Harmine Acts as an Indirect Inhibitor of Intracellular Protein Aggregation

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ABSTRACT: Protein aggregation and oxidative stress are two pathological hallmarks of a number of protein misfolding diseases, including Huntington’s disease (HD). Whether protein aggregation precedes elevation of oxidative stress or follows it remains ambiguous. We have investigated the role of harmine, a beta-carboline alkaloid, in aggregation of a mutant huntingtin fragment (103Q-htt) in a yeast model of HD. We observed that harmine was able to decrease intracellular aggregation of 103Q-htt, and this reduction was higher than that observed with trehalose, a conventional protein stabilizer. The presence of harmine also decreased prion formation. Decreased protein aggregation was accompanied by reduction in oxidative stress. However, harmine had no effect on aggregation of the mutant huntingtin fragment in vitro. Thus, based on experimental data, we conclude that the antioxidant harmine lowers aggregation-induced elevation in oxidative stress, which slows down intracellular protein aggregation.

INTRODUCTION

Huntington’s disease (HD) is an autosomal dominant neurodegenerative disorder. Mutation in HTT (huntingtin) gene results in polyglutamine expansion at the N-terminus of huntingtin protein, leading to its misfolding and aggregation, and ultimately cell death.1 The length of the CAG (coding for glutamine) repeat (at the 5’-end of huntingtin/IT-15 gene) varies from 6 to 36 in healthy individuals and between 38 and 182 in HD patients. The misfolded protein drives inappropriate interactions with transcription factors and proteins involved in cell signaling and maintenance of cell integrity.2,3 Aggregation of mutant huntingtin generates oxidative stress within the cell,4-8 that is, an imbalance in the amount of reactive oxygen species (ROS) and antioxidative action of the cell. ROS have the ability to damage all biomolecules, including lipid, protein, carbohydrates, and DNA, either directly or indirectly.9 In neurological disorders such as multiple sclerosis, stroke, and neuroinfection, and in neurodegenerative diseases such as alzheimer’s, Parkinson’s, and Huntington’s, oxidative stress is thought to be a principal mechanism in the progression of the disease.10,11 Examination of HD postmortem tissues has demonstrated an increase in multiple markers of oxidative stress,12 which suggests that oxidative damage is increased during the course of the disease. Oxidative stress leads to caspase-mediated neuronal cell death and is considered to be a potential cause of observed neuropathological changes.13 Antioxidants may play an important role in protecting against a number of human diseases.14-19 Various studies have shown the role of antioxidants in neuroprotection.18,20-22 Protopanaxtriol is a plant extract isolated from Panax ginseng mayer and has shown a protective effect against 3-nitropropionic acid (3-NP)-induced oxidative stress in a rat model of HD.20 Protopanaxtriol restores mitochondrial complex enzyme II and SOD (superoxide dismutase) activity and directly scavenges superoxide anions and hydroxyl radicals.20 Several plant extracts or secondary metabolites have shown strong antioxidant activity and protection against oxidant-induced damage in the case of neurodegenerative disorders.14,21,22 Among these plant metabolites is harmine, a plant-derived beta-carboline alkaloid with one indole nucleus and a six-membered pyrrole ring.23 Beta-carboline alkaloids can act as scavengers of ROS.24-26 Harmine increases superoxide dismutase and catalase activities and decreases carbonyl formation in mitochondria in MPTP-treated mice brains as compared to control.27 The alkaloid is also able to decrease Cu2+-induced oxidation of low density lipoproteins.28 Harmine increases hippocampal levels of the brain-derived neurotrophic factor in rat brains,29 which has been implicated in a number of neurodegenerative disorders.30 Harmine is also an inhibitor of monoamine oxidase.31 The alkaloid is a potent ATP-competitive inhibitor of DYRK1A (dual-specificity tyrosine-phosphorylation-regulated kinase 1A), whose overexpression is
a risk factor in β-amyloidosis, neurofibrillary degeneration, and a number of malignant conditions. 

Studies indicate that the basic cellular machinery is well conserved and aggregation of proteins depends on the conserved pattern of folding, despite the species barrier. Many yeast models faithfully recapitulate disease-relevant phenotypes which have been further validated in mammalian systems and human patients. The function of wild-type huntingtin is missing in yeast, HD is modeled in this organism by its prion form ([RNQ+]), and aggregation-induced toxicity of mutant huntingtin protein carrying longer stretches of polyQ tracts is observed only in yeast cells of this strain. Cells expressing 103Q-htt showed puncta. We wanted to study whether harmine has aggregation inhibitory properties or if its role is limited to reduction of oxidative stress, and thus, an indirect effect on protein aggregation.

