Original Article

Establishment of an In-Vitro Alzheimer's Disease Model Through Amyloid-β(25–35) Neurotoxicity in SH-SY5Y Cells

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ABSTRACT

Background: Alzheimer's disease (AD) is a progressively neurodegenerative condition marked by amyloid-β (Aβ) accumulation and neuronal death. Despite substantial research, effective disease-modifying medications remain unavailable, underlining the need for dependable preclinical models to test novel therapeutic techniques. In order to enable future screening of possible anti-Alzheimer's drugs, the current work set out to create an in vitro Alzheimer's disease model with an ideal dose of Aβ (25–35) that causes repeatable neuronal cytotoxicity in SH-SY5Y cells.

Methods: The experiment was conducted at Ziauddin University, Karachi. SH-SY5Y neuroblastoma cells were treated to Aβ (25–35) at doses ranging from 0–80 µM during 24 hours. Cell viability was assessed using the MTT test, and the concentration that reduced survival by 50–60% was determined as the best cytotoxic dose for model development. To confirm cell death and observe membrane integrity and nuclear morphology, cells had been stained with propidium iodide (PI) and DAPI, then studied by fluorescence microscopy.

Results: Aβ (25-35) treatment produced a clear, concentration-dependent reduction in viability. At 40 µM, cell survival decreased to ~54%, meeting the target threshold of 50–60% cytotoxicity. Fluorescence microscopy confirmed these findings, with PI staining highlighting membrane-compromised cells and DAPI revealing nuclear condensation and fragmentation. Together, these outcomes validate 40 µM Aβ (25–35) as a reliable concentration for establishing a standardized in vitro model of AD.

Conclusion: This study created an in vitro Alzheimer's disease model utilizing SH-SY5Y cells exposing them to Aβ(25-35), with 40 µM causing ~50-60% cytotoxicity and neurotoxicity. This model may serve as a core basis for future in vitro AD research.

Keyword: *Alzheimer Disease; Amyloid beta-Peptides; SH-SY5Y Cells; Neurotoxicity Models; Apoptosis; Cell Survival; Neuroprotection.*

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INTRODUCTION

Alzheimer's disease (AD) is a progressive form of neurodegenerative that gradually impairs memory and an array of cognitive functions, including language, learning capacity, visuospatial processing, reasoning, and behavior[1]. Alzheimer's disease and other neurodegenerative conditions demonstrate several distinctive microscopic features. Key changes include abnormal buildup of proteins such as Aβ and tau, impaired synaptic signaling, disrupted protein turnover, cytoskeletal disorganization, altered energy regulation, and changes in DNA and RNA integrity, along with activation of inflammatory pathways. Together, these disruptions ultimately lead to neuronal loss. Oxidative stress is also commonly present in Alzheimer's and related dementias. However, it is often treated as a downstream effect rather than being recognized alongside the principal pathological hallmarks.[2]

Despite decades of work delineating its core lesions, extracellular amyloid-β (Aβ) deposits, intracellular tau pathology, synaptic failure, neuroinflammation, and progressive neuronal loss, currently available disease-modifying

therapies remain limited.[3] This translational gap underscores the need for robust, scalable cellular systems that recapitulate key aspects of AD biology to enable mechanism testing and preclinical screening.[4]

Among in-vitro approaches, Aβ-driven neurotoxicity remains a pragmatic entry point because it models early injury pathways implicated in synaptic compromise and neuronal death.[5,6] The Aβ25–35 fragment is widely used for this purpose; compared with full-length Aβ species, Aβ25–35 aggregates rapidly and reproducibly under defined conditions, yielding stable oligomeric assemblies with potent neurotoxicity across neuronal preparations.[7] Its effects on oxidative stress, mitochondrial dysfunction, calcium dysregulation, and activation of apoptotic cascades mirror well-described pathomechanisms in AD brains and animal models.[8]

The human neuroblastoma line SH-SY5Y provides a complementary cellular context for Aβ-induced injury. SH-SY5Y cells possess neuronal characteristics, support neurite extension, and demonstrate a predictable response to Aβ exposure and maintain the throughput and genetic consistency needed for experimental reproducibility.[9] When paired with viability assays such as MTT and complementary morphological evaluation, these cells provide a dependable system for validating in vitro Alzheimer's disease (AD) models, balancing biological relevance with experimental practicality.[10,11]

