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Enhancing Stability: Molecular Dynamics Simulations of a Novel Inhibitor Targeting Sodium-Dependent Noradrenaline Transporter (SLC6A2) for Parkinson's Disease Management

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Abstract: Parkinson's disease (PD), a progressive neurodegenerative disorder, is characterized by the loss of dopaminergic neurons, leading to motor and non-motor dysfunctions. The sodium-dependent noradrenaline transporter (SLC6A2), a critical protein responsible for norepinephrine reuptake, has gained attention as a promising drug target in PD management. This study investigates the molecular dynamics (MD) of a novel inhibitor, Lead1, in complex with SLC6A2 and compares its stability with a known inhibitor, Ibogamine. Simulations performed over 300 nanoseconds revealed enhanced stability and favorable binding interactions in the Lead1-SLC6A2 complex. Analysis of root mean square deviation (RMSD), root mean square fluctuation (RMSF), and hydrogen bond interactions demonstrated superior binding conformation and stability for Lead1. These findings highlight the potential of Lead1 as a novel therapeutic agent targeting SLC6A2 for PD treatment

IndexTerms – Parkinson's disease, Sodium-dependent noradrenaline transporter (SLC6A2), molecular dynamics simulations, inhibitor stability, Lead1, Ibogamine.

I. INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disorder that significantly impacts motor and cognitive functions, affecting millions of individuals worldwide. Characterized by the selective degeneration of dopaminergic neurons in the substantia nigra pars compacta, PD leads to a marked depletion of striatal dopamine levels, which is pivotal in regulating motor control and behavior. The resultant deficiency in dopamine manifests through hallmark motor symptoms, such as resting tremors, muscle rigidity, and bradykinesia, while non-motor symptoms, including depression, anxiety, cognitive impairments, and autonomic dysfunction, further complicate the clinical picture [1]. Current therapeutic strategies for managing PD predominantly revolve around dopamine replacement therapies, primarily utilizing levodopa and dopamine agonists. While these treatments can transiently alleviate motor symptoms, they do not address the underlying neurodegenerative processes and often lead to diminished efficacy and the emergence of motor complications with long-term use. This highlights the pressing need for novel therapeutic strategies that can modulate neurotransmission pathways beyond dopamine replacement, particularly those that focus on non-motor symptoms and the multifactorial nature of PD [2]. Within this context, the sodium-dependent noradrenaline transporter (SLC6A2), also known as the norepinephrine transporter (NET), has emerged as a compelling drug target for PD management. SLC6A2 plays a vital role in the reuptake of norepinephrine from the synaptic cleft into presynaptic neurons, thereby maintaining norepinephrine homeostasis in the central nervous system [3]. Dysregulation of norepinephrine signaling has been implicated in various non-motor symptoms associated with PD, including mood disorders, cognitive decline, and sleep disturbances, thus positioning SLC6A2 as a potential target for therapeutic intervention aimed at ameliorating these non-motor manifestations of the disease [4]. In the pursuit of effective therapeutics targeting SLC6A2, this study focuses on a novel inhibitor, designated Lead1, which was identified through a comprehensive virtual screening process. The stability and binding interactions of Lead1 when complexed with SLC6A2 are compared to those of a known inhibitor, Ibogamine. By utilizing advanced molecular dynamics (MD) simulations over a period of 300 nanoseconds, this research investigates the binding stability and conformational dynamics of the Lead1-SLC6A2 complex. The analysis includes metrics such as root mean square deviation (RMSD), root mean square fluctuation (RMSF), and hydrogen bond interactions to elucidate the relative efficacy of Lead1 as a therapeutic candidate. The findings of this study underscore the potential of Lead1 in providing enhanced stability and favorable binding interactions with SLC6A2, thereby serving as a promising novel therapeutic agent for the management of PD. These insights not only advance our understanding of SLC6A2 as a target for drug development in PD but also contribute to the broader discourse on innovative treatment modalities aimed at addressing both motor and non-motor symptoms of this complex neurodegenerative disorder

II. MATERIALS AND METHODS

2.1 Homology Modeling of SLC6A2: Homology modeling was employed to predict the 3D structure of SLC6A2, absent in the Protein Data Bank (PDB). The human SLC6A2 crystal structure (PDB ID: 6DZZ) served as a template using the MODELLER server, achieving 87% sequence coverage and 53.12% sequence identity. These metrics ensure structural reliability, critical for accurate protein conformation representation. The model was validated using MODELLER server, achieving 87% sequence coverage and 53.12% sequence identity. These metrics ensure structural reliability, critical for evaluation tools, confirming its reliability and stability [5].

