Supporting Information

Curcumin inhibits the Al(III) and Zn(II) induced amyloid fibrillation of β - lactoglobulin in vitro

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Table 1

Structural integrity of native β-lg in absence and presence of curcumin, metal, Curcumin-metal mixture as determined by circular dichroism (CD).

β-lg	% α-Helix	% β-sheet	% β-turn	% Random coil
Native	16.844	25.798	17.908	39.539
Heated-β-lg	14.007	27.844	17.827	40.323
Curcumin-β-lg	13.694	27.98	17.751	40.406
Al-β-lg	12.67	28.85	17.92	40.5
Al-curcumin-β-lg	15.065	27.1	18.009	39.74
Zn-β-lg	13.361	28.235	17.899	40.504
Zn-curcumin-β-lg	14.85	27.315	17.78	39.969

Experimental results:

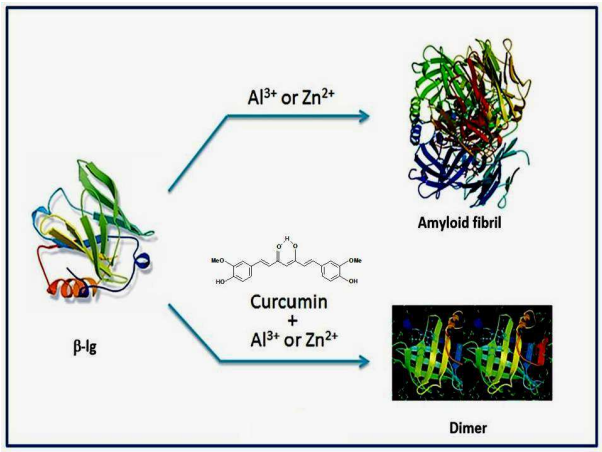

Fig. S1 Schematic representation of Al^{+3} and Zn^{+2} induced amyloid fibrillation of heat stressed β-lg in absence and presence of curcumin at 70°C for 2h.

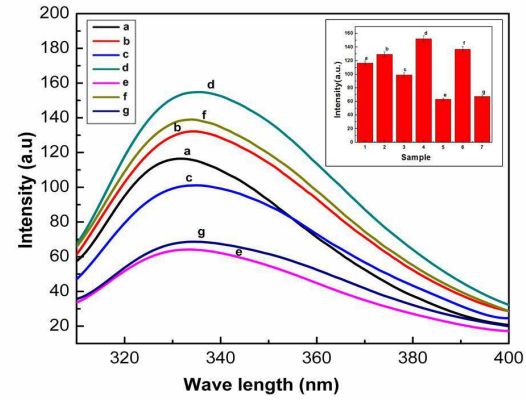

Fig. S2 (A) Intrinsic tryptophan fluorescence spectra of (a) native β-lg, heat treated (70°C for 2h): (b) β-lg, (c) curcumin-β-lg, (d) Al- β-lg, (e) Al-curcumin (1:1)-β-lg, (f) Zn-β-lg, and (g) Zn-curcumin(1:1)-β-lg at pH 7.0 in 10 mM Na-phosphate buffer. The excitation wavelength was 295 nm and emission wavelength range was 300–400 nm. Protein concentration was 0.25 mg/ml. Bar diagram of the end-point fluorosence intensity versus sample in fluorosence assay to study the aggregation of β-lg, Standard deviations are within the range of ±3.0 (inset). The experiment has been repeated three times.

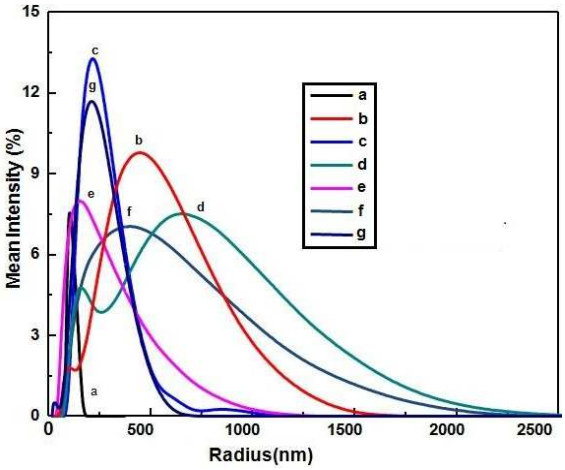

Fig. S2 (B) Number-particle size distribution profile of native β-lg (curve a), heat treated β-lg (curve b) in the presence Curcumin (curve c), Al- β-lg (curved), Al-curcumin (1:1)-β-lg (curve e), Zn-β-lg) (curve f and Zn-curcumin(1:1)-β-lg(curve g). Samples (b to g) were heated at 70 °C for 2h, in 10 mM Na-phosphate buffer (pH 7.0) and in all systems β-lg concentration was 54.35 µM. Each of these spectra is an average of 48 scans.

