained from Qiagen (Germany). Tenofovir drug was received as a gift from Bakhtar Bioshimi Company (Iran).

2.2. Cloning of TAT Coding Sequence in a Prokaryotic Expression Vector

The coding sequence of the TAT gene from the pNL4.3 vector (the first exon, AF324493.2) was obtained by polymerase chain reaction (PCR) using the designed forward (5′-AAGCTAGCATGGAGCCAGTA-3′ and reverse (5′-AACTCGAGCTTTGATAGAGAAGCTT-3′) primers. PCR was performed by thermo cycler under standard conditions (95°C for 30s, 60°C for 1 min and 72°C for 1min: 30 cycles; and final extension: 72°C for 5 min) and the product was detected by DNA agarose gel electrophoresis. The digested PCR product was ligated into the pET24a (+) expression vector in *Nhe*I/ *Xho*I restriction sites. The pET24a-TAT was used to transform 100 µl of *E. coli* DH5α strain by the standard heat shock method. Then, the kanamycin-resistant clones were picked and grown overnight in Luria-Bertani (LB) medium (Sigma, Germany) containing 25µg/ml Kanamycin (Sigma, Germany). Next, the recombinant pET24a--TAT was extracted from DH5α cells by FavorPrep ™ plasmid extraction mini-kit. Eventually, for confirming the recombinant pET24a-TAT, the extracted pET24a-TAT was digested by *Nhe*I/ *Xho*I restriction enzymes, and visualized by DNA gel electrophoresis. Moreover, the TAT gene sequence was confirmed by PCR and sequencing.

2.3. Expression of the Recombinant TAT Protein

For protein expression, the *E. coli Rosetta* strain was transformed using the recombinant prokaryotic plasmid (pET24a-TAT) with an N-terminal 6X His-tag. A single colony of transformant was cultured in fresh liquid LB medium containing kanamycin 25 µg/ ml at 37°C for 16 hr. After the inoculation of bacteria in Ty2x medium, when the bacterial cells reached mid-log growth (OD_{600}: 0.7-0.8), the expression of recombinant TAT protein was induced by the addition of isopropyl-D-thiogalacto-pyranoside (IPTG) to a final concentration of 1mM. The bacterial cells were harvested by centrifugation at 6000 rpm for 5 min at different incubation periods (2, 3 & 4 hr). Finally, the cell pellets were analyzed by 13.5% SDS-PAGE (SDS gel apparatus; BioRad,

USA) and stained by coomassie brilliant blue (Sigma, Germany).

2.4. HIV-1 TAT Protein Purification

The recombinant TAT protein was purified under native conditions using nickel-nitrilotriacetic acid (Ni-NTA)-agarose column *via* Qiagen protein purification protocol. Briefly, the cell pellets were resuspended in lysis buffer A (10 mM Imidazole, pH=8, Sigma, Germany), placed on ice for 1 hr, and sonicated for 5 min. Then, the supernatant was collected by centrifugation at 10000 rpm for 20 min at 4°C. Next, the cell lysate was applied on the Ni-NTA column (Qiagen, Germany) and after three washes by buffer B (30 mM Imidazole, and pH=8), the TAT protein was eluted by 300 mM imidazole elution buffer (pH=8). All fractions were collected and analyzed by SDS-PAGE 13.5%. Finally, the purified protein was dialyzed against PBS1X (pH=7.2), and its concentration was estimated by the Bradford method and NanoDrop spectrophotometry. Endotoxin contamination with LPS was evaluated by Limulus Amebocyte Lysate (LAL assay, QCL-1000, USA) that was less than 0.5 EU/mg for TAT protein. The recombinant protein was kept at -70˚C until use.

2.5. Western Blotting

The protein samples (before IPTG induction, after IPTG induction, the purified protein) were run on 13.5% SDS--PAGE, and then transferred into the nitrocellulose membrane (Millipore, USA). The peroxidase conjugated-anti-His antibody (Qiagen, Germany) was used to confirm TAT protein expressed under standard procedures. Finally, the immune reactive protein bands were visualized using a peroxidase substrate named as 3, 3'-diaminobenzidine (DAB, Sigma, Germany).

