POLYPHENOLS AND THEIR EFFECT ON THE AGGREGATION OF ALS-LINKED Cu-Zn SUPEROXIDE DISMUTASE (SOD1)

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by

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CERTIFICATE

This is to certify that the thesis entitled **'Polyphenols and their effect on the aggregation of ALS-linked Cu-Zn superoxide dismutase (SOD1)"** being submitted by Ms. **Nidhi Kaur Bhatia** to the Indian Institute of Technology Delhi for the award of the degree of **Doctor of Philosophy** in chemistry is a record of a bona fide research work carried out by her. Ms. Nidhi Kaur Bhatia has worked under my guidance and supervision and has fulfilled the requirements for the submission of this thesis, which to my knowledge has reached the requisite standard.

The results contained in this dissertation have not been submitted in part or full to any other University or Institute for the award of any degree or diploma.

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is fatal and progressive neurodegenerative disease which is associated with the aggregation of Cu-Zn Superoxide Dismutase (SOD1). Unfortunately, effective therapeutics against ALS has not yet been developed. In the past few years, small molecules like polyphenols have been identified as potential anti-amyloidogenic agents and their biological properties are governed by the relative concentrations of their different conformational and ionic forms. Therefore, a thorough understanding of the conformational properties and mechanism of action of these polyphenols, which may serve as a potential inhibitor against SOD1 aggregation is needed. The thesis entitled **'Polyphenols and their effect on the aggregation of ALS-linked Cu-Zn superoxide dismutase (SOD1)'** is concerned with the understanding of physicochemical properties of three naturally occurring polyphenols (curcumin, quercetin, and baicalein) in nearly aqueous solution and their role as an anti-amyloidogenic agent against aggregation of ALS-linked SOD1. An attempt has also been made to understand the mechanism of their action.

The thesis is composed of seven chapters. **Chapter 1** (Introduction) provides an overview of SOD1 and its implication in ALS pathogenesis. detail review of aggregation of SOD1 and strategies are known so far, to inhibit SOD1 aggregation is also presented. The chapter then describes polyphenols and their therapeutic role against many chronic diseases as an anti-bacterial, anti-carcinogenic and anti-amyloidogenic agent. This chapter finally deals with the origin of the scientific problem associated with polyphenols and aggregation of SOD1 and the outline of the present research work done in this thesis in the context of addressing these problems. **Chapter 2** (Materials and Methodologies) deals with chemical procurement and protein expression, purification and various analytical techniques used for the investigation of aggregation of SOD1 and physicochemical properties of curcumin, baicalein, and quercetin. **Chapter 3** (Effect of pH and temperature on conformational equilibria and aggregation behaviour of curcumin in aqueous binary mixtures of ethanol) provides a detailed study on the effect of various factors like temperature, pH and solvent composition on keto-enol conformational equilibria and aggregation of curcumin. **Chapter 4** (Curcumin binds to the pre-fibrillar aggregates of

Cu/Zn superoxide dismutase (SOD1) and alters its amyloidogenic pathway resulting in reduced toxicity) describes, in details, the inhibition of DTT-induced SOD1 fibrillation by curcumin at physiological pH and temperature using ThT binding assay, DLS, AFM, TEM, ATR-FTIR. Binding parameters with native SOD1 were also estimated by tryptophan quenching experiments using steady state and time-resolved fluorescence spectroscopy. Docking studies demonstrated that putative binding sites of curcumin are the aggregation-prone regions of SOD1. MTT- assay was performed on THP1 cells to compare the cytotoxicity of SOD1 aggregates formed in the presence and absence of curcumin. **Chapter 5** (Effect of pH and temperature on physicochemical properties and aggregation behaviour of quercetin and baicalein in nearly aqueous media) deals with the detailed investigation of the effect of different solution conditions (pH and temperature) on physicochemical and aggregation behaviour of two naturally occurring flavonoids having same core structure (quercetin and baicalein) but varying hydroxyl groups in nearly aqueous media. **Chapter 6** (Quercetin and baicalein act as a potent anti-amyloidogenic and fibril destabilizing agents for SOD1 fibrils) presents a detailed study on the effect of quercetin and baicalein on the DTT-EDTA induced fibrillation of SOD1 at 37 °C and pH 7.4. The anti-amyloidogenic and disaggregating effect of both the flavonoids against SOD1 fibrillation was revealed by using ThT fluorescence, TEM and SDS PAGE. Binding parameters of these polyphenols were also estimated by quenching of tryptophan fluorescence and UV absorption spectra of quercetin and baicalein in the presence of varying concentration of SOD1. Putative binding sites of quercetin and baicalein were predicted by docking studies. The MTT assay was performed to determine the toxicity of SOD1 fibrils formed without and with flavonoids. **Chapter 7** (Summary and future perspectives) **contains** salient highlights of this work. In a nutshell, our findings have been instrumental in discovering and understanding the role of naturally occurring polyphenols (curcumin and quercetin) in the inhibition of aggregation of immature forms of SOD1 and in exploring the physicochemical properties of these polyphenols. Thus, our studies will help in providing the pathway for effective therapeutics against ALS and also have utility in terms of the enhancement of the bioavailability and therapeutic potential of polyphenols.

