Supplementary Information

for

A natural polyphenol activates and enhances GPX4 to mitigate amyloid-β induced ferroptosis in Alzheimer's disease

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[±] Equal contribution

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Materials and reagents. All chemicals and solvents were purchased from Merk or Spectrochem and utilised without additional purification, except as otherwise noted. Reagents used for protein expression and purification were obtained from HiMedia or Invitrogen and used as per the manufacturer's instructions. Glutathione Peroxidase Assay Kit was purchased from Cayman Chemicals, USA (Item No. 703102). Rabbit origin GPX4 polyclonal antibody was purchased from ELABS science (Cat no. E-AB-64550) and Ravbbit Origin GAPDH antibody was purchased from Invitrogen. HRP-conjugated secondary antibody of goat origin was ourchased from Invitrogen and used as per manufacturer's protocol. 2,2-Diphenyl-1-picrylhydrozyl (DPPH), 2,2'-azinobis(3-ethylbenzothiazoline-6-6sulfonic acid) (ABTS), 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide (MTT) and metal salts were procured from Merck Millipore and used without further purification. Sterile T25 flasks (Eppendorf), 96 well plates (Thermos Fischer Scientific), and confocal dishes (SPL Lifesciences) were procured and used without further sterilization. The absorbance and fluorescence in well plates were recorded in Spectramax i3 (Molecular devices) plate reader. The isothermal titration calorimetry (ITC) measurements were carried out in the Malvern MicroCal-PEAQ ITC instrument. Olympus Fluoview-3000 confocal laser scanning microscope was used for all immunofluorescence studies and Leica Dmi8 fluorescent microscope for live cell imaging which was further analysed by ImageJ software. All plotting and data analysis was performed in Origin(Pro), Version 2022 and GraphPad Prism 8.0 software. The molecular docking studies were performed using AutoDock Vina 2.0 and UCSF Chimera and Discovery Studio Visualizer (DSV) were used for residual analysis of docked poses.

UV-Visible absorption spectroscopy. PPs of 10 mM stock solution were prepared in water and used for further experiments. Measurements were conducted in HEPES buffer (10 mM, pH 7.4) at RT. The metal ions (Fe3+, Cu2+, Zn2+ and Al3+) were titrated with increasing

concentration from 10 μM to 100 μM with incubation time of 2 min. The change in absorbance spectra of PP was recorded and analysed for metal-PP binding. The absorbance values were fitted using the Benesi-Hildebrand equation.

$$\frac{1}{|A_0 - A|} = \frac{1}{|A_\alpha - A|} + \frac{1}{|A_\alpha - A| K_B [Q]}$$

Where, K_B is the binding constant, A_0 and Aα are the peak absorbance of PP independently and in the presence of metal ion with highest concentration (100 μM).

Isothermal Titration Calorimetry (ITC) measurements. ITC measurements were performed using a Malvern MicroCal-PEAQ ITC machine. Solutions of PPs and $FeCl_3$ were prepared in HEPES buffer (10 mM, pH 7.4). 26 consecutive injections of 1.5 μL aliquots of Fe^{3+} into PPs were performed to confirm continual mixing. The ITC cells' reference power and temperature were 5 cal/s and 25 °C, respectively. An interval of 150 s was maintained betwee measurements. Heat of dilution was subtracted to correct obtained isotherms. The thermodynamic parameters and dissociation constant were calculated by nonlinear least squares fitting of the binding isotherms using a single site binding model in MicroCal PEAQ-ITC analytical software.

DPPH radical scavenging assay. To test the radical scavenging ability of lead PP, the DPPH radical quenching experiment was performed. Sodium ascorbate was used as a positive control. DPPH (100 μM) was incubated in MeOH:H_2O (1:1) at 37 °C for 30 min. independently or with 0 to 100 μM concentration of PP. Microplate reader was used to measure the absorbance at 540 nm.

ABTS radical scavenging assay. Using the 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay, the reactive nitrogen species radical scavenging capacity of compounds was evaluated. A 12-hour reaction between ABTS and potassium persulfate produced the deep

green ABTS radical cation ($ABTS.^{+}$). At 37 °C for 30 min, PBS containing 100 μM of $ABTS.^{+}$ was incubated independently or with 10 μM of PPs. Microplate reader was used to measure the absorbance at 734 nm.

Ferric ascorbate assay. To monitor the redox silencing of Fe^{3+} and quenching of ROS by PP, we performed Fe^{3+} ascorbate assay. Coumarin-3-carboxylic acid (3-CCA, 50 μM) and Fe^{3+} (20 μM) were incubated independently and in the presence of PPs in PBS (10mM pH= 7.4) at physiological conditions under high concentrations of ascorbate (200 μM). Fluorescent active 7-OH-CCA was formed (λ_{em} = 450 nm) upon interaction of •OH which was monitored at a regular interval of 10 min upto saturation.

DNPH assay for protein oxidation. BSA (1 mg/mL), Fe^{2+} (0.1 mM), and H_2O_2 (2.5 mM) in PBS buffer (pH = 7.4, 50 mM) was incubated independently and in presence of PP for 24 h at 37 °C. Carbonyl groups was quantified using DNPH. 500 μL of DNPH (10 mM in 0.5 M H_3PO_4) was mixed with500 μL of PP solution, and incubated in dark for 15 min after which 250 μL of trichloroacetic acid (50% w/v) was added and the reaction mixture was incubated at 20 °C. Sample was centrifuged for 5 min, supernatant was discarded and the pellet was washed with 1 mL (3X) of ethanol/ethyl acetate (1/1; v/v). Pellet was then suspended again in 6 M guanidine-HCl with absorbance being recorded at 370 nm. The values were represented in % protein oxidation relative to control sample which contained no PP.

TBARS assay for lipid peroxidation. Lipid peroxidation assay was performed using α-phosphatidylcholine as a model lipid and Fe^{2+}-H_2O_2 system to generate ROS. Malondialdehyde (MDA), side product of lipid peroxidation was measured using thiobarbituric acid (TBA). TBA reacts with MDA to form thiobarbituric acid reactive substances (TBARS) that shows absorbance at 532 nm. To a solution of Fe^{2+} (0.1 mM), α-phosphatidylcholine (10 mM), and PP (100 mM), Asc (2 mM) was added, and samples were incubated for 30 min at 37°C.

Samples were the treated with TBA, and absorbance at 532 nm was measured. The values were represented in % lipid peroxidation relative to control sample which contained no PP.

$A\beta_{42}$ expression and purification. $A\beta_{42}$ plasmid construct was gifted generously by Prof. James S. Nowick, University of California, USA. $A\beta_{42}$ protein was expressed and purified from E. coli. The $A\beta_{42}$ plasmid was transformed into *E. coli* BL21 strain and cultured in Luria-Bertani (LB) broth at 37 °C, with continuous shaking (180 rpm) overnight with ampicillin (100 µM). Then 1% of primary culture was inoculated to 1 L of LB broth pre-treated with ampicillin, incubated until it reaches OD of 0.4 to 0.45. This was followed by induction with 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and further cultured for 4 h. Then cells were pelleted by centrifugation at 7000 x g in 4 °C and the pellet was resuspended in 10 mM Tris/HCl and 1 mM EDTA in water (pH 8.0). This was followed by sonication and centrifugation at 38,000x g to obtain the pellet, which was later resuspended in Tris buffer containing 8M urea. This was followed by HPLC purification and lyophilization to obtain the white $A\beta_{42}$ powder. The protein was characterised with MALDI and LC-MS and stored at -80 °C until further use.