2.2 Preparation of SLC6A2 and Ligands: Protein preparation was conducted using the Schrödinger Protein Preparation Wizard, optimizing structures under physiological pH (~7.4) by adding hydrogens and reconstructing missing side chains. Ligand preparation utilized the Schrödinger LigPrep module, generating all ionization and tautomeric states relevant for docking studies, ensuring accurate representation of ligand-protein interactions [6,7].

2.3 Molecular Docking: Molecular docking was performed using the Glide protocol (version 7.4) in three stages: HTVS, SP, and XP modes. This approach identified optimal ligand poses and assessed binding affinities. Lead1 displayed superior binding affinity compared to Ibogamine, highlighting its potential as a lead compound for PD therapeutic development [8].

2.4 MD Simulations: MD simulations were conducted using Desmond (version 7.2) with the OPLS 2005 force field and SPC water model. Simulations of 300 nanoseconds for Lead1-SLC6A2 and Ibogamine-SLC6A2 complexes ensured comprehensive conformational exploration. Stability metrics included potential energy fluctuations, RMSD (global structural changes), RMSF (residue mobility), and hydrogen bonding interactions. These analyses provided critical insights into the stability and interaction dynamics of the complexes, supporting Lead1's therapeutic potential. Figure 1 illustrates the modeled structure of SLC6A2, including the 3D structure (A), binding site residues highlighted in the alignment of SLC6A2 and SLC6A4 (B), structural evaluation using ProQ (C) and ProSA (D), and validation through the Ramachandran plot (E) and its statistical analysis (F). Figure 2 depicts the docking interactions of SLC6A2 with different ligands: Ibogamine (control) (A), Netarsudil (B), and Lead1 (C), with Lead1 exhibiting the most favorable binding affinity[9].

III. RESULTS AND DISCUSSION

3.1 Stability of the Lead1-SLC6A2 Complex: MD simulations provided valuable insights into the stability and interaction dynamics of the Lead1-SLC6A2 complex compared to the Ibogamine-SLC6A2 complex. The analysis revealed several key findings that underscore the favorable characteristics of Lead1 as a potential therapeutic agent for PD.

3.2 Consistent potential energy: The Lead1-SLC6A2 complex demonstrated remarkable stability throughout the MD simulation, with minimal fluctuations in potential energy[9]. This stability indicates an energetically favorable binding interaction, suggesting that the complex remains in a low-energy conformation critical for sustained pharmacological efficacy[11]. Conversely, the Ibogamine-SLC6A2 complex exhibited more pronounced energy fluctuations, indicating potential instability that could compromise its therapeutic effectiveness (Figure 3A, 3B).

3.3 Lower RMSD values: Root mean square deviation (RMSD) analysis revealed that the Lead1-SLC6A2 complex maintained significantly lower average RMSD values compared to the Ibogamine-SLC6A2 complex. Lead1 retained a stable conformation throughout the simulation, reflecting strong interactions with the target protein. In contrast, the higher RMSD values of the Ibogamine-SLC6A2 complex indicated greater conformational changes over time, highlighting the superior conformational rigidity of Lead1, a critical attribute for a stable inhibitor (Figure 3C, 3D).

3.4 Reduced RMSF values: Root mean square fluctuation (RMSF) analysis at the residue level showed that Lead1 exhibited lower fluctuations at key binding site residues of SLC6A2 compared to Ibogamine. These reduced RMSF values suggest that Lead1 forms a more rigid and stable interaction, minimizing the potential for conformational changes that could lead to reduced binding affinity (Figure 3E, 3F).

3.5 Enhanced hydrogen bonding: The Lead1-SLC6A2 complex consistently formed multiple hydrogen bonds with crucial residues in the binding pocket. These interactions were maintained throughout the simulation and occurred more frequently than in the Ibogamine-SLC6A2 complex, which showed intermittent hydrogen bonding. The robust hydrogen bond network established by Lead1 contributes to its enhanced stability and binding affinity, underscoring its therapeutic potential (Figure 3G–3J).