The present investigation was attempted to construct an in vitro AD model by exposing SH-SY5Y cells to Aβ (25–35) and discovering the peptide concentration that generates around 50 60% loss in viability. The specific objectives were to determine the concentration–response relationship after 24 hours of exposure, define an appropriate "injury threshold" for evaluating neuroprotective interventions via the MTT assay, and verify cytotoxic effects through microscopic assessment of characteristic morphological changes. This strategy was intended to yield a standardized and reproducible model that can be applied in future mechanistic research and therapeutic screening in AD.

METHODOLOGY

This experimental study was conducted at Ziauddin University, Karachi, over a period of six months following approval from the Institutional Ethical Review Committee. This study was exempt from ethical review as it involved only cell line experiments (Ref: 8011123SKMM).

Cell Culture

Human neuroblastoma SH-SY5Y cells (ATCC, USA) were cultured in Advanced Dulbecco's Modified Eagle Media (DMEM/F12 medium; Gibco, USA) with 10% fetal bovine serum (FBS; Sigma-Aldrich, USA), 100 U/mL penicillin, and 100 µg/mL streptomycin at 37°C in a humidified environment with 5% CO_2. Cells were sub-cultured at ~80% confluence then utilized between passages 3-10 to reduce phenotypic drift[12]. SH-SY5Y cells were differentiated before treatment to achieve a more neuron-like phenotype. Cells were first exposed to retinoic acid (RA) for a few days to stimulate neuronal maturation, then administered brain-derived neurotrophic factor (BDNF) to improve neurite outgrowth and function. This approach enhances the physiological relevance of the model for Aβ-induced toxicity research.

Experimental Design and Grouping

Cells were seeded in 96-well plates at a density of 1 × 10^4 cells/well and allowed to adhere overnight. The following groups were established:

- **Group 1 (Control/Vehicle):** Cells treated with vehicle (sterile distilled water) only.
- **Group 2–7 (Model groups):** Cells treated with Aβ25–35 at final concentrations of 2.5, 5, 10, 20, 40, or 80 µM and incubated for 24 h.

Each condition was performed in multiple replicates, and the experiment was independently repeated three times to ensure reproducibility.

Cell Viability Assay (MTT)

Cell viability was evaluated using the MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) test (Sigma-Aldrich, USA). A 96-well plate was seeded with cells and left to grow there for 24 hours. Cells were exposed to Aβ-25-35 at varied doses for 24 hours to determine its effects. After the incubation and removal of the culture media, the cells were treated with 0.5 mg/ml MTT in phosphate-buffered saline (PBS) for three hours in the dark. The formed purple formazan crystals were later dissolved in 100 µL of DMSO, and their absorbance at 570 nm was measured with a spectrophotometer. Absorbance was determined at 570 nm using a microplate reader (Bio-Rad, USA). Equation 13 was used to calculate cell viability (%).

Percentage viability = (absorbance of test/absorbance of control) x 100"

DAPI with Propidium Iodide (PI) Staining

To validate Aβ(25–35)-induced cytotoxicity, dual staining with propidium iodide (PI) and 4′,6-diamidino-2-phenylindole (DAPI) was carried out. A PI and DAPI stock solution (1 mg/mL) was diluted to a working dosage of 1µg/mL before use. After being seeded onto 24-well plates, SH-SY5Y cells were left to adhere for the entire night. The following day, cells were treated with 40 µM Aβ(25–35) for 24 hours to establish the AD model. After the procedure, the cells were incubated with the PI functioning solution for 30 minutes at room temperature. Excess dye was extracted by washing with phosphate-buffered saline (PBS), and stained cells were viewed using a fluorescence microscope. Red PI-positive nuclei signified cell death and loss of membrane integrity. To view intact nuclei and evaluate overall nuclear morphology, DAPI labeling was used concurrently. The viability data from the MTT experiment were supported by supplementary evidence of Aβ-induced cell damage from PI and DAPI labeling.