Tau expression and purification. The plasmid construct for full-length tau was a generous gift from Dr. Sharad Gupta, IIT Gandhinagar, India. Tau protein was expressed and purified from E. coli as reported earlier. Briefly, the tau plasmid was transformed into E. coli BL21 strain and cultured in LB broth at 37 °C continuous shaking (180 rpm) overnight with ampicillin (100 µM) and chloramphenicol (50 µM). 1% of primary culture was inoculated to 1 L of LB broth with antibiotics, incubated until it reaches OD of 0.6, induced with 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and further cultured for 4 h. Then cells were pelleted by centrifugation at 7000 rpm in 4 °C and the pellet was resuspended in 20 mM phosphate buffer (pH 7.4) and boiled to heat precipitate unwanted proteins. The tau protein present in supernatant was purified by Ni-NTA affinity column chromatography and was

measured by Bradford assay, and further characterised by SDS-PAGE. Protein was stored at -80 °C until further use.

Preparation of Aβ$_{42}$ fibrils. Aβ$_{42}$ peptide (~1 mg) was dissolved in hexafluoro-2-propanol (HFIP) in 1.5 mL and incubated for 1 h at room temperature. Residual HFIP was removed by purging N_2 gas. The processed Aβ$_{42}$ was dissolved in 1% DMSO and PBS (pH= 7.4) for preparation of monomeric form. The Aβ$_{42}$ concentration was determined using absorption at 280 nm (ε = 1450 cm^{-1} M^{-1}). Aβ42 fibrillar aggregates were prepared from monomeric form for 48 h.

Thioflavin T fluorescence assay. Aβ$_{42}$ peptide was dissolved in PBS buffer (10 mM, pH= 7.4) containing 1% DMSO to obtain a final concentration of 10 μM, which is used as a stock solution. ThT assay (λ_{ex} = 444 nm, λ_{em} = 484 nm) was used to assess the PP's capacity to inhibit Aβ$_{42}$ aggregation. Newly reconstituted Aβ$_{42}$ (10 μM) was incubated for 48 h at 37 °C in phosphate buffer saline (PBS, pH=7.4 and 10 mM) both alone and with compounds (10 M). The determined quantity of ThT was dissolved in filtered PBS (pH= 7.4, 10 mM). Finally, ThT (10 μM) was added to the appropriate samples and allowed to incubate for 10 min. For Fe-induced Aβ$_{42}$ aggregation, 10 μM of Aβ$_{42}$ was incubated with 10 μM of $FeCl_3$ for 5 min before the start of kinetic experiment. The kinetic curve is averaged from three data sets and fitted into the kinetic equation.

The time dependent ThT fluorescence data was modelled using the following equation:

$$y = y_0 + \frac{y_{max} - y_0}{1 + e^{\frac{-(t-t_1)k}{2}}}$$

where y stands for the fluorescence intensity at time t, y_0 and y_{max} stand for the fluorescence intensities at the initial and maximum points, respectively; $t_{1/2}$ stands for the time needed to reach half the maximum fluorescence intensity (halfway from nuclei to fibrils); and is the apparent first-order rate constant of aggregation. By using $t_{1/2}$-2k, the lag time (T_{lag}) was

calculated. Tau (5 μM) was treated with different molar ratios of lead compounds for 2.5 hours at 37 °C to induce aggregation in aggregation buffer (20 mM phosphate buffer, 185 μM arachidonic acid).

GPX4 enzymatic assay. Cayman's GPX4 Inhibitor Screening Assay Kit was used for measuring GPX4 activity indeependently and in presence of inhibitors and activators. By using a coupled reaction with glutathione reductase, the test indirectly detects GPX4 (GR). GR and NADPH recycle oxidised glutathione (GSSG), which is created during the reduction of hydroperoxide by GPX4:

$$\text{R-O-O-H} + 2\text{GSH} \xrightarrow{\text{GPX4}} \text{R-O-H} + \text{GSSG} + H_2O$$

$$\text{GSSG} + \text{NADPH} + H^+ \xrightarrow{\text{GR}} 2\text{GSH} + NADP^+$$

The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbanceat 340 nm (A_{340}). The rate of decrease in A_{340} is directly proportional to GPX4.

The % inhibition/activation was calculated by the following equation,

$$\frac{(Initial\ enzyme\ activity - inhibitor\ or\ activator\ \ activity)}{Initial\ enzyme\ activity} \times 100$$

Cell culture and MTT assay. SH-SY5Y were cultured using DMEM/F-12 (Dulbecco's Modified Eagle Medium/Nutrient Mixture F 12) medium (Gibco, Invitrogen) with 10% of FBS (fetal bovine serum) and 1% PS (pen-strep) under the ambient cell growing condition (37 °C and 5% CO_2 atmosphere). The SH-SY5Y cells were seeded in a 96 well plate with a cell density of 25,000 cells per well and incubated for 24 h. After that, the media was changed, and the cells were treated with the PP of varying concentration and further incubated for 48 h in the ambient cell growing condition. After 48 h, 10 μL of 3-(4,5-Dimethylthiazol-2-yl)-2,5-

diphenyltetrazolium bromide (MTT) (5 mg/mL) was added to the cells and incubated for 2.5 h, and the media was replaced by DMSO/MeOH (1:1) solution to dissolve the formazan crystals. The absorbance was recorded at 570 nm, and the cell viability (%) was calculated relative to the control untreated cells.

Intracellular ROS quantification. The (2′,7′-dichlorofluorescin diacetate) DCFDA test was used to track the intracellular ROS level and PP's capacity to quench them. SH-SY5Y cells were plated in a 48-well plate (15,000 cells per well) and incubated at 37 °C in a 5% CO_2 environment with DMEM F12 containing 10% FBS and 1% PS. After being replaced with low serum DMEM F12 (2.5% FBS), the cultured media was treated with DCFDA (20 μM) for 30 minutes. After a gentle PBS wash, the cells were exposed to independent and combined treatments of H_2O_2 (100 M) for 4 hours. Microplate reader was used to do the total fluorescence well scan (λ_{em} =530 nm), and GraphPad Prism 8.0 software was used to plot and analyse the data. b) For the intracellular ROS measurement in presence of $A\beta_{42}$, Fe and ascorbate, the PP were incubated in presence and absence of Fe ascorbate system post incubation of DCFDA (20 μM) for 30 mins. The cells were gently washed after 6 hours of treatment and fluorescence well scan was performed (λ_{em}= 530 nm) as mentioned above to monitor the intracellular ROS generated by the Fenton reaction.

Neuronal cell rescue assay from $A\beta_{42}$ and $A\beta_{42}$ -Fe induced toxicity. Neuronal cells rescue from $A\beta_{42}$ induced toxicity was performed in presence and absence of PP's independently via MTT assay. The cells were cultured in 96 well plate and incubated for 24 h. The media was further replaced with low serum (2.5%) followed by treatment with $A\beta_{42}$, $A\beta_{42}$+Fe (1:5) independently and in presence of PP's and further incubated for 30 h at ambient cell growing conditions. After that, MTT solution (5mg/mL) was added to the experimental cells and incubated for further 3 h. The media was replaced with 100 μL of DMSO: MeOH (1:1) and the absorbance at 570 nm was monitored *via* multiplate reader.