3.6 Docking Interactions: Figure 2 highlights the docking interactions of the sodium-dependent noradrenaline transporter (SLC6A2) with different ligands: (A) Ibogamine (control), (B) Netarsudil, and (C) Lead1. Lead1 demonstrated the most favorable binding affinity, further supporting its candidacy as a lead compound. These findings strongly support the hypothesis that Lead1 provides a stable and effective interaction with SLC6A2, establishing it as a promising therapeutic candidate for PD management. The enhanced stability and consistent interactions observed for Lead1 underscore its potential to address both motor and non-motor symptoms associated with PD. Future studies should explore the pharmacokinetics and clinical implications of these findings to advance Lead1 into later stages of drug development.

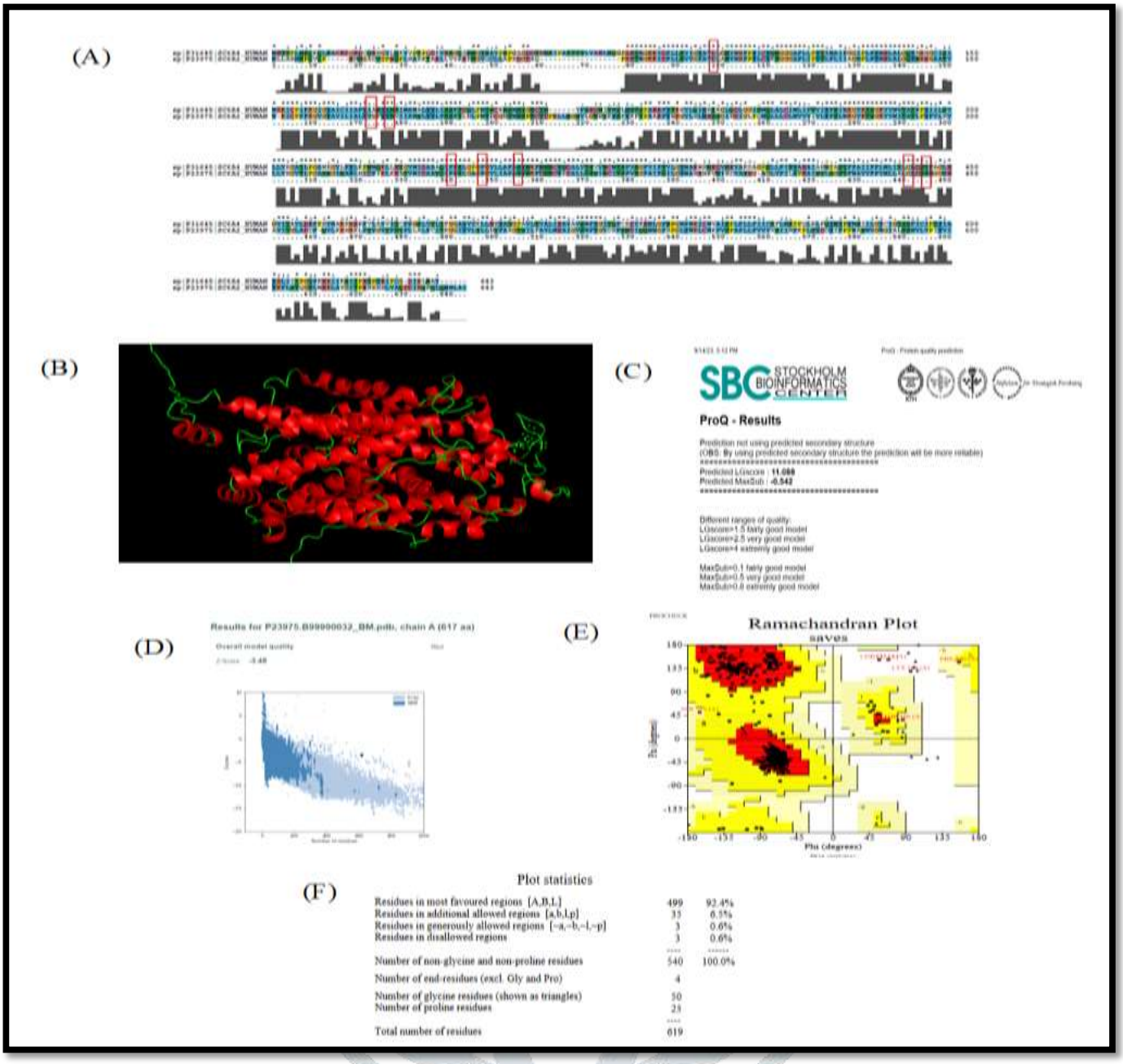


Figure 1. Modeled structure of the sodium-dependent noradrenaline transporter (SLC6A2). (A) Modeled structure of SLC6A2; (B) Multiple sequence alignment of SLC6A2 and SLC6A4 from Homo sapiens, with the binding site residues highlighted in a red box; (C) Structural evaluation using ProQ; (D) Structural evaluation using ProSA; (E) Ramachandran plot generated during PROCHECK evaluation; (F) Statistical summary of the Ramachandran plot analysis.

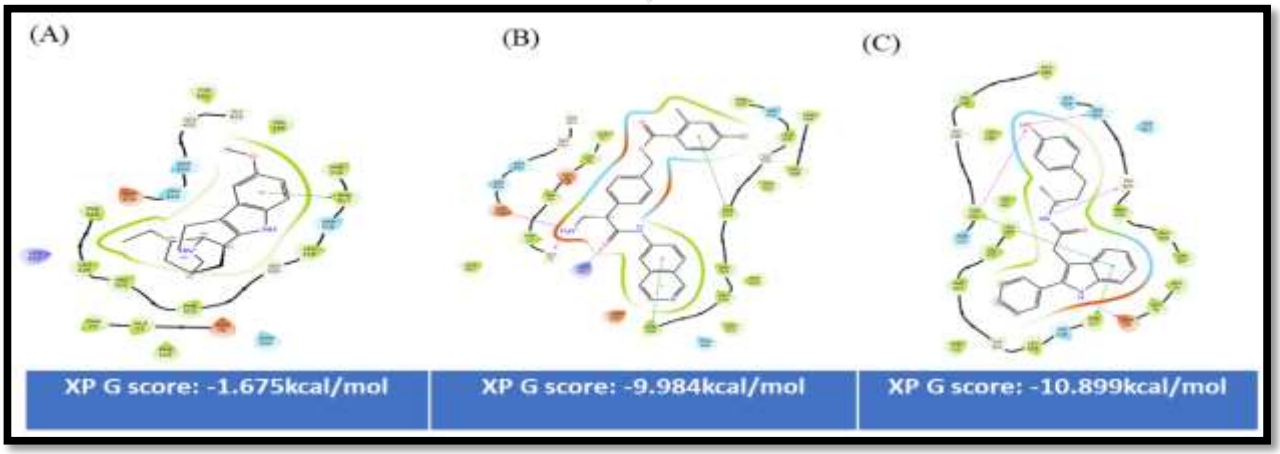
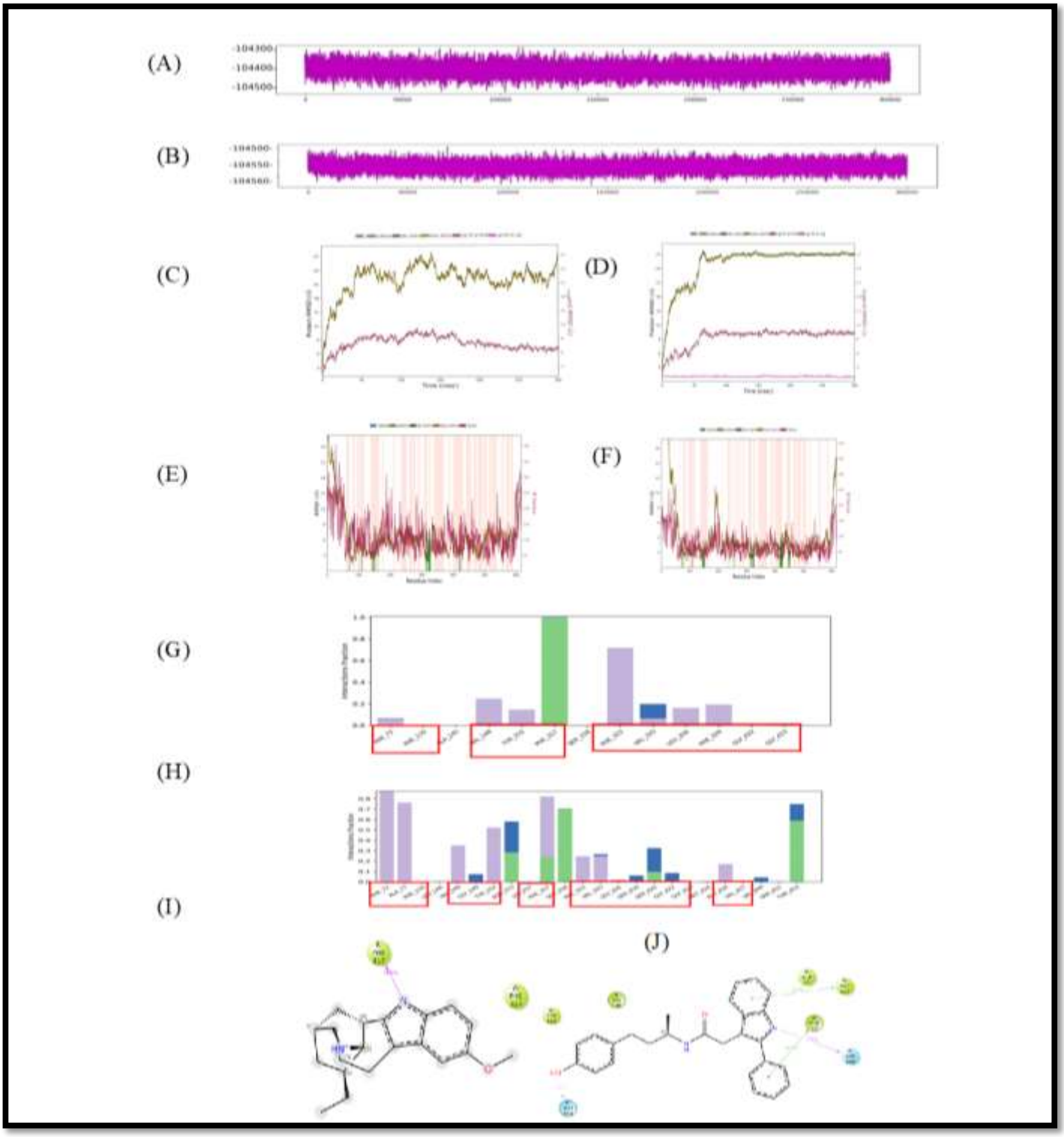