2.6. Cytotoxicity Assay

MTT assay was used for the determination of the cell toxicity of TAT protein, and Tenofovir (anti-HIV-1 drug) alone and in combination with TAT protein. Briefly, HeLa cells (1×10^4 cells/ well) were seeded in 96-well microtiter plates and incubated for 24 hr at 37°C. After replacement with fresh medium (DMEM medium containing FBS 10%), the cells were treated with 100 µl of TAT protein (0.25, 0.5, 1 & 2 µg), Tenofovir drug (0.75, 1.25, 5, 10, 20 & 30 µM), and the combination of TAT protein (2 µg) with Tenofovir drug (0.75, 1.25, 5, 10, 20 & 30 µM) for 48 hr. Then, the MTT solution was added and incubated for 3 hr at 37°C. Next, the produced formazan was dissolved in isopropanol (Sigma, Germany), and the absorbance was measured by ELISA reader in 570 nm wavelength. Finally, the cytotoxicity rate was determined by Equation 1. The negative control was untreated HeLa cells.

Equation 1:

Toxicity percentage: $[1 - (OD_{Sample} / OD_{Negative\ control})] \times 100$

2.7. The Inhibitory Effects of TAT Protein, Tenofovir, and TAT Combined with Tenofovir on HIV-1 Replication

The anti-HIV-1 activity was studied using a single-cycle replication assay. Generation of single cycle replicable (Scr) HIV-1 was performed according to the previous study [41]. Briefly, HEK-293T cells were seeded in a 6-well plate and co-transfected with different plasmids of pSPAX2, pMD2G and pmzNL4-3 using Lipofectamine 2000 transfection reagent (Fermentas, Germany) according to manufacturer's instruction. Then, culture supernatants of transfected cells were harvested, filtered and further centrifuged at 50000 g for 3hr. Finally, the virion pellet was resuspended in DMEM media and stored at -70°C. The infectious titer was determined by the enzyme-linked immunosorbent assay (ELISA) [41, 42].

After the preparation of virions, the inhibitory effects of TAT and Tenofovir drug against HIV-1 were studied by Scr assay. For this purpose, the HeLa cells were seeded in a 6 well plate (5×10^5 cells/well). After one day, the cells were infected with Scr HIV-virions (600 ng P24/well). After 6 hr, the cells were washed twice with pre-warmed DMEM for removing unbound virions, and then, 15×10^3 cell/200 µl of infected HeLa cells were seeded to each well of the 96-well plate. The HIV inhibitory effects of TAT protein and Tenofovir drug were evaluated at different concentrations. After 72 hr, the cell supernatants were analyzed for p24 antigen load according to the p24 capture ELISA kit (Biomerieux, USA) protocol. The HIV inhibitory rate was measured by Equation 2.

Equation 2:

$$\frac{\text{Average P24 produced by treatment} - \text{average P24 produced by control}}{\text{average P24 produced by control}} \times 100$$

2.8. Statistical Analysis

All of thetests were performed in triplicate and two independent experiments. The results were expressed as the mean ± standard deviation (SD). Statistical analysis was done by Graph Pad Prism version 8.3.0 using One-way ANOVA. A *p*-value of lower than 0.05 was considered to be statistically significant.

3. RESULTS

3.1. TAT Protein Expression and Purification

At first, the recombinant pET24a-TAT vector was confirmed by PCR amplification or enzyme digestion as a clear band of ~210 bp on agarose gel electrophoresis (data not shown). For protein expression, the transformation of the *E.-coli Rosetta* strain was done using the pET24a-TAT vector. A single colony of transformant was cultured until OD_{600} reached 0.77. Then, IPTG (1mM) was added for induction of protein expression at different times (2, 3 & 4 hr). The expression of TAT protein was observed at 4 hr after IPTG induction. As calculated, the recombinant TAT_{1-72} protein should migrate as a clear band of ~10 kDa, but it is higher

than this size. The higher size may be related to hydrophobic or negative charge amino acids (Fig. **1A**) Then, the recombinant TAT protein was purified by Ni-NTA column based on the interaction between immobilized Ni^{2+} ion and the histidine side chain of protein under native conditions. Accordingly, the supernatant of the cell lysate was purified by competition of imidazole with 6xHis-tag of TAT protein at constant pH (Fig. **1B**). Finally, the recombinant protein was identified by the peroxidase conjugated-anti-His antibody using western blotting (Fig. **2**). The yield of protein of about 150-200 was obtained µg/ml using Bradford kit and Nano-Drop spectrophotometry.