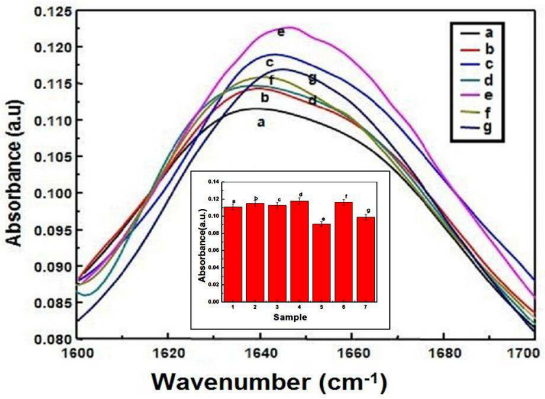

Fig. S3 (A) FTIR spectra of the amide-I region for (a) native β-lg, (b) β-lg, (c) curcumin-β-lg, (d) Al-β-lg, (e) Al-curcumin (1:1)-β-lg, (f) Zn-β-lg, and (g) Zn-curcumin(1:1)-β-lg. Samples (b to g) were heated at 70°C for 2h, pH 7.0 in 10 mM Na-phosphate buffer and protein concentrations were 20 mg/ml. Each spectrum is an average of 32 scans in D_2O solvent at 25°C. Bar diagram of the end-point FTIR intensity versus sample in FTIR spectroscopy assay to study the aggregation of β-lg, Standard deviations are within the range of ±3.0 (inset).

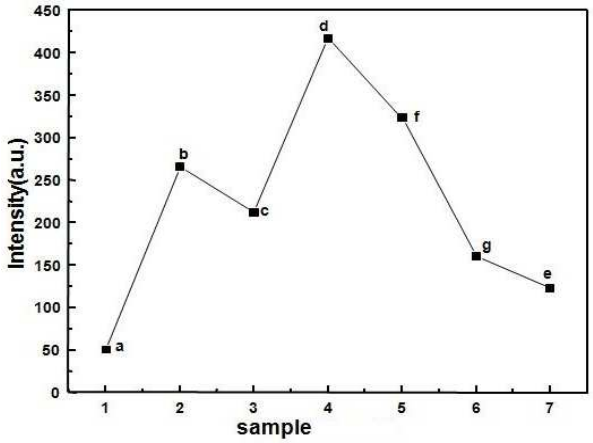

Fig. S3 (B Rayleigh Light Scattering data (turbidity measurements): (a) native β-lg, heat treated (70°C for 2h): (b) β-lg, (c) curcumin-β-lg, (d) Al-β-lg, (e) Al-curcumin (1:1)-β-lg, (f) Zn-β-lg, and (g) Zn-curcumin(1:1)-β-lg at pH 7.0. The samples were excited and emitted at 350 nm. Data were presented as mean of three different experiments performed in triplicate.

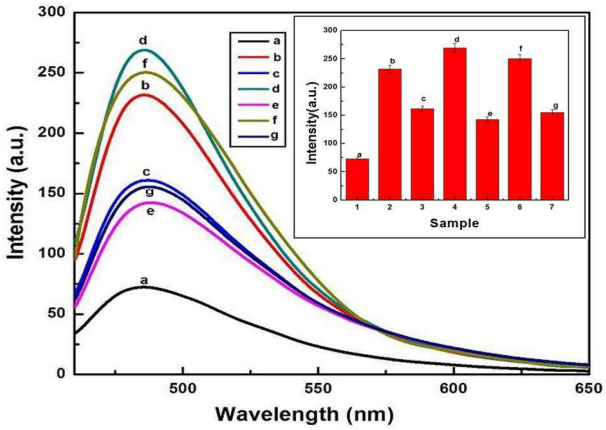

Fig. S4 (A) Fibrillation measurements were monitored by ThT fluorescence of (a) native β-lg, heat treated (70°C for 2h): (b) β-lg, (c) curcumin-β-lg, (d) Al-β-lg, (e) Al-curcumin(1:1)-β-lg, (f) Zn-β-lg, and (g) Zn-curcumin(1:1)-β-lg in the wavelength range 460–600 nm after excitation at 450 nm. Protein concentration was 13.6 μM. Bar diagram of the end-point ThT intensity versus sample in ThT assay to study the aggregation of β-lg, standard deviations are within the range of ±3.0 (inset). The experiment has been done three times.