सार

एमीट्रोफिक पार्श्व स्क्लेरोसिस (एएलएस) घातक और प्रगतिशील न्यूरोडिजेनरेटिव बीमारी है जो Cu-Zn सुपरऑक्साइड डिसमूटेज (एसओडी 1) के एकत्रीकरण से जुड़ा हुआ है। दुर्भाग्यवश, एएलएस के खिलाफ प्रभावी चिकित्सीय विकास अभी तक विकसित नहीं हुआ है। पिछले कुछ वर्षों में, पॉलीफेनॉल जैसे छोटे अणुओं को संभावित एंटी-एमीलाइडोजेनिक एजेंटों के रूप में पहचाना गया है और उनके जैविक गुणों को उनके विभिन्न संरचनात्मक और आयनिक रूपों के सापेक्ष सांद्रता द्वारा नियंत्रित किया जाता है। इसलिए, इन पॉलीफेनॉल की संरचना के गुणकारी गुणों और तंत्र की पूरी तरह से समझ, जो एसओडी 1 एकत्रीकरण के खिलाफ संभावित अवरोधक के रूप में कार्य कर सकती है, की आवश्यकता है। थीसिस 'पॉलीफेनॉल और एलएस-लिंक्ड क् Cu-Zn सुपरऑक्साइड डिमूटेज (एसओडी 1) के एकत्रीकरण पर उनके प्रभाव को लगभग जलीय घोल में तीन स्वाभाविक रूप से होने वाले पॉलीफेनॉल (कर्क्यूमिन, क्वार्सेटिन और बाइकेलिन) के भौतिक रसायन गुणों की समझ से संबंधित है। एलएस-लिंक्ड एसओडी 1 के एकत्रीकरण के खिलाफ एंटी-एमीलाइडोजेनिक एजेंट के रूप में उनकी भूमिका। उनकी कार्रवाई के तंत्र को समझने के लिए भी एक प्रयास किया गया है।

थीसिस सात अध्यायों से बना है। अध्याय 1 (परिचय) एसओडी 1 और एएलएस रोगजन्य में इसके निहितार्थ का एक सिंहावलोकन प्रदान करता है। एसओडी 1 और रणनीतियों के एकत्रीकरण की विस्तृत समीक्षा अब तक ज्ञात है, एसओडी 1 एकत्रीकरण को बाधित करने के लिए भी प्रस्तुत किया जाता है। अध्याय में पॉलीफेनॉल और कई पुरानी बीमारियों के खिलाफ एंटी-बैक्टीरिया, एंटी-कैंसरजन्य और एंटी-एमीलाइडोजेनिक एजेंट के रूप में उनकी चिकित्सीय भूमिका का वर्णन किया गया है। अंततः यह अध्याय पॉलीफेनॉल से जुड़े वैज्ञानिक समस्या की उत्पत्ति और एसओडी 1 के एकत्रीकरण और इन समस्याओं को हल करने के संदर्भ में इस शोध में किए गए वर्तमान शोध कार्य की रूपरेखा से संबंधित है। अध्याय 2 (सामग्रियों और पद्धतियों) रासायनिक खरीद और प्रोटीन अभिव्यक्ति, शुद्धिकरण और विभिन्न विश्लेषणात्मक तकनीकों से संबंधित है जो एसओडी 1 के एकत्रीकरण और कर्क्यूमिन, बाइकेलिन और क्वार्सेटिन के भौतिक रसायन गुणों की जांच के लिए उपयोग किए जाते हैं। अध्याय 3 (पीएच का प्रभाव और इथेनॉल के जलीय बाइनरी मिश्रण में कर्क्यूमिन के समेकित संतुलन और तापमान के एकत्रीकरण व्यवहार पर तापमान) केटो-एनोल संरचनात्मक संतुलन और curcumin के एकत्रीकरण पर तापमान, पीएच और