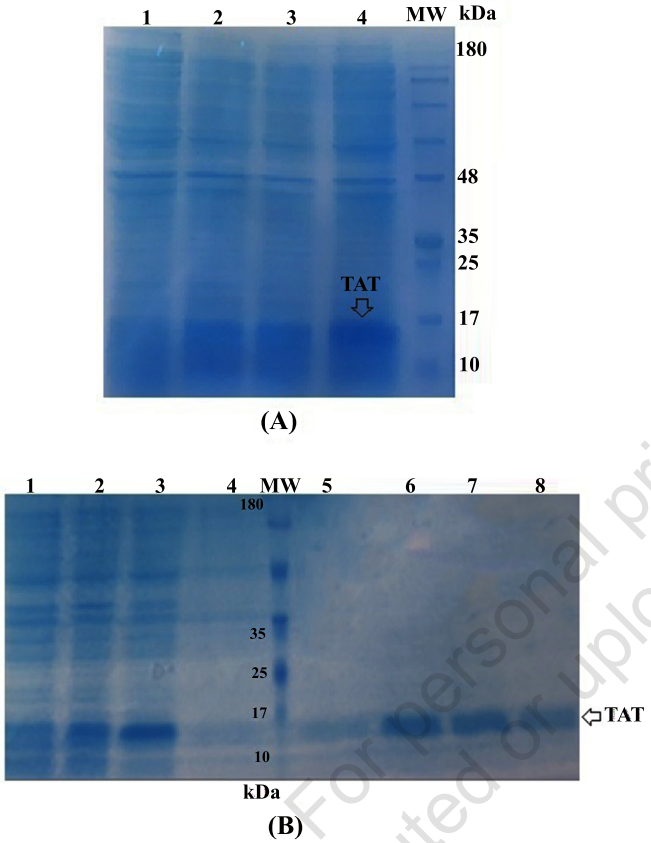

Fig. (1). Generation of TAT protein in the *E. coli Rosetta* strain confirmed by SDS-PAGE: A) Expression of TAT_{1-72} protein: Before IPTG induction (Lane 1), 2 hr after IPTG induction (Lane 2), 3 hr after IPTG induction (Lane 3), and 4 hr after IPTG induction (Lane 4); B) Purification of TAT_{1-72} protein: Before IPTG induction (Lane 1), 4 hr after IPTG induction (Lane 2), Supernatant of cell lysate (Lane 3), Pellet of cell lysate (Lane 4), Different elution fractions (Lane 5-8). MW: Molecular weight marker (prestained protein ladder, 10-180 kDa, Fermentas). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

3.2. *In vitro* Cytotoxicity Study

The cytotoxicity of the recombinant TAT protein, Tenofovir drug, and TAT+Tenofovir on HeLa cells was investigated by MTT assay at different concentrations. The un-treated HeLa cells were considered as a negative control. With regard to the results, all of the treatments exhibited clear dose-dependent cytotoxicity against HeLa cells (Fig. **3A**). The main result was that TAT protein (2 µg) combined with different doses of Tenofovir could reduce Tenofovir cytotoxicity. It was interesting that TAT protein at a constant dose could further decrease Tenofovir toxicity at higher doses. It may be related to a possible interaction between Tenofovir and TAT protein, especially at higher doses of the drug (Fig. **3B**).