Immunofluorescence study

RSL3/Aβ+Fe induced GPX4 level reduction. SH-SY5Y cells were cultured in confocal dishes with a confluence of 70% were treated with RSL3/$A\beta_{42}$+Fe independently and in presence of polyphenols and incubated for 30 h. The cells were gently washed with warm PBS and fixed with 4% paraformaldehyde for 15 min. The cells were washed twice with PBS for 5 mins, followed by treatment with permeation buffer (PBS with 0.1% Triton X-100) for 10 mins. The permeation buffer was removed and rinsed with PBS for a couple of times. The samples were blocked using 10% HS in dPBS for 45 min in room temperature. The blocking solution was removed, followed by treatment with primary antibody specific for GPX4 with a desired dilution (1:200) in 4 °C for 16 h. The samples were washed with PBS twice, followed treatment with secondary antibody conjugated to Alexa Fluor-488 for 1.5 h in room temperature. The cells were gently washed, followed by counterstaining with DAPI (1 μM) for 5 min and imaged using confocal microscope. The fluorescence signal corresponding to the protein were quantified using ImageJ software.

TA induced Nrf2 activation. SH-SY5Y cells were cultured in confocal dishes until the confluency reached 75%. Further the cells were treated with TA for 30 h at the ambient cell growing conditions. The cells were gently rinsed with warm PBS and fixed with 4% PFA, followed by treatment with permeabilization and blocking solution, as mentioned above. The cells were treated with antibody specific to Nrf2 for 16 h, followed by treatment with secondary antibody conjugated to Alexa Fluor-488 for 1.5 h in room temperature. The cells were gently rinsed with PBS and counterstained with DAPI as mentioned above. The fluorescence signal corresponding to Nrf2 accumulation and translocation has been quantified.

Western blot experiments. Western blot experiment was performed to analyse the change in the protein levels from cell lysates. SH-SY5Y cells were cultured in 6 well plate and allowed to adhered for 24 h. Further, the cells were treated RSL3 (1μM) independently and in the

presence of TA (20 μM) and incubated for 16 h. The cells were harvested using trypsin and the protein was extracted with 60 μL RIPA lysis buffer containing protease inhibitor cocktail (1X) and kept for gentle shaking in 4 °C for 30 min. The cells were centrifuged at 12000 g for 15 min and the supernatant were collected and quantified using Bradford assay. The protein lysates were subjected to SDS-PAGE with 12% resolving gel. The gel was transferred to PVDF membrane after its activation. The blot paper was blocked with 5% skim milk for 1.5 h at RT and then washed thrice with TBST. The blots were incubated with primary antibody (1:1000) for 16 h at 4 °C. The blots were washed thrice with TBST for 5 min each time and then incubated with anti-rabbit HRP-conjugated secondary antibodies for 12 h at 4 °C. Finally, the blots were washed thrice using TBST and developed with ECL reagent and imaged with ChemiDoc instrument. The western blots were quantified using ImageJ, and the data were plotted with GraphPad Prism.

Table S1. Binding parameters of different PPs with $FeCl_3$.

PP	Binding constant, K_B (M^{-1})
TA	6.6×10^5
EGCG	5.1×10^5
GA	4.2×10^3
MEG	2.7×10^3
GEN	1.5×10^4
EA	1.9×10^5
2-HF	9.8×10^3
SA	-
4-HF	-

Table S2. Binding parameters of TA with various metal ions.

Metals	Binding constant, K_B (M^{-1})
Fe^{3+}	1.2×10^5
Cu^{2+}	1.6×10^3
Zn^{2+}	2.9×10^3
Al^{3+}	9.9×10^3
Na^+	-
K^+	-
Ca^{2+}	-

Table S3. Kinetic parameters of Aβ aggregation in presence of increasing concentrations of TA and EGCG.

System	T_{lag}	k	System	T_{lag}	k
Aβ	9.20	0.51	Aβ	8.95	0.48
Aβ–TA = 1:1	9.67	0.43	Aβ–EGCG = 1:1	9.40	0.42
Aβ–TA = 1:2	10.39	0.38	Aβ–EGCG = 1:2	9.82	0.36
Aβ–TA = 1:3	10.74	0.29	Aβ–EGCG = 1:3	10.11	0.36
Aβ–TA = 1:5	12.70	0.22	Aβ–EGCG = 1:5	13.95	0.10

Table S4. Kinetic parameters of Fe-induced Aβ aggregation in presence of increasing concentrations of TA and EGCG.

System	T_{lag}	k	System	T_{lag}	k
Aβ	8.72	0.72	Aβ	8.68	0.77
Aβ–TA = 1:1	7.93	0.62	Aβ–EGCG = 1:1	8.83	0.49
Aβ–TA = 1:2	8.53	0.59	Aβ–EGCG = 1:2	8.81	0.44
Aβ–TA = 1:3	9.17	0.36	Aβ–EGCG = 1:3	9.28	0.41
Aβ–TA = 1:5	10.12	0.20	Aβ–EGCG = 1:5	10.98	0.17

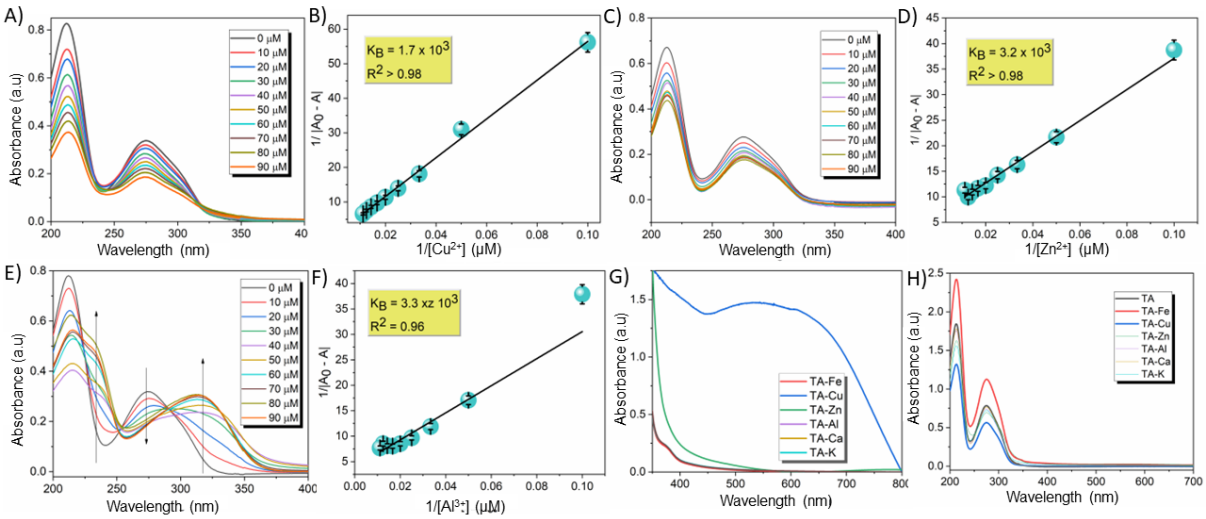

Fig. S1. UV-Visible spectra of A) $CuCl_2$ C) $ZnCl_2$ E) $AlCl_3$ in the presence of increasing concentrations of metal ions. The graph derived by fitting the data from using Benesi-Hilderbrand equation. B) $CuCl_2$ D) $ZnCl_2$ F) $AlCl_3$. G) Formation of a new peak at 600 nm in case of TA-Fe^{3+} binding. H) UV-Vis spectra of TA in the presence of biologically relevant metal ions.