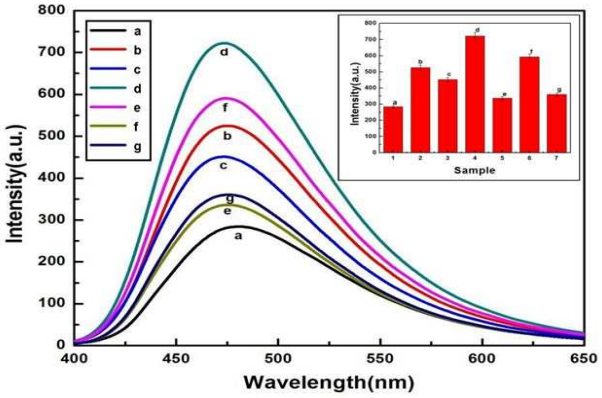

Fig. S4 (B) ANS fluorescence of heat stressed β-lg (70°C for 2 h) in the absence and presence of metal ions, curcumin, metal ion-curcumin mixtures in 10 mM phosphate buffer at pH 7.0, excitation was done at 380 nm and emissions were measured in the wavelength range 400–600 nm. Lines a–g correspond to (a) native β-lg, heat treated: (b) β-lg, (c) curcumin-β-lg, (d) Al-β-lg, (e) Al-curcumin(1:1)-β-lg, (f) Zn-β-lg, and (g) Zn-curcumin(1:1)-β-lg. Protein concentrations during ANS fluorescence measurements were 0.25 mg/ml. Bar diagram of the end-point ANS intensity versus sample in ANS assay to study the aggregation of β-lg, standard deviations are within the range of ±3.0 (inset). The experiment has been done three times.

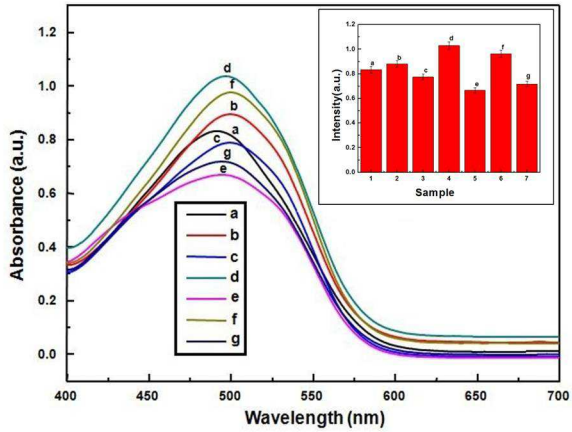

Fig. S5 (A) Congo red absorption spectra of (a) native β-lg, heat treated: (b) β-lg, (c) curcumin-β-lg, (d) Al-β-lg, (e) Al-curcumin (1:1)-β-lg, (f) Zn-β-lg, and (g) Zn-curcumin(1:1)-β-lg. The absorption spectra were recorded from 400 to 600 nm. The protein concentration was 0.25 mg/ml. Bar diagram of the end-point congo red intensity versus sample in congo red assay to study the aggregation of β-lg, standard deviations are within the range of ±3.0 (inset). The experiment has been repeated three times.