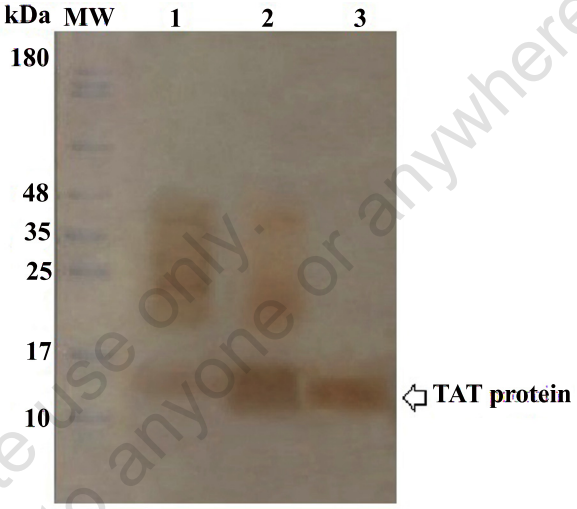

Fig. (2). Generation of TAT protein in the *E. coli Rosetta* strain confirmed by Western blotting: Before IPTG induction (Lane 1), 4 hr after IPTG induction (Lane 2), and purified TAT protein (Lane 3); MW: Molecular weight marker (prestained protein ladder, 10-180 kDa, Fermentas). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

3.3. *In vitro* Anti-HIV-1 Activity

The anti-HIV-1 efficacy of the recombinant TAT protein, Tenofovir, and TAT + Tenofovir was evaluated at different concentrations after 72 hr incubation in the HeLa cell line. The anti-HIV-1 effects of all treatments displayed a dose-dependent manner. In addition, the TAT protein at a dose of 2 µg (low toxic) could suppress Scr-HIV replication in infected HeLa cells (~ 30%) that was comparable with Tenofovir drug (~ 40% at 20 µM). It was interesting that TAT protein (2 µg) could enhance anti-HIV potency of Tenofovir drug (20 µM) up to 66%. Indeed, the inhibitory effect of Tenofovir drug was enhanced after combination therapy with TAT protein (Fig. **4**).

4. DISCUSSION

HIV TAT protein could play a critical role in inducing extrinsic or intrinsic pathways of HIV-infected cell apoptosis, leading to the reduction of HIV diffusion and its pathogenesis. On the other hand, TAT protein induced an anti-apoptotic pathway in HIV-infected cells leading to the promotion of virus diffusion and pathogen spreading in the body. Thus, the opposite roles for TAT protein were reported [43]. Moreover, TAT protein could suppress the expression of cellular Mn-containing superoxide dismutase and the activity of glu-