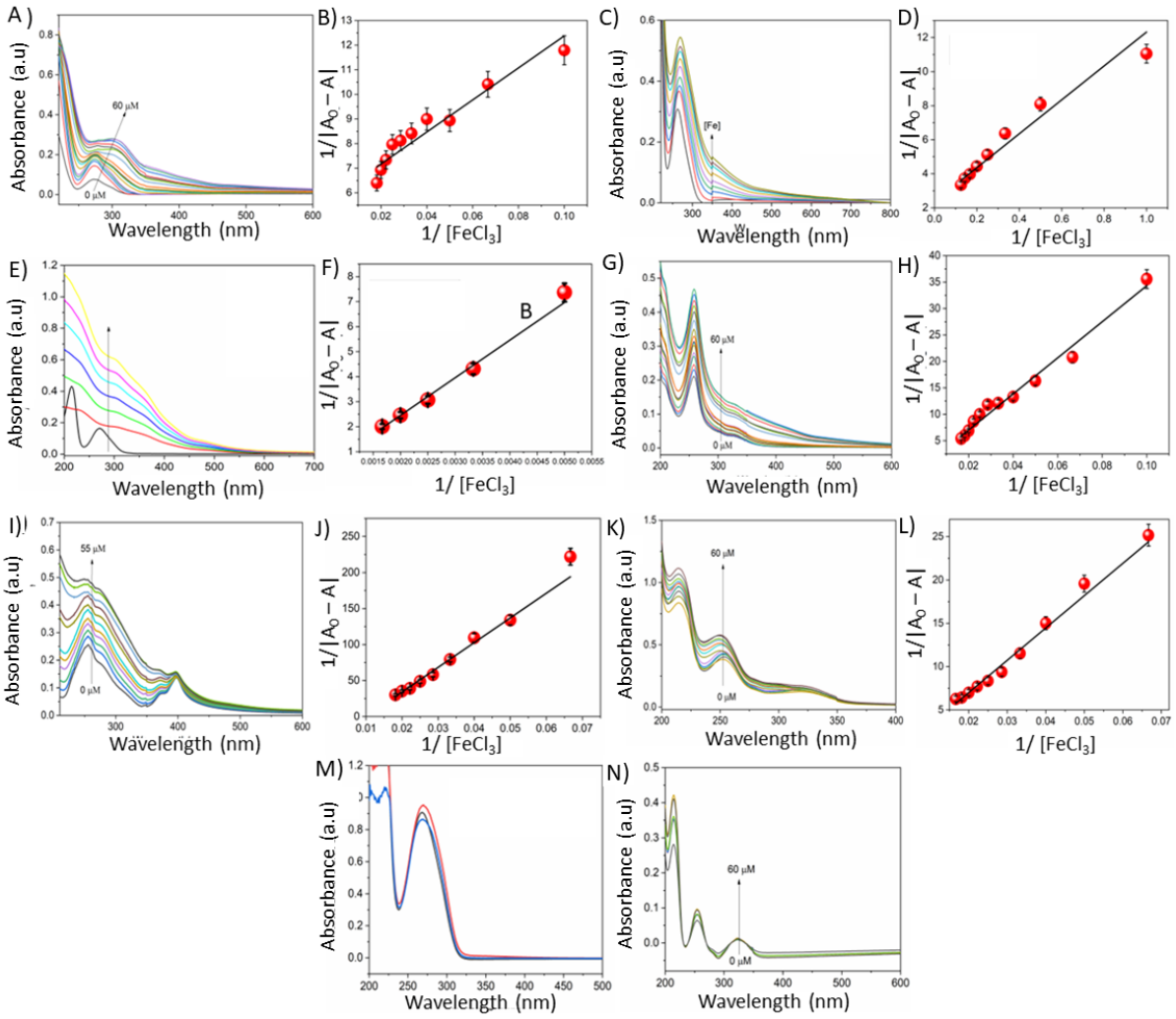

Fig. S2. UV-Visible spectra of A) EGCG C) GA E) MeG G) GEN I) EA K) 2-HF M) SA N) 4-HF with $FeCl_3$ in the presence of increasing concentrations of metal ions. The graph derived by fitting the data from UV-Visible spectra using Benesi-Hilderbrand equation B) EGCG D) GA F) MeG H) GEN J) EA L) 2-HF M) SA N) 4-HF.

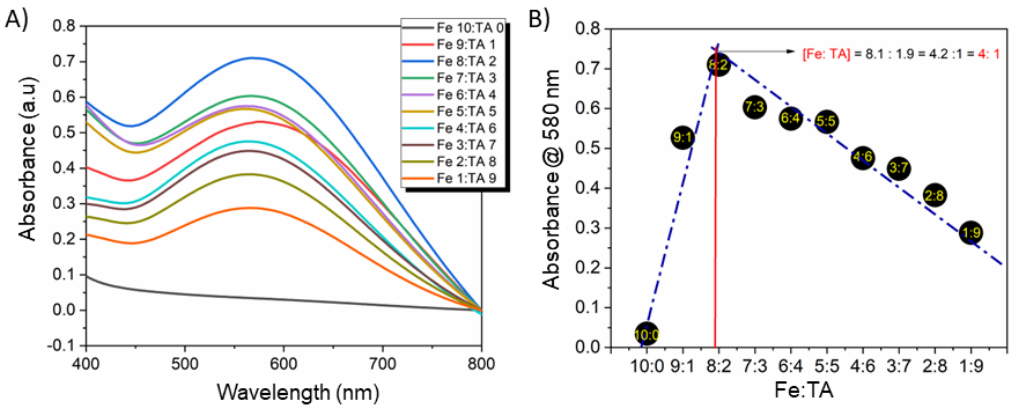

Fig. S3. A) Formation of new peak of TA-Fe complex at 580 nm. B) Job's plot obtained from A) to elucidate the stoichiometry of binding.

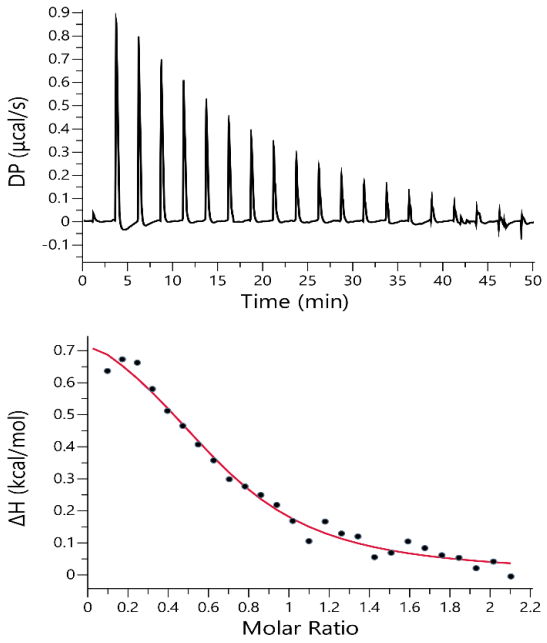

Fig. S4. ITC binding isotherm for binding of TA to $FeCl_3$. The upper panel shows the raw heat data acquired from successive injections of $FeCl_3$ into the compound solution. The lower panel depicts the integrated binding isotherms as a function of the binary complex at 298 K. Fitting was obtained from the data points with single binding site model.