Statistical Analysis

The data analyses were performed with SPSS version 22 (SPSS, Inc., Chicago, IL, USA). Data was expressed as mean ± SEM. For the in vitro experiments, the comparisons were done by the student t-test and one-way ANOVA, as applicable. The p-value <0.05 was considered statistically significant.

RESULTS

Aβ25–35 Induced Dose-Dependent Cytotoxicity in SH-SY5Y Cells

The MTT assay demonstrated that Aβ25–35 considerably reduced SH-SY5Y cell viability in a dose-dependent way (Table-1). At lower dosages (2.5–10 µM), viability fell moderately, ranging from ~86% to 75% viability in comparison to untreated controls. Viability decreased to 64.97 ± 1.54% at 20 µM, a more noticeable decrease. At higher dosages (40 and 80 µM), survival was lowered further, reaching 54.27 ± 2.84% and 52.74 ± 3.81%, respectively ($p < 0.05$ versus control). These data indicate that 40 µM generates an approximate 50–60% loss in viability, making it a good damage set-point for model formation.

Table-1. Effect of Aβ25–35 on SH-SY5Y cell viability (MTT assay)

Aβ25–35 (µM)	Mean Viability (%)	SEM
0 (Control)	100.00	0.00
2.5	85.88	2.33
5	82.41	1.79
10	75.09	3.29
20	64.97	1.54
40	54.27	2.84
80	52.74	3.81

A concentration–response curve of Aβ25–35 on SH-SY5Y cell viability is presented in Figure 1. The plot illustrates a clear, dose-dependent decline in viability compared with the untreated control. At low concentrations (2.5–10 µM), only moderate reductions were observed, whereas higher concentrations (20–80 µM) caused substantial loss of viability. Notably, treatment with 40 µM Aβ25–35 reduced viabilities to approximately 54%, aligning with the targeted 50–60% cytotoxicity threshold used for model development.