विलायक संरचना जैसे विभिन्न कारकों के प्रभाव पर एक विस्तृत अध्ययन प्रदान करता है । अध्याय 4 (Curcumin पूर्व-फाइब्रिलर योग के लिए बांधता है क्यू / जेएन सुपरऑक्साइड विघटन (एसओडी 1) और इसके अमीलाइडोजेनिक मार्ग को बदलता है जिसके परिणामस्वरूप कम विषाक्तता होती है) विवरण में, डीटीटी प्रेरित एसओडी 1 फाइब्रिलेशन का अवरोध शारीरिक पीएच और तापमान पर टीसी बाध्यकारी परख, डीएलएस, एएफएम, टीईएम, एटीआर का उपयोग करके तापमान से होता है। -FTIR। देशी एसओडी 1 के साथ बाध्यकारी पैरामीटर का अनुमान स्थिर राज्य और समय-हल फ्लोरोसेंस स्पेक्ट्रोस्कोपी का उपयोग करके ट्राइपोफान क्वेंचिंग प्रयोगों द्वारा भी किया गया था। डॉकिंग अध्ययनों से पता चला है कि कर्क्यूमिन की रखरखाव बाध्यकारी साइटें एसओडी 1 के एकत्रीकरण-प्रवण क्षेत्र हैं। टीटीपी 1 कोशिकाओं पर एमटीटी-परख प्रदर्शन किया गया था ताकि curcumin की उपस्थिति और अनुपस्थिति में गठित एसओडी 1 योग की साइटोटोक्सिसिटी की तुलना की जा सके। अध्याय 5 (पीएच का प्रभाव और भौतिक रसायन गुणों पर तापमान और लगभग जलीय मीडिया में क्वार्सेटिन और बाइकेलिन का एकत्रीकरण व्यवहार) भौतिक रसायन पर दो अलग-अलग समाधान स्थितियों (पीएच और तापमान) के प्रभाव की विस्तृत जांच से संबंधित है और दो स्वाभाविक रूप से होने वाले फ्लेवोनोइड्स के एकत्रीकरण व्यवहार लगभग मूल संरचना (क्वार्सेटिन और बाइकेलिन) होने पर लगभग जलीय मीडिया में हाइड्रोक्साइल समूह अलग-अलग होते हैं। अध्याय 6 (क्वार्सेटिन और बाइकेलिन एसओडी 1 फाइब्रिल के लिए एक शक्तिशाली एंटी-एमिलाइडोजेनिक और फाइब्रिल अस्थिर एजेंटों के रूप में कार्य करते हैं) एसओडी 1 के डीटीटी-ईडीटीए प्रेरित फाइब्रिलेशन पर 37 डिग्री सेल्सियस और पीएच 7.4 पर क्वार्सेटिन और बाइकेलिन के प्रभाव पर विस्तृत अध्ययन प्रस्तुत करते हैं। एसओडी 1 फाइब्रिलेशन के खिलाफ फ्लैवोनोइड्स के एंटी-एमीलाइडोजेनिक और असंतुलित प्रभाव को टीटी फ्लोरोसेंस, टीईएम और एसडीएस पेज का उपयोग करके पता चला था। इन पॉलीफेनॉल के बाध्यकारी मानकों का भी एसओडी 1 की विभिन्न एकाग्रता की उपस्थिति में ट्राइपोफान फ्लोरोसेंस और क्वार्सेटिन और बाइकेलिन के यूवी अवशोषण स्पेक्ट्रा को बुझाने का अनुमान लगाया गया था। Quercetin और baicalein की रखरखाव बाध्यकारी साइटों डॉकिंग अध्ययन द्वारा भविष्यवाणी की गई थी। एमटीटी परख flavonoids के बिना और उसके साथ गठित एसओडी 1 फाइब्रिल की विषाक्तता निर्धारित करने के लिए किया गया था। अध्याय 7 (सारांश और भविष्य के दृष्टिकोण) में इस काम की मुख्य हाइलाइट्स शामिल हैं। संक्षेप में, हमारे निष्कर्ष एसओडी 1 के अपरिपक्व रूपों के एकत्रीकरण और इन पॉलीफेनॉल के भौतिक रसायन गुणों की खोज में प्राकृतिक रूप से

होने वाले पॉलीफेनॉल (कर्क्यूमिन और क्लोरोजेनिक एसिड) की भूमिका को खोजने और समझने में महत्वपूर्ण भूमिका निभा चुके हैं। इस प्रकार, हमारे अध्ययन एएलएस के खिलाफ प्रभावी चिकित्सकीय मार्ग के मार्ग प्रदान करने में मदद करेंगे और जैव उपलब्धता और पॉलीफेनॉल की चिकित्सकीय क्षमता के संवर्द्धन के संदर्भ में उपयोगिता भी करेंगे।

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LIST OF ABBREVIATIONS

ALS	Amyotrophic Lateral Sclerosis
ANS	1-anilinonaphthalene-8-sulphonate
AAS	Atomic absorption spectrometry
AFM	Atomic Force Microscopy
ATR-FTIR	Attenuated total reflection fourier transform infrared spectroscopy
CD	Circular dichroism
DTT	Dithiothreitol
DFT	Density functional theory
DLS	Dynamic light scattering
EDTA	Ethylenedinitrilotetraacetic acid disodium salt (dihydrate)
ESPT	Excited state proton transfer
IPTG	Isopropyl-b-D-thio galactose
IMAC	Immobilized metal ion affinity chromatography
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide
MCR	Multivariate curve resolution
MALDI-TOF	Matrix-assisted laser desorption/ionization–time of flight
NTA	Nitrilotriacetic acid
NMR	Nuclear magnetic resonance
NDP	Nucleation dependent polymerization
NEM	N-ethylmaleimide
RMSD	Root mean square deviation
SOD1	Superoxide dismutase 1
SEC	Size exclusion chromatography
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel Electrophoresis

TEM	Transmission electron microscopy
TDFT	Time dependent density functional theory
ThT	Thioflavin T
UV	Ultra violet
WT	Wild type