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.