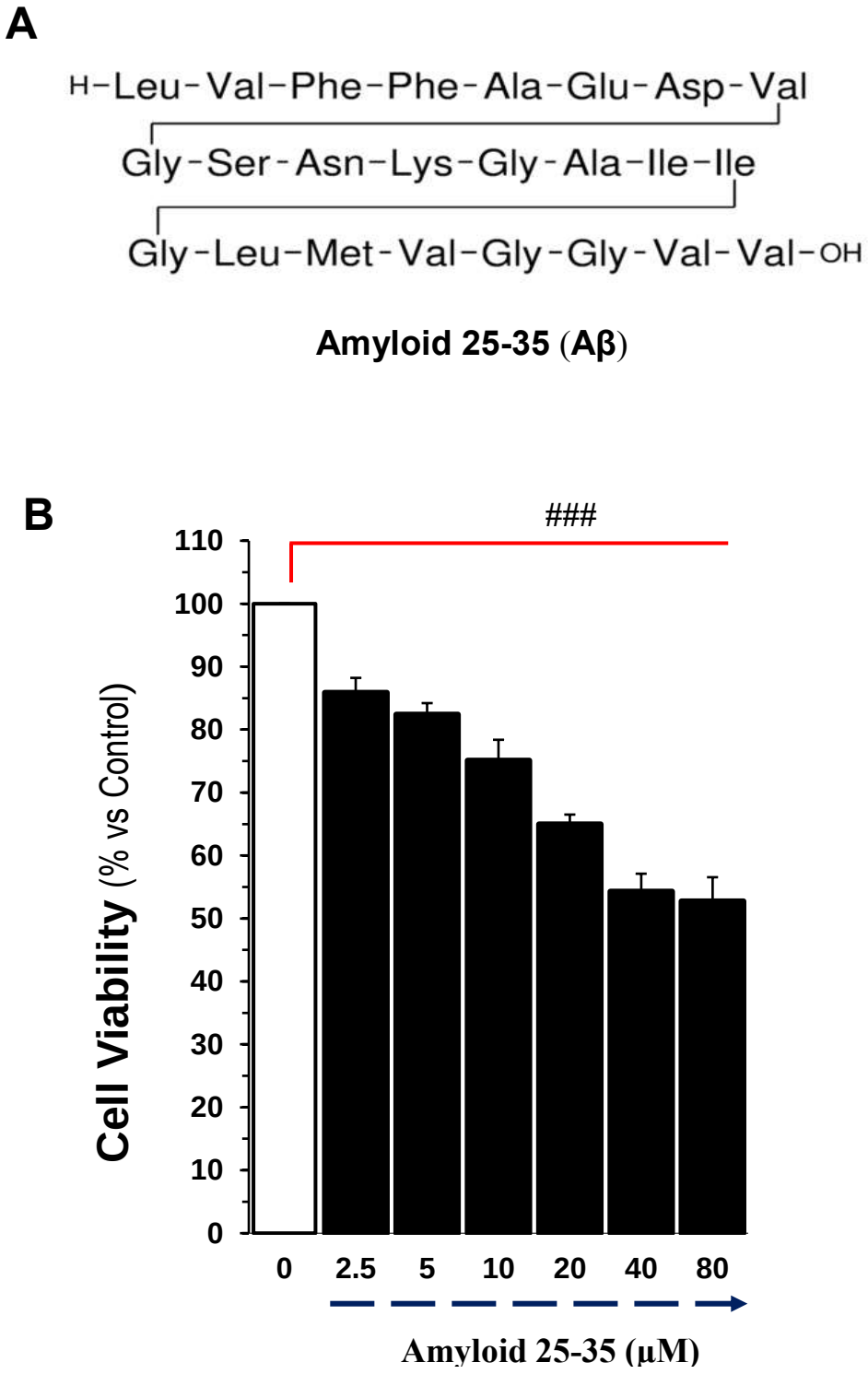

Fig.1 Dose-dependent cytotoxicity of Aβ25–35 in SH-SY5Y cells. Cells were treated with Aβ25–35 (0–80 µM) for 24 h, and survivability was established by MTT assay. Data are displayed as mean ± SEM. ###P<0.001, an significant shift compared to Aβ25–35 treated sample.

PI/DAPI Staining Confirms Aβ (25–35)-Induced Cell Death

Fluorescence imaging with PI and DAPI staining provided visual confirmation of Aβ (25–35)-induced cell death in SH-SY5Y cultures. In untreated control cells, DAPI staining revealed intact, uniformly distributed nuclei, while PI uptake was negligible, consistent with preserved membrane integrity. By contrast, cells exposed to the 40 µM Aβ (25–35) displayed a marked increase in PI-positive nuclei, visible as strong red fluorescence which is indicative of membrane disruption and cell death consistent with necrotic/apoptotic cell populations. DAPI counterstaining revealed nuclear condensation and fragmentation in the treated group, features characteristic of apoptotic and necrotic changes. These findings corroborate the MTT assay results and validate 40 µM Aβ (25–35) as the optimal concentration to induce significant neurotoxicity, confirming its suitability for establishing a reproducible in vitro Alzheimer's disease model.

