Inhibition of Thermal and Shear induced Aggregation of Albumin (Bovine serum albumin) by *Centella asiatica* extract

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Protein aggregation phenomena owing to its numerous disadvantages in the pharmaceutical industry as well as being the seed cause of neurodegenerative diseases [1], remains one of the important topics in scientific research. Various natural sources are now being focused for solving the protein aggregation issue as no side effects are accompanied. *Centella asiatica* (CA) is one such plant that is a perennial medicinal herb known for its neuroprotection property and memory enhancement. Its anti-oxidative action, free radical scavenging and modulation of cholinergic activities are some of the properties which attribute to the neuroprotection ability [2]. Apart from this, it is very likely that components of the CA could bind to the bigger macromolecule protein and peptides. This small molecule binding then aids to the native structure stabilization when exposed to various external stimuli. Therefore, in light of the above possibility, we checked the inhibition action of CA extract against thermal and shear induced aggregation of a model protein, BSA at 60°C and shear force of 300^{s-1}. Three types of extract were tested namely CA ethyl acetate extract (CEE), CA methanolic extract (CME) and CA water extract (CWE), which were extracted sequentially with increasing polarity of the solvent. The aggregation kinetics was monitored through Thioflavin T (Th-T) assay and dynamic light scattering (DLS) and the aggregate morphology were analysed via AFM. The interaction of the extract and the protein was thermodynamically investigated through stern volmer quenching experiment. The characterization of the extract was performed through the high resolution liquid chromatography mass spectrometry (HRLCMS) and the antioxidative action via DPPH free radical scavenging assay. 300 µg/ml CWE, CME and CEE conferred scavenging activity on DPPH radical with the inhibitory percent of 42.48, 53.35 and 16.87 % respectively. From the stern volmer quenching experiment, the Gibbs free energy for both the CWE and CEE extracts were found to be -29.512 kJ/ mol and -25.986 kJ/ mol respectively showing that both interactions lead to an exothermic reaction. From this study, it was found that CA extract could significantly inhibit the aggregation of BSA and CEE and CWE extracts were found to perform the best.

Keywords: Protein aggregation, *Centella asiatica*, Shear, Thermal

Thioflavin T is a benzothiazole dye that binds to the amyloid fibrils (aggregates) with the cross β-sheet structures [30, 31]. The Th-T fluorescence intensity of the three crude extracts namely CME, CWE and CEE at $300s^{-1}$, 60°C are shown in figure 1 (A, B and C). From the figure, we can observe that Th-T fluorescence intensity decreases with respect to the control where no extracts were added. The decrease in intensity can be observed in all three extracts with the maximum and almost similar inhibition shown by CEE and CWE extract followed by CME extract. The same pattern is observed in the case of BSA samples exposed to both thermal and shear but with lesser intensity figure 2. To determine the behaviour

of interaction between the ligand and the macromolecule, here the tryptophan intrinsic fluorescence of BSA was analysed in presence of the crude extract which quenches the fluorescence with increasing concentrations. The quenching mechanism was then determined by using the Stern Volmer equation. The number of binding sites was determined as 1.165 for CWE and 0.994 for CEE respectively. The binding constant comes in the range of 1.49×10^5 and 3.59×10^4 for CWE and CEE respectively.

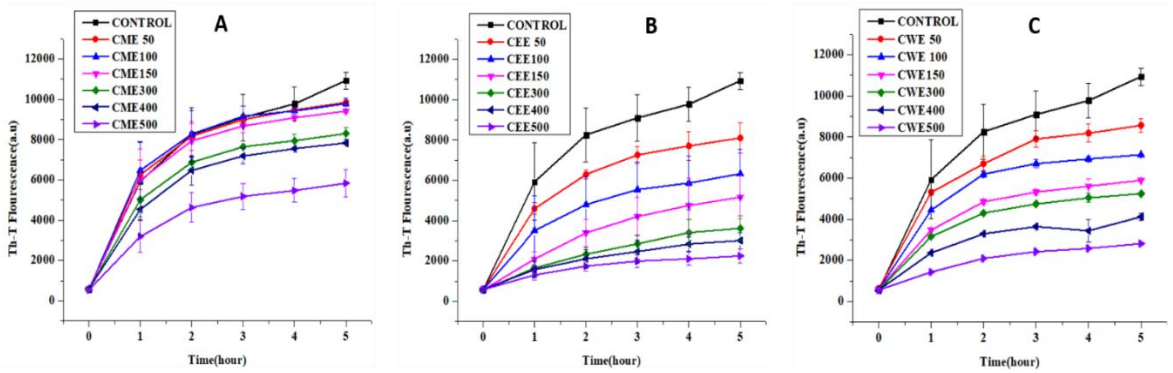

Figure 1: Thioflavin T fluorescence assay of thermal treated BSA with different concentrations (50 -500 µg/mL) of the three different extracts; A) CME, B) CEE and C) CWE.

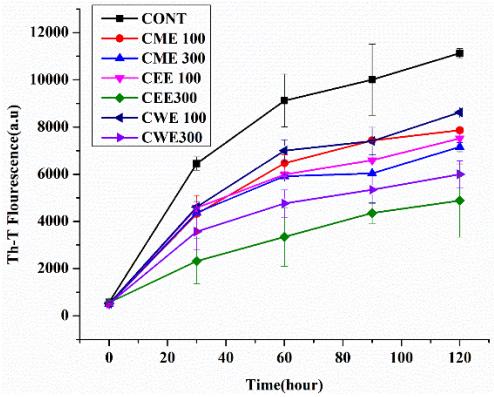

Figure 2: Thioflavin T fluorescence assay of <u>shear and thermal</u> treated BSA with different concentrations (100 -300 µg/mL)of the three different extracts (CME, CWE and CME)

References

1. Ross, C.A. and M.A. Poirier, Protein aggregation and neurodegenerative disease. Nature medicine, 2004. 10(7): p. S10-S17.
2. Arora, R., et al., Comparison of three different extracts of Centella asiatica for anti-amnesic, antioxidant and anticholinergic activities: in vitro and in vivo study. Biomedicine & Pharmacotherapy, 2018. 105: p. 1344-1352.