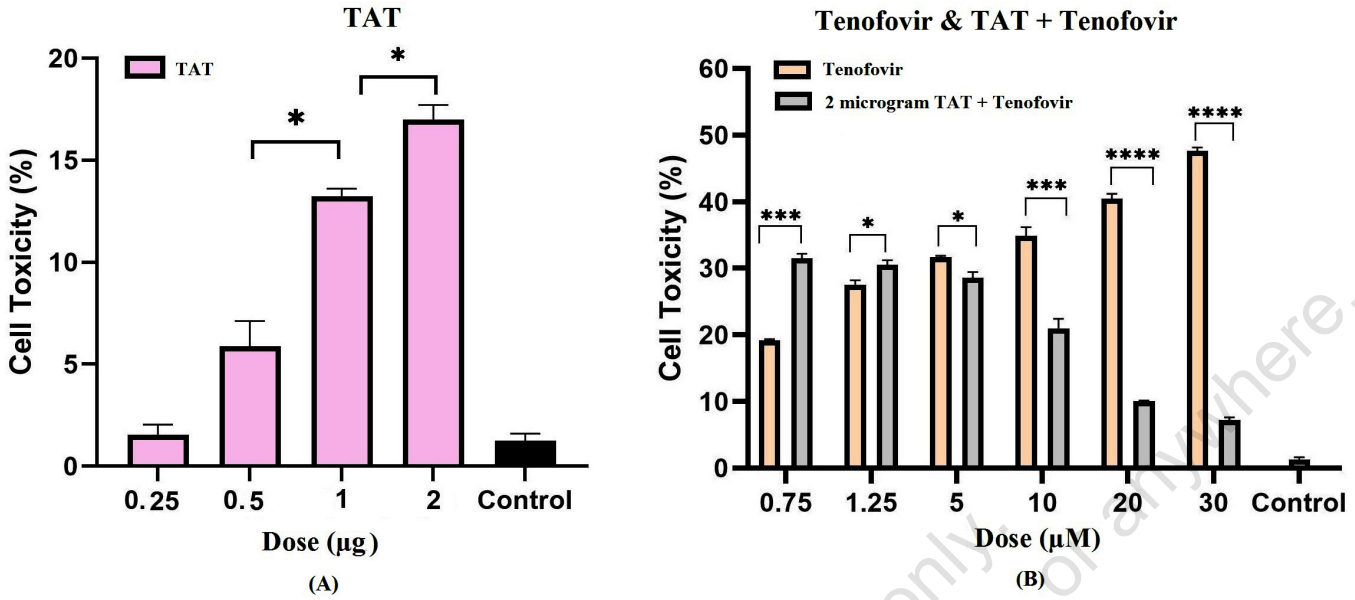

Fig. (3). MTT assay of the treated HeLa cells with different compounds as compared to the untreated cells (control): A) TAT treatment; B) Tenofovir as well as TAT+Tenofovir treatments. The error-bars represent the standard deviation from the mean (N= 3); ns: non-significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

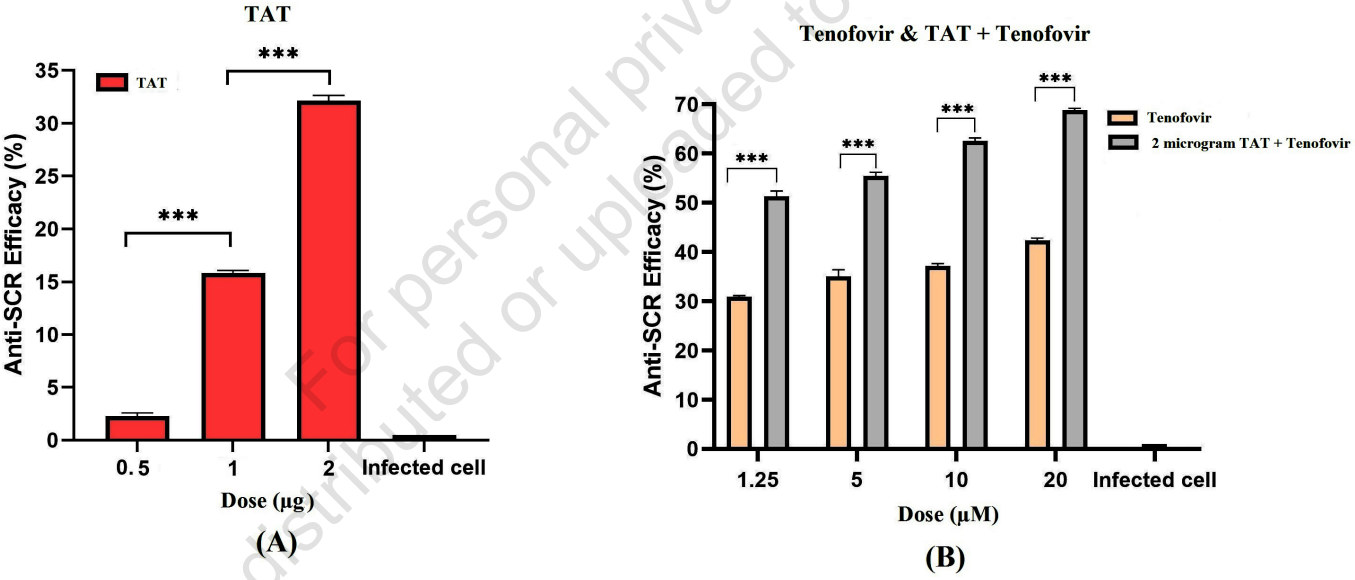

Fig. (4). Anti-HIV-1 activity of treatments in different doses on infected HeLa cells after 72 hr incubation: A) TAT treatment; B) Tenofovir as well as TAT+Tenofovir treatments.The error-bars represent the standard deviation from the mean (N= 3); ns: non-significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