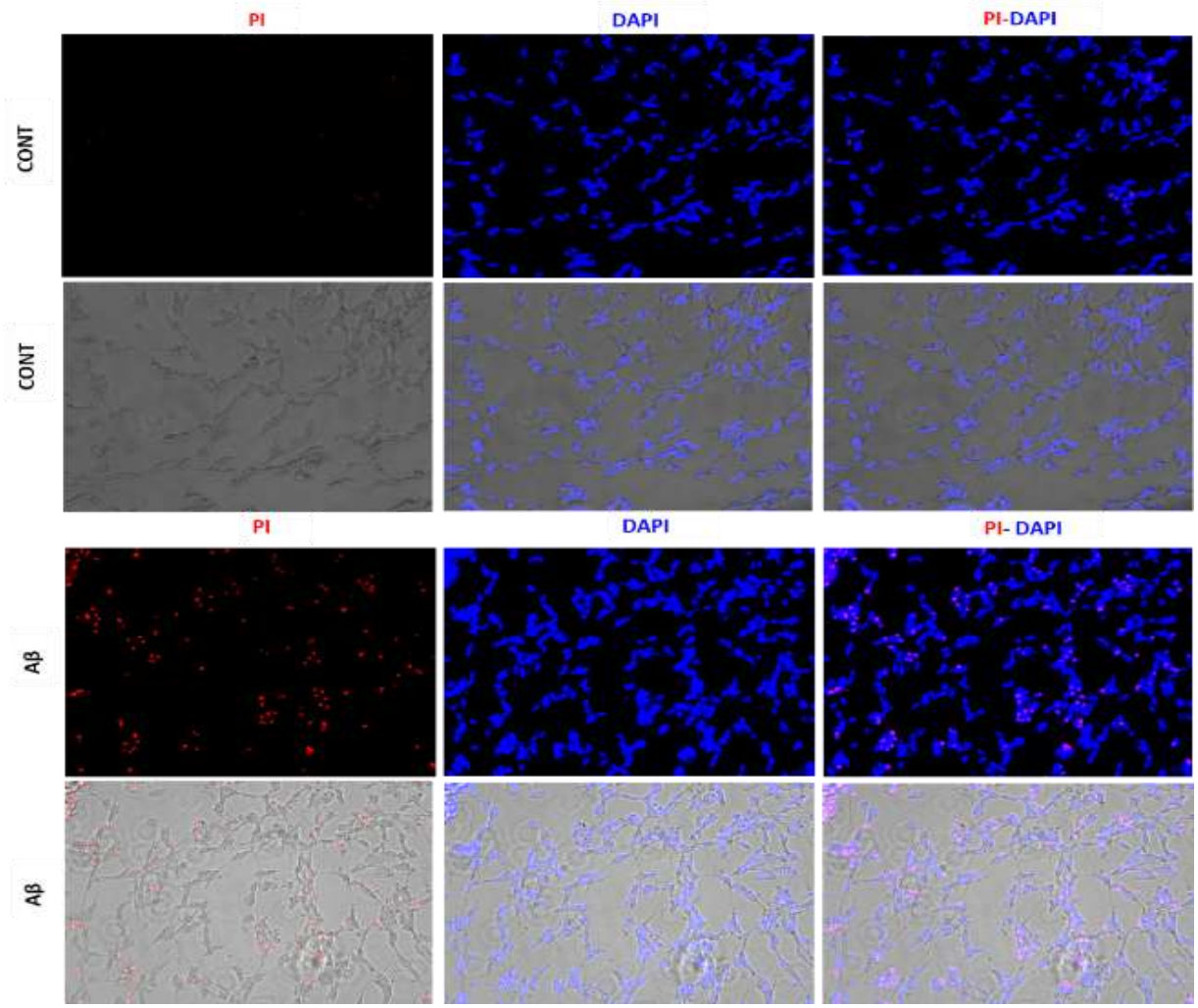

Fig.2 Propidium iodide (PI) and DAPI staining of SH-SY5Y cells. In the control group, nuclei appeared intact and were clearly visualized with DAPI staining (blue), while PI fluorescence was minimal, reflecting normal morphology and preserved cell viability. In contrast, SH-SY5Y cells exposed to 40 µM Aβ(25–35) for 24 hours showed intense PI uptake (red), marking cells with disrupted membranes. DAPI counterstaining further revealed nuclear condensation and fragmentation. Together, these observations demonstrate substantial cell death at 40 µM and support the use of this concentration as the standard dose for establishing the AD model.

DISCUSSION

This study created a consistent and reproducible in vitro model of Alzheimer's disease (AD) by treating SH-SY5Y neuroblastoma cells with 40 μM of Aβ25-35, a peptide fragment with neurotoxic properties. The study found a concentration-dependent decline in cell viability, including considerable neuronal loss at values over 20 μM. At 40-80 μM, viability decreased to approximately 50%, indicating an optimal window for mimicking AD-related neurotoxicity ($p < 0.05$). Previous studies have shown that Aβ25-35 mimics major clinical features of Alzheimer's disease, such as oxidative stress, mitochondrial dysfunction, and apoptotic signaling in neuronal cells[14,15,16].