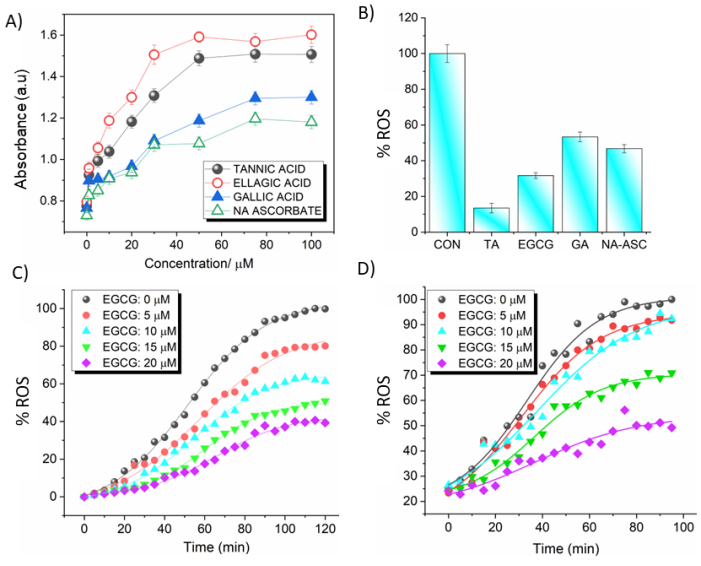

Fig. S5. A) DPPH assay of different PPs using ascorbate as a control B) ABTS radical quenching assay of PPs, C) ability of increasing concentration of EGCG to keep Fe^{3+} in a redox dormant state and the corresponding experiment performed in presence of Aβ (D).

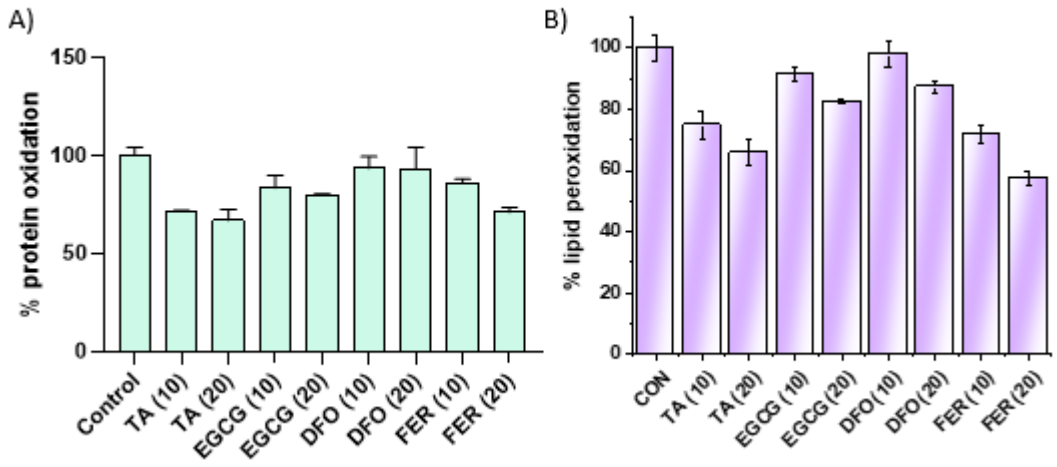

Fig. S6. A) Inhibition of protein oxidation by compounds monitored by the 2,4-dinitrophenylhydrazine (DNPH) assay. B) Inhibition of lipid peroxidation by compounds monitored by the thiobarbituric acid reactive substance (TBARS) assay and H concentration-dependent inhibition of lipid peroxidation by TA and EGCG. The numbers in the parentheses represent the concentration of the compounds in μM.

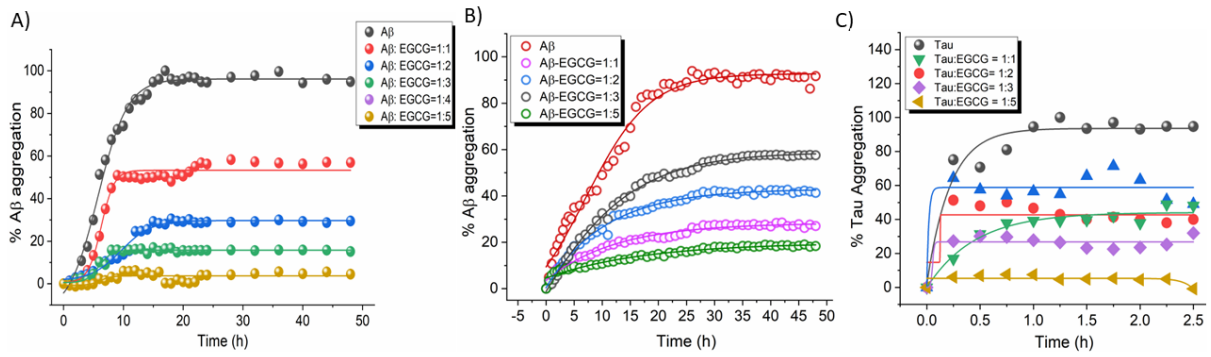

Fig. S7. Screening of PPs for their ability to inhibit $A\beta_{42}$ and tau aggregation monitored using ThT assay (λ_{ex} = 444 nm, λ_{em} = 484 nm). [$A\beta_{42}$] = 10 μM, [Tau] = 5 μM. Kinetics of $A\beta_{42}$ aggregation (A) and Fe-induced $A\beta_{42}$ aggregation (B) in the presence of increasing concentration of EGCG (10 to 50 μM). C) Kinetics of tau aggregation in the presence of an increasing concentration of TA (5 to 25 μM). AA: Arachidonic acid.

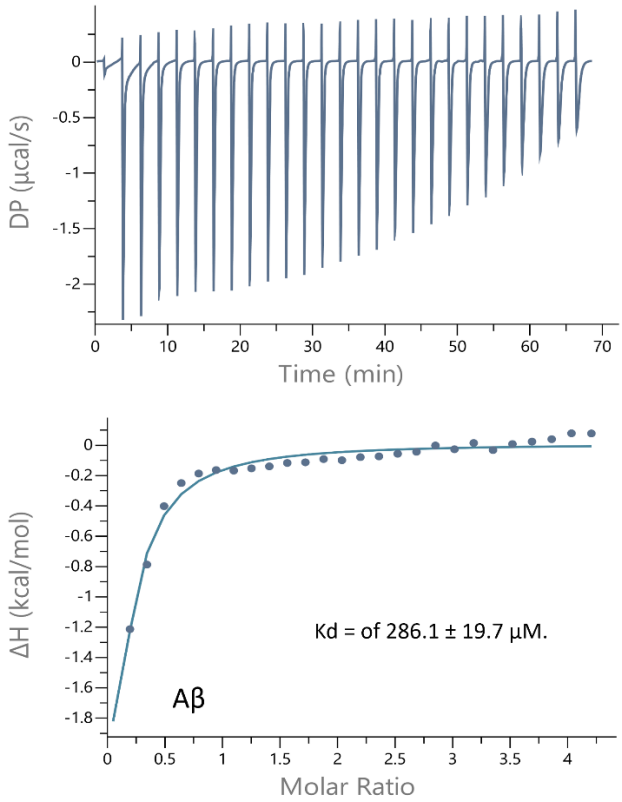

Fig. S8. ITC binding isotherm for binding of Aβ to $FeCl_3$. The upper panel shows the raw heat data acquired from successive injections of $FeCl_3$ into the compound solution. The lower panel depicts the integrated binding isotherms as a function of the binary complex at 298 K. Fitting was obtained from the data points with single binding site model.