Figure 2. Docking interactions of the sodium-dependent noradrenaline transporter (SLC6A2): (A) Interaction with Ibogamine (control).; (B) Interaction with Netarsudil.; (C) Interaction with Lead1.



III. SUMMARY AND CONCLUSION

The study underscores the intricate interplay between the treatment regimens and Malate Dehydrogenase (MDH) activity across age groups in male albino rats, shedding light on the biochemical dynamics underlying nicotine exposure and the protective effects of Nardostachys jatamansi extract (NJEt). The findings reveal significant alterations in MDH activity, with nicotine-induced oxidative stress potentially contributing to metabolic dysfunction, particularly in the aging cohort. Conversely, NJEt demonstrated a notable ability to mitigate these changes, highlighting its therapeutic potential as an antioxidant and neuroprotective agent. This research provides compelling evidence for the age-dependent effects of oxidative stress and the modulatory role of herbal interventions. The differential responses observed in young versus old rats emphasize the need to consider age as a critical factor in the development of therapeutic strategies. Future investigations should explore the molecular mechanisms underpinning these changes, including gene expression analysis of MDH isoforms and pathways involved in mitochondrial function. Furthermore, extending this study to other tissue types and employing advanced imaging techniques could offer a more comprehensive understanding of the systemic effects of NJEt and nicotine. By addressing these gaps, the potential of Nardostachys jatamansi as a natural therapeutic agent can be further validated, paving the way for its clinical application in managing oxidative stress-related conditions

V. ACKNOWLEDGMENT

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VI CONFLICT OF INTEREST

The authors do not have any conflict of interest.

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