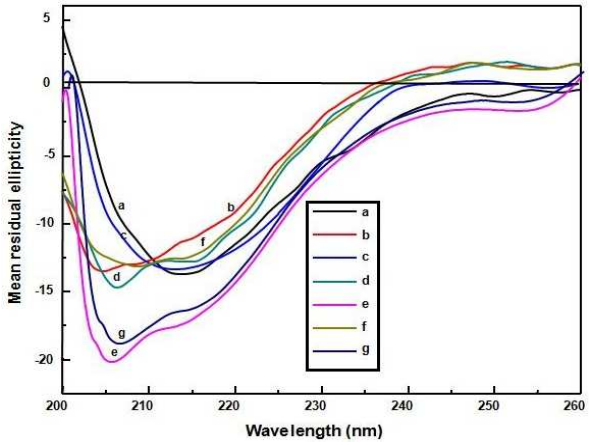

Fig. S5 (B) Inhibitory effect of curcumin on Al(III)-induced conformational alteration of β-lg. CD spectra of β-lg (13.6 µM) samples incubated for 2 hours at 70°C in the absence (b) and in the presences of Al(III)and Zn(II) (d, f), curcumin (c) and both curcumin-Al(III) and curcumin-Zn(II) (e, g) respectively. The far-UV CD spectra were recorded between 200 nm and 260 nm and the path length was 1 mm. Each spectrum is an average of three scans.

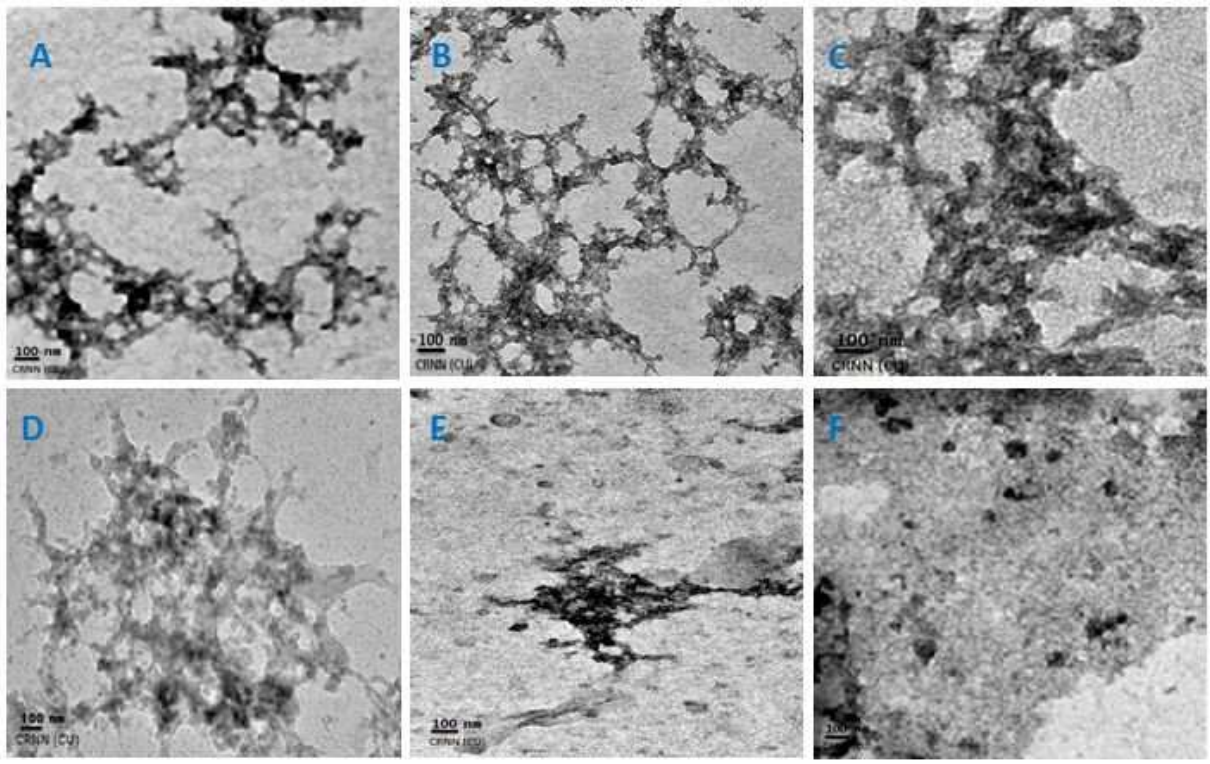

Fig. S6 Selected TEM images of β-lg aggregates in the absence and presence and of metal ion, curcumin and metal ion curcumin mixture incubated at 70°C at pH 7.0: β-lg alone heated (A), amyloid fibrillar formation network in presence of Al(III) (B), fibrilar aggregation in presence of Zn(II) (C), fewer and less fibrialr aggregates in presence of curcumin (D), amorphous aggregates in presence of Al(III)-Curcumin and Zn(II)-curcumin mixture (E, F). All images were taken after 6 h incubation and protein concentration were taken 10 µM.