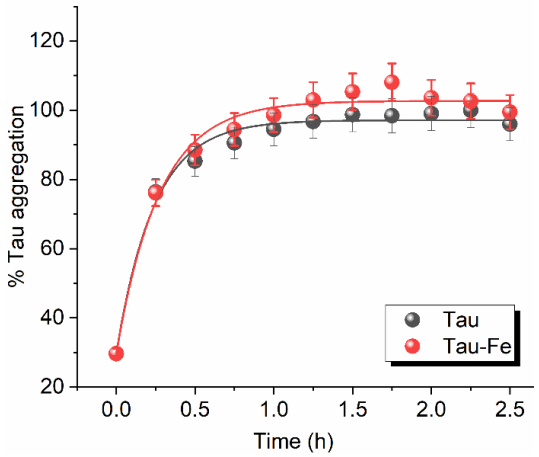

Fig. S9. Kinetic study of aggregation of tau (5 μM) in presence of 5 μM of $FeCl_3$.

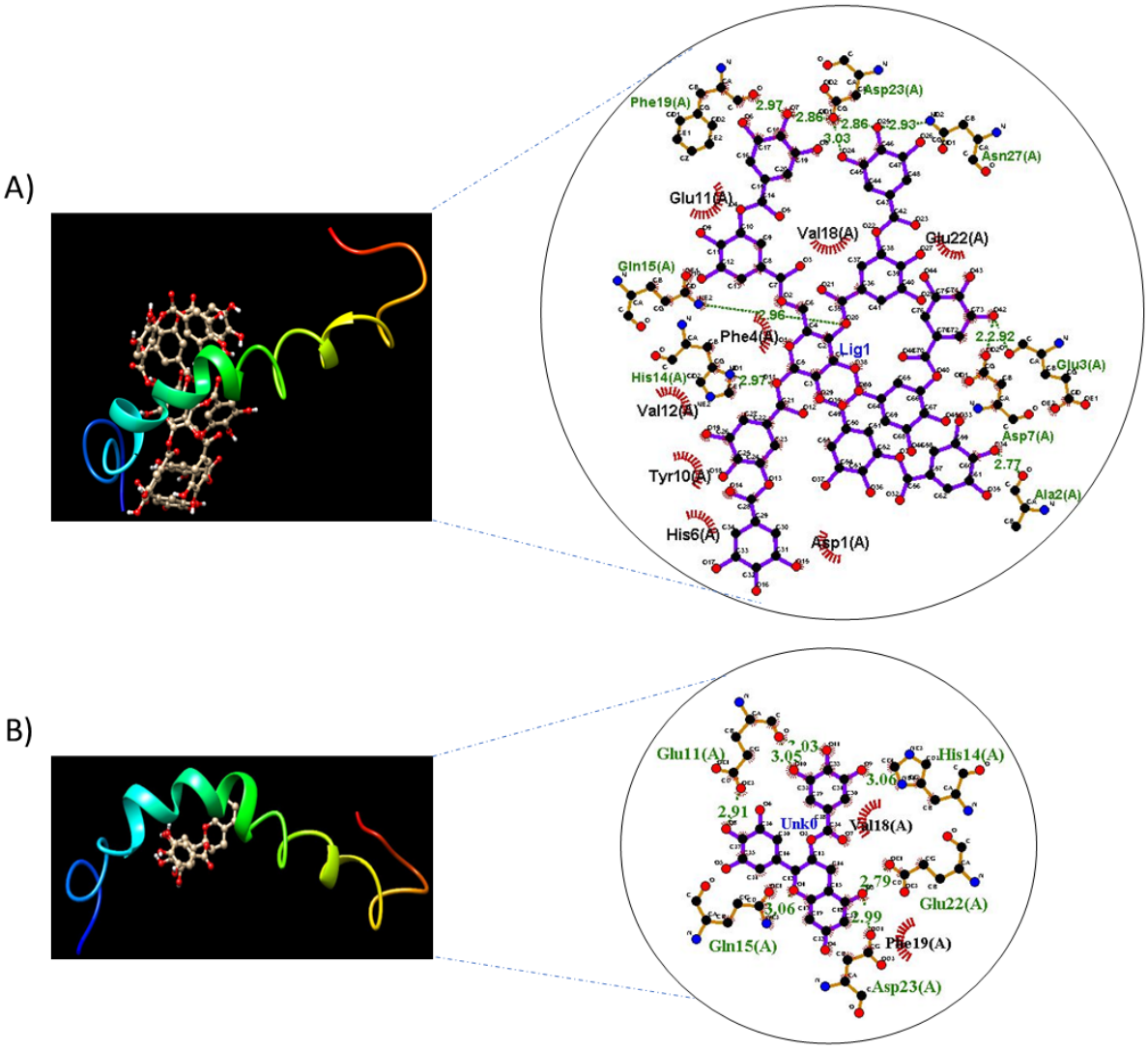

Fig. S10. Docked poses of TA (A) and EGCG (B) with the monomeric form of $A\beta_{42}$. Right panel shows the various hydrogen bonding and hydrophobic interactions with amino acid residues of protein that contribute to the strong interaction.