The dose-dependent nature of Aβ25–35 toxicity aligns with the notion that amyloid accumulation in the human brain exerts a threshold effect, whereby modest deposition may be tolerated but progressive aggregation triggers irreversible cellular injury.[17] Importantly, the intermediate concentration range (20–40 μM) identified in this study provides a balance between sufficient neuronal damage and model stability, ensuring reliability for subsequent pharmacological or mechanistic investigations. This range optimizes "signal-to-noise ratio" for detecting neuroprotective effects and has been highlighted in prior studies utilizing PC12 and SH-SY5Y cells, underscoring the external validity of our observations.[13, 18,19,20,21]

The PI staining findings are consistent with previous reports demonstrating that Aβ (25–35) induces marked membrane disruption and nuclear damage in neuronal cell lines, visualized through red PI uptake and chromatin condensation. Such morphological evidence of cell death has been widely used to complement metabolic assays like MTT, offering a more direct assessment of membrane integrity and apoptotic changes. Studies using SH-SY5Y and PC12 cells have similarly shown that exposure to Aβ peptides leads to a concentration-dependent increase in PI-positive cells, confirming their utility in validating AD models in vitro.[13,22,23,24] In agreement with this body of evidence, the current study shows that 40 μM Aβ (25–35) produces reproducible cytotoxic effects, validating its use as a reliable dose for model development and for testing potential neuroprotective interventions. Such dual validation strengthens the robustness of this model and enhances its translational relevance for preclinical drug discovery.

Despite these strengths, significant restrictions must be recognized. Aβ25-35 is a shortened peptide, unlike the full-length Aβ1-42 found in Alzheimer's disease pathology. Although Aβ25-35 is commonly used due to its ability to aggregate and induce consistent toxicity, it may not fully represent the range of amyloid pathology seen in patients[25,26]. Similarly, while SH-SY5Y cells are a popular neural model, they cannot accurately duplicate the anatomical and functional complexity of cortical or hippocampal neurons. As a result, when applying these findings to clinical settings, they should be regarded with caution.

The 24-hour endpoint was chosen for the Aβ25-35-induced in vitro disease model as it provides sufficient time for the peptide to induce measurable cytotoxic effects, including oxidative stress, mitochondrial dysfunction, and morphological changes in SH-SY5Y cells, while avoiding excessive cell death that could interfere with subsequent analyses. However, this relatively short duration limits the temporal stability of the model, preventing assessment of longer-term effects such as chronic accumulation of amyloid beta or progressive neuronal degeneration.

Future studies could address this limitation by evaluating extended time points to better mimic the gradual pathology observed in Alzheimer's disease. Consequently, the interpretation and application of the current model should be considered within the context of acute cytotoxicity, and extrapolation to chronic disease processes should be done cautiously.

Future work should focus on complementing this model with more advanced experimental systems, including three-dimensional cultures, co-culture with glial populations, or human neurons derived from induced pluripotent stem cells, in order to more closely reflect the cellular environment of the AD brain. Furthermore, incorporating additional outcome measures such as reactive oxygen species (ROS) production, mitochondrial function, apoptotic signaling, synaptic markers, or proteostasis pathways

would provide a broader understanding of Aβ-induced toxicity and enhance the future scope of the study.

CONCLUSION

This study establishes and validates a simple, reproducible, and quantifiable cellular AD model based on Aβ25–35 exposures in SH-SY5Y cells. By defining the concentration range that consistently produces neuronal loss, the model serves as a practical platform for screening neuroprotective agents and exploring disease mechanisms. This model may serve as a reference framework for standardizing future in vitro AD studies.

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None.

Author Contributions

Saviya Kashif and **Abdul Hameed** contributed to the study conception, data collection, and initial drafting of the manuscript. **Rehan Imad** assisted in data analysis, interpretation of results, and manuscript revision. **Mati-ur-Rehman** supervised the study, provided critical feedback, and approved the final version of the manuscript.

Ethical Approval

This study is approved by the Ethics Review Committee of Ziauddin University, Karachi, Pakistan (Ref: 8011123SKMM)

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None.

Conflict of Interests

None.

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