

**STUDIES ON MODULATION OF GELSOLIN
AMYLOIDOGENESIS *IN VITRO***

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STUDIES ON MODULATION OF GELSOLIN AMYLOIDOGENESIS *IN VITRO*

By

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Certificate

This is to certify that the thesis titled “**Studies on modulation of gelsolin amyloidogenesis *in vitro***” being submitted by Ms. Prabha Arya to the Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology Delhi, New Delhi for the award of the degree of ‘**Doctor of Philosophy**’ is a record of the bonafide research work carried out by her, prepared under our supervision in conformity with the rules and regulations of ‘Indian Institute of Technology Delhi’. The research report and the results presented in the thesis have not been submitted to any other University or Institute for the award of any other degree or diploma.

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ABSTRACT

Several pathological conditions in humans have been attributed to amyloid deposition. Amyloidosis is cause of number of neurodegenerative and systemic diseases like Alzheimer's disease, Creutzfeldt-Jakob disease and Parkinson's disease etc. Besides several other uncured or partially cured diseases such as AIDS, Hepatitis and cancers; amyloid diseases also remain a major nemesis to mankind. Gelsolin is an actin modulating protein which nucleates, caps and severs actin protein is abundantly present in all cells. Gelsolin amyloidosis resulting from a point mutation (D187N/Y) causes familial amyloidosis disease prevalent in Finland, Japan and southern American continent. Under *in vivo* conditions, mutant gelsolin gets cleaved through a cascade of proteases and releases an 8kDa fragment that forms amyloid deposits in the extracellular space. However, the intact gelsolin protein has not been reported to form amyloid under any condition.

Although several amyloid intervention strategies have been reported for structurally diverse proteins, the molecular mechanisms involved are not fully understood. Growing body of experimental data have reported the presence of amino acid stretches called the 'amyloidic' stretches within protein molecules. These stretches typically get exposed upon unfolding, followed by assembly with identical stretches from other protein molecules. Hence, in the present study induction of amyloidosis in the intact gelsolin protein and its amyloidogenic variants has been attempted utilizing different physico-chemical parameters. Another focus of the study was to identify small molecule modulators against gelsolin amyloidosis. For this, library of bio-active compounds was created and computationally screened against the amyloidic stretch of gelsolin protein.

The identified compounds were tested for their effect on the in vitro amyloidosis. It is shown here that the amyloid intervention of AGel (gelsolin's core amyloidogenic stretch with D187N FAF mutation) by two natural compounds, curcumin and emetine follow two distinct pathways. Curcumin accelerates the amyloid fibril formation and reduces the formation of potentially toxic oligomeric intermediates. On the contrary, emetine totally inhibits fibrillogenesis in AGel and produces non-fibrillar and non-toxic aggregates. Although both the natural compounds modulate amyloid fibrillation differently, they show remarkable reduction in amyloid toxicity in HT 22 and PC 12 cells. Based on substantial experimental evidences from multiple spectroscopic probes, atomic force microscopy, transmission electron microscopy and cytotoxicity studies on neuronal (SHS5Y) and cancerous (HeLa) cell lines, it is found that targeting the toxic stretch in proteins could modulate amyloidogenesis and hence could be an alternate strategy for preventing amyloid toxicity. The results were also confirmed on the disease-associated amyloidogenic fragment of gelsolin (*f*AGel). The identified compounds were also nanoencapsulated for increasing their solubility and bio-availability and were found giving similar results.

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