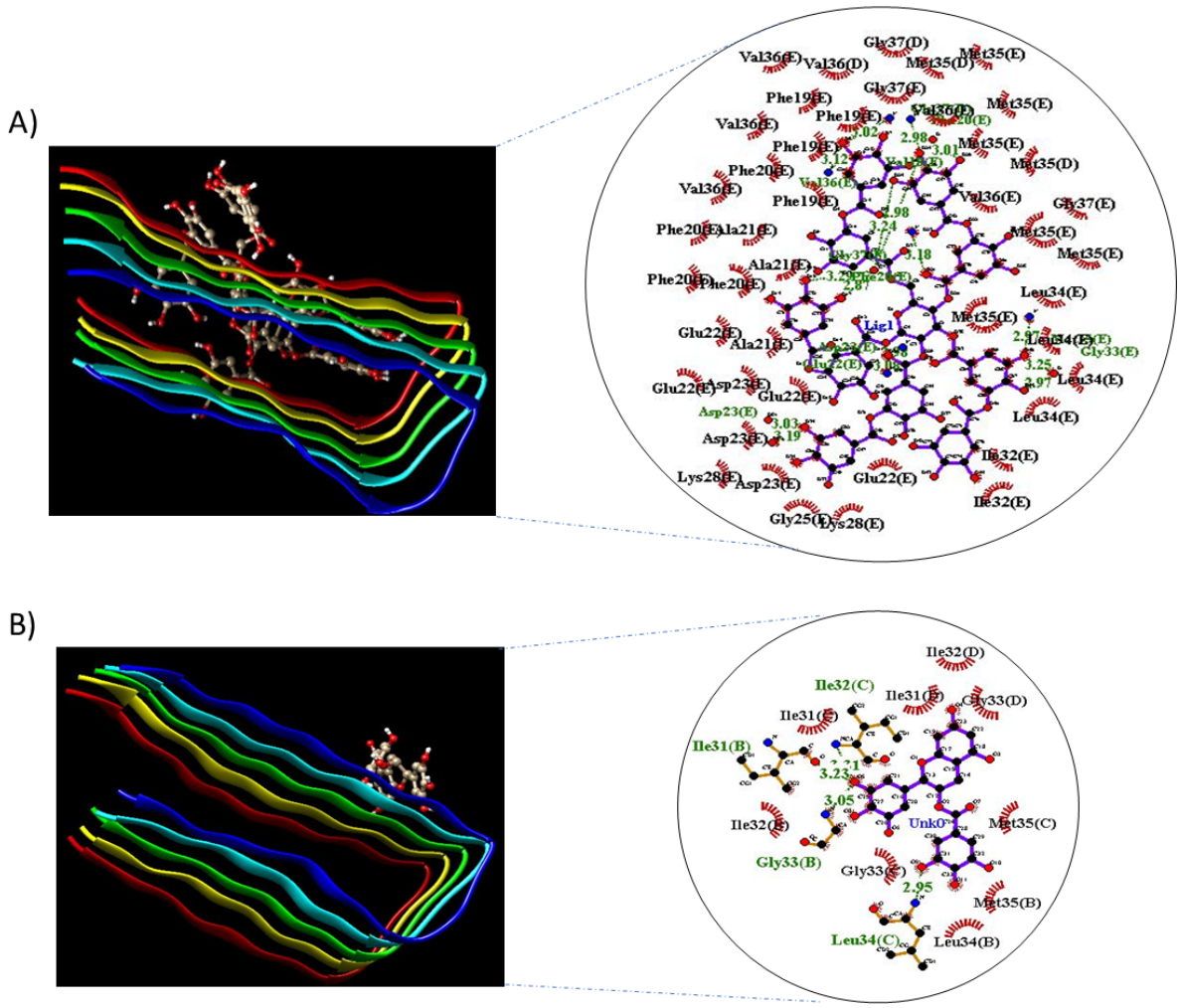

Fig. S11. Docked poses of TA (A) and EGCG (B) with the fibrillar form of $A\beta_{42}$. Right panel shows the various hydrogen bonding and hydrophobic interactions with amino acid residues of protein that contribute to the strong interaction.

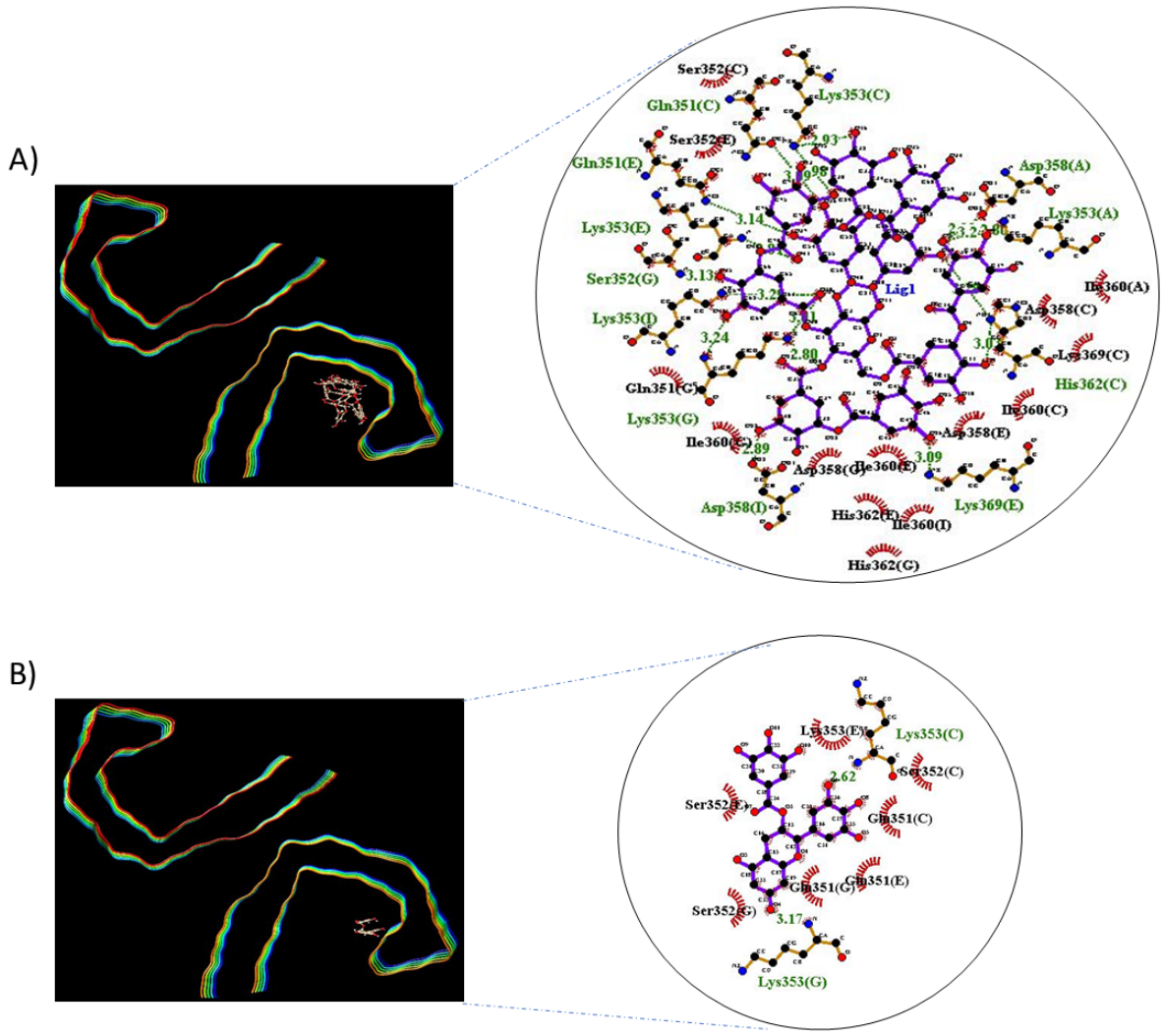

Fig. S12. Docked poses of TA (A) and EGCG (B) with the tau protofibril. Right panel shows the various hydrogen bonding and hydrophobic interactions with amino acid residues of protein that contribute to the strong interaction.

Fig. S13. Binding of EGCG to GPX4. EGCG binds at a site that does not conform to either the inhibitor or activator site of GPX4.

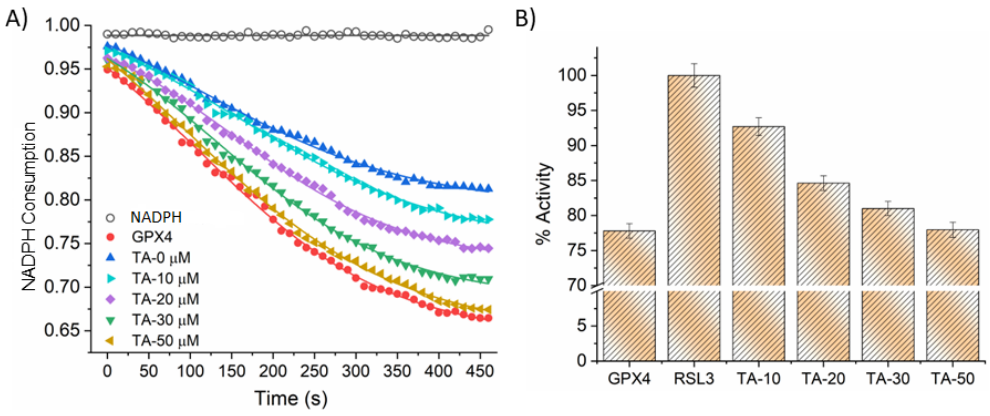

Fig. S14. A) TA reverts back the inhibition of enzyme activity caused by RSL3 in a concentration-dependent manner. [RSL3] = 20 μM. B) The end-point bar diagram of the enzyme activity inhibited in presence of RSL3 and restored by TA in a concentration dependent manner.