tathione peroxidase in the HeLa cell line. Therefore, it could increase oxidative stress and induce apoptosis pathways [44, 45]. TAT protein activated NF-κB in HeLa cells resulting in promoting inflammation cascades, as well [46]. Bennasser *et al*. showed that HIV-1 TAT protein had a suppressor of RNA silencing function in its amino acids 30-72 to combat host cellular immunity [47].

In this study, we intended to clarify the anti Scr-HIV activity of the TAT protein on infected HeLa cells for the first

time. As a matter of fact, various strategies were applied for the improvement of TAT protein expression and simple purification in the previous studies [48]. As known, *E. coli* strain is a common cellular host for foreign protein expression due to its fast growth rate, inexpensive expression system, owning T7 as a powerful promoter and also highly selective and efficient T7 RNA polymerase [48-54]. In addition, BL21 (DE3) strain is a type of *E. coli* strain that has a T7 RNA polymerase coding gene in its genome for expression of the coding gene inserted in downstream of the T7 promoter. It seems that BL21 (DE3) strain is a good choice for TAT expression, but as mentioned by the previous studies, it could not express TAT protein efficiently. This consequence was due to the lack of tRNAs, which was necessary for seven rare expression codons (AGA, AGG, AUA, CUA, GGA, CCC and CGG) in the coding sequence of TAT protein. Therefore, the *E. coli Rosetta* strain was used for the expression of the recombinant TAT protein. This *E. coli* strain derived from BL21 (DE3) strain could provide tRNAs for the expression of these seven rare codons [50, 55, 56]. We also used the *E. coli Rosetta* strain as an expression host for the generation of the recombinant TAT protein. Our results showed the expression of TAT protein on SDS-PAGE at four hours after IPTG induction. On the other hand, affinity chromatography based on immobilized metal ions such as Cu^{2+}, Co^{2+}, Ni^{2+} and Zn^{2+} was known as a powerful method for protein purification. This purification system was based on the interactions between an immobilized metal ion on a matrix and specific amino acid sequences. One of these sequences was six histidine residues, which have highly efficient interaction with immobilized nickel ion. The protein purification could be done under native or denaturing conditions based on protein solubility. The protein purification under native conditions could keep the tertiary structure of the protein, thus the biological activity of the protein remained intact [57, 58]. In this study, we purified His-tagged TAT protein using Ni-NTA column under native conditions. After the preparation of the recombinant TAT protein, its toxicity and anti-HIV-1 effects were evaluated *in vitro* as compared to Tenofovir, a commercial anti-HIV drug. We used a safe Scr-HIV virion for the evaluation of anti-HIV effects of TAT, Tenofovir, and TAT + Tenofovir. Tenofovir is a nucleotide analog with potent anti-retroviral activity. This drug could be considered as a unique type of nucleoside analog reverse-transcriptase inhibitors (NRTIs). According to a previous study, the NRTIs could influence mitochondrial functions in the liver and skeletal muscle cell line at doses above 300 μM [59]. In addition, Tenofovir could promote TNF-α release, mitochondrial stress and cell apoptosis in the HK-2 cell line [60]. Inhibition of human DNA polymerase α, β, γ and ε was suggested as a mechanism of NRTIs toxicity in the HeLa cell line [61]. A study indicated that the reduced doses of antiretroviral drugs, including tenofovir disoproxil fumarate and efavirenz, may decrease toxicity while maintaining efficiency [62]. Moreover, it was reported that long-acting drugs can play a similar role with a short-term AIDS vaccine [63]. As known, Scr-HIV-1 virions have frameshift mutation or deletion across the *pol* gene, which encodes RT (reverse transcriptase) and IN (integrase) enzymes. Therefore, Scr-HIV virions contain the structural features of wild type HIV-1, but their genome cannot code the functional RT or IN proteins, thus they have lost their infectivity potential [41, 64, 65]. In this