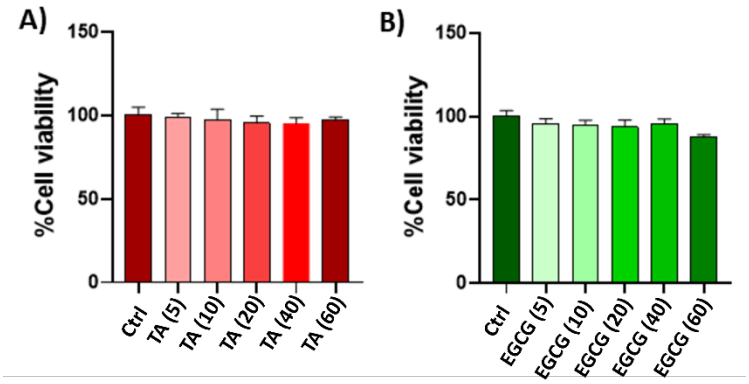

Fig. S15. MTT assay for monitoring the toxicity of the PP A) Tannic acid B) EGCG. The numbers in the parentheses represent the concentration of the compounds in µM.

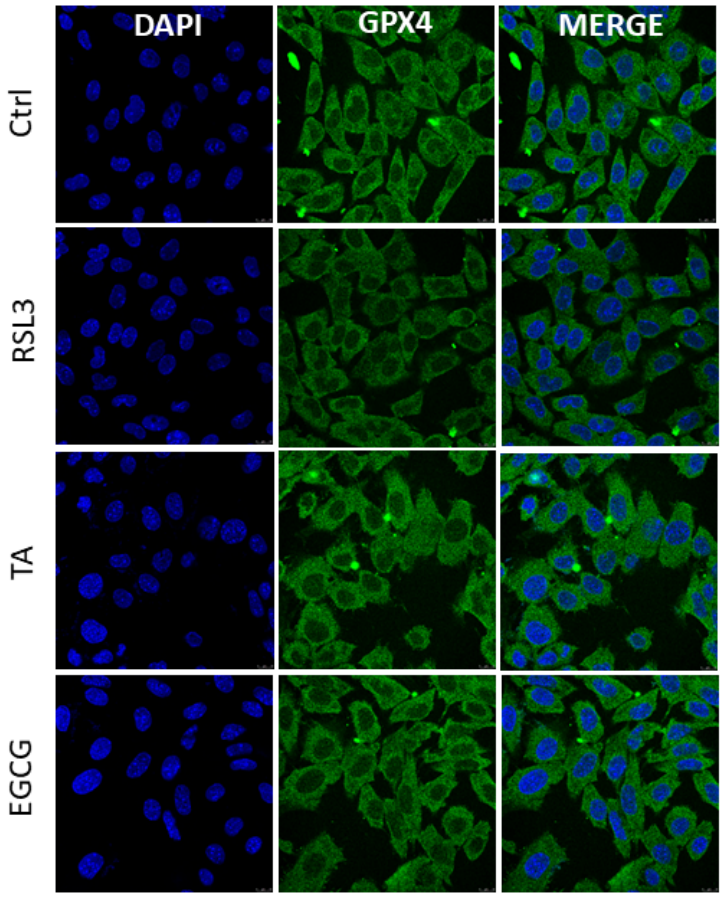

Fig. S16. Immunofluorescence assay to monitor the cellular GPX4 levels under ferroptotic conditions.

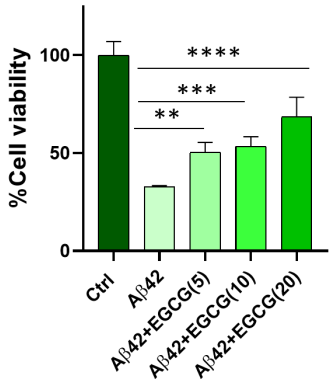

Fig. S17. Ability of EGCG to recuse cells from Aβ induced toxicity. The numbers in the parentheses represent the concentration of the compounds in μM.

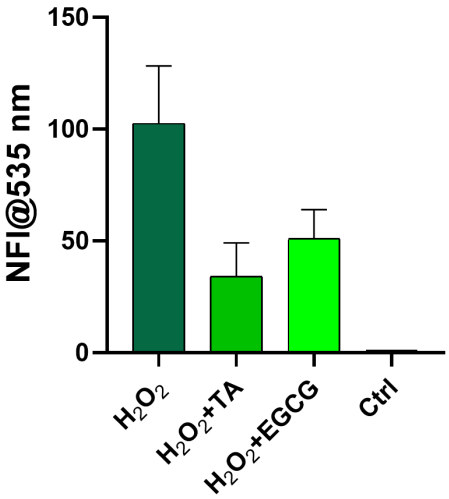

Fig. S18. DCFDA study to monitor the intracellular ROS induced in presence of H_2O_2 and the ability of TA (20μM) and EGCG (20μM) to rescue.

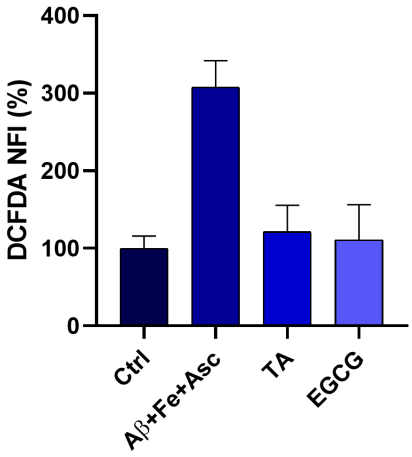

Fig. S19. DCFDA study to monitor the intracellular ROS induced Fenton type reaction and the ability of TA (20 µM) and EGCG (20 µM) to rescue.

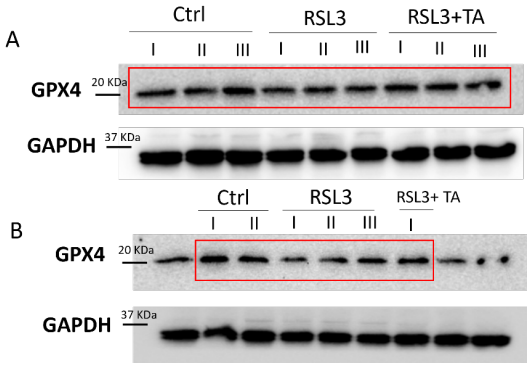

Fig. S20. Western blot analysis of GPX4 levels (presented in Fig. 5E,F) in the presence of RSL3 (1 µM) independently and in the presence of TA (20 µM). I-III represents biological replicates, while A and B represent technical replicates. The data points used for quantification are marked.

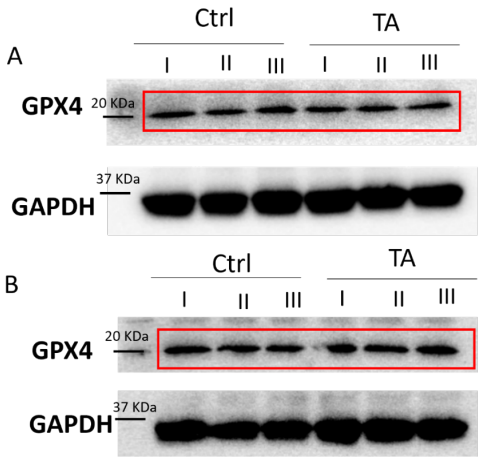

Fig. S21. Western blot analysis of GPX4 levels (presented in Fig. 7C, D) in the presence of TA (20 μM). I-III represents biological replicates, while A and B represent technical replicates. The data points used for quantification are marked in red.