study, due to the bilateral effects of Tenofovir and TAT protein, the combination therapy of Tenofovir and TAT protein was considered on the infected HeLa cell line, as well. Our results indicated that all of the treatments displayed a dose-dependent anti-HIV-1 inhibitory effect. Moreover, TAT protein indicated significant Scr-HIV inhibition on the infected-HeLa cell line comparable with anti-HIV activity of Tenofovir, the *Food and Drug Administration* (FDA) approved anti-HIV drug. It was interesting that TAT protein could significantly increase the anti-HIV activity of Tenofovir drug. It was shown that the combination of M48U1 (6.7 μM) and Tenofovir (20 μM) synergistically inhibited HIV infection in activated PBMCs and human cervicovaginal histocultures [66]. Herein, the combination of TAT protein (2 μg) with the same dose of Tenofovir (20 μM) could significantly increase anti-HIV-1 activity, as well (~66%). In contrast, TAT protein could significantly reduce cytotoxicity of Tenofovir drug on the HeLa cell line *in vitro,* especially at high doses. The reports suggested that the cytotoxicity potential of the TAT protein was due to the occupation of the intrinsic and extrinsic pathways of apoptosis, simultaneously [43, 67]. Herein, at the selected doses of TAT, it did not show high toxicity as compared to Tenofovir drug at certain doses. A study showed the cytotoxicity of Tenofovir drug at doses of 1.5, 3, 4.8, 14.5 and 28.8 on HK-2 cells was about 40-60% at 48 hr after treatment. Tenofovir final doses of 4.8 and 14.5 μM showed additional growth in toxicity, especially at 72 hr after treatment (~ 50-55% at 48 hr and ~ 55-60% at 72 hr). Our study showed approximately 30-35% toxicity for the same doses of Tenofovir on HeLa cells at 48 hr after treatment. Thus, the type of cells affects the cellular mechanism of drug toxicity [60]. The combination therapy of Tenofovir in different doses with the fixed--dose of TAT (2 μg) displayed a synergistic effect on HeLa cell viability. This achievement may be attributed to the overlapping of the TAT protein and the Tenofovir cytotoxicity mechanism. Some micro- or nanoparticles were used for the delivery of anti-HIV drugs. These delivery systems could increase anti-viral potency of drugs and decrease their toxicity [68-75]. For instance, Tenofovir-platelet microparticles increased HIV-1 inhibitory effects as compared to Tenofovir alone. Moreover, this formulation decreased the toxicity of Tenofovir due to the slow release of the drug [76]. However, the mechanisms of delivery systems are almost clear for reducing the toxicity and increasing anti-HIV-1 activity; but the anti-HIV activity of TAT protein along with Tenofovir drug was evaluated for the first time and will need further studies on their mechanisms of action.

CONCLUSION

In the current study, TAT protein could be successfully expressed in *E. coli Rosetta* strain and purified using affinity chromatography under native conditions. In addition, the combination therapy of the TAT protein with a reverse transcriptase inhibitor, Tenofovir drug, showed higher an-

ti-HIV-1 effects than TAT or Tenofovir, alone. However, further studies are needed to clarify their mechanism for this synergistic effect.

LIST OF ABBREVIATIONS

HIV	=	Human Immunodeficiency Virus
AIDS	=	Acquired Immunodeficiency Syndrome
HAART	=	Highly Active Antiretroviral Therapy
TAT	=	Transactivator of Transcription
DC	=	Dendritic Cells
IPTG	=	Isopropyl-D-thiogalacto-pyranoside
PBS	=	Phosphate-Buffered Saline
MTT	=	3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
DAB	=	3,3'-diaminobenzidine
PS	=	Penicillin/ Streptomycin
Scr	=	Single-Cycle replicable
FDA	=	Food and Drug Administration
ELISA	=	Enzyme-linked Immunosorbent Assay

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This article does not contain any studies with human participants or animals performed by any of the authors.

HUMAN AND ANIMAL RIGHTS

No animal and human were used in this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the results and findings of this study are available within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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