RESULTS AND DISCUSSION

Trehalose Reduces Aggregation of 103Q-htt and Increases Survival of Yeast Cells. Saccharomyces cerevisiae BY4742 cells were transformed with pYES2-25Q-htt-EGFP or pYES2-103Q-htt-EGFP. This strain has Rnq1 protein in the prion form ([RNQ+]'), and aggregation-induced toxicity of mutant huntingtin protein carrying longer stretches of polyQ tracts is observed only in yeast cells of this strain. Cells expressing 103Q-htt showed fluorescent puncta which confirmed the formation of aggregates (Figure 1a). Trehalose has been used to stabilize proteins under a variety of stress conditions in vitro and in cells. The disaccharide has no effect on expression of the wild-type huntingtin fragment, 25Q-htt (Figure S1a–c), and has been shown to
Figure 2. Harmine attenuates aggregation of 103Q-htt in yeast cells. (a) Postinduced yeast cells were pelleted down, washed, mounted on glass slides, and viewed under a fluorescence microscope (Nikon E600 Eclipse, Nikon Corporation, Japan). Bar = 10 μm. (b) Number of cells exhibiting diffused fluorescence were counted to quantify the extent of solubilization. Values shown are percentage of each set and are mean ± sem of three independent experiments; *p < 0.05, **p < 0.01, and ***p < 0.001 against untreated cells (in the absence of trehalose and harmine). (c) Native PAGE analysis of soluble fractions of cell lysates expressing 103Q-htt in the absence and presence of trehalose (4%, w/v) and different concentrations of harmine. The gel was scanned with an image scanner (Typhoon Trio, GE Healthcare), using λex 532 nm and λem 610 nm. Lower panel shows densitometric analysis of the bands. Band intensity of 103Q-htt in untreated cells (absence of trehalose and harmine) was assigned an arbitrary value of 100%. Values shown are mean ± sem of three independent experiments; *p < 0.05, **p < 0.01 against untreated cells (in the absence of trehalose and harmine). Equal amount of protein was loaded in each well. The Coomassie stained gel is shown in Figure S5a. (d) Western blotting of soluble fractions of cell lysates expressing 103Q-htt in the absence and presence of trehalose (4%, w/v) and different concentrations of harmine using a polyglutamine antibody. Lower panel shows densitometric analysis of the bands. Band intensity of 103Q-htt in untreated cells (absence of trehalose and harmine) was assigned an arbitrary value of 100%. Values shown are mean ± sem of three independent experiments; **p < 0.01, ***p < 0.001 against untreated cells (in the absence of trehalose and harmine). Equal amount of protein was loaded in each well. The Ponceau S-stained membrane is shown in Figure S5b. (e) Filter retardation assay of cell lysate expressing 103Q-htt in the absence and presence of trehalose (4%, w/v) and different concentrations of harmine. Equal amount of protein was filtered through each slot. Lower panel shows densitometric analysis of the triplicate dots. Intensity of dot for 103Q-htt in untreated cells (absence of trehalose and harmine) was assigned an arbitrary value of 100%. Values shown are mean ± sem of three independent experiments; *p < 0.05, ***p < 0.001 against untreated cells (absence of trehalose and harmine). (f) Estimation of ROS in cells expressing 103Q-htt using DHE (λex 535 nm, λem 635 nm). Values shown are mean ± sem of three independent experiments; *p < 0.05, **p < 0.01, ***p < 0.001 against untreated cells (in the absence of trehalose and harmine). (g) Viability of yeast cells expressing 103Q-htt in the absence and presence of trehalose (4%, w/v) and different concentrations of harmine. Values shown are mean ± sem of three independent experiments; **p < 0.01, ***p < 0.001 against untreated cells (in the absence of trehalose and harmine). (h) Native PAGE analysis of soluble fractions of cell lysates overexpressing Rnq1-EGFP in the absence and presence of harmine. The gel was scanned with an image scanner (Typhoon Trio, GE Healthcare), using λex 532 nm and λem 610 nm. Lower panel shows densitometric analysis of the bands. Band intensity of Rnq1-EGFP in untreated cells (absence of harmine) was assigned an arbitrary value of 100%. Equal amount of protein was loaded in each well. The Coomassie stained gel is shown in Figure S6. (i) Filter retardation assay of cell lysates overexpressing Rnq1 in the absence and presence of harmine using a Rnq1 antibody. Triplicate dots are shown. Lower panel shows densitometric analysis of the dots. Intensity of dot for Rnq1 in untreated cells (absence of harmine) was assigned an arbitrary value of 100%. Values shown are mean ± sem of three independent experiments; ***p < 0.001 against untreated cells (absence of harmine).
have a beneficial effect in cells and animal models of HD.\textsuperscript{45} Fluorescence microscopy suggested that the cells in which the expression of 103Q-htt was induced in the presence of 4% w v\textsuperscript{−1} trehalose showed diffusible fluorescence as compared to cells which were untreated (Figure 1a). It was observed that in the presence of trehalose, approximately 25% of total cells had diffused expression of 103Q-htt as compared to untreated cells, where it was negligible (Figure 1b). Native PAGE analysis showed a significant increase in the fraction of soluble 103Q-htt when cells were grown in the presence of trehalose (Figures 1c and S2a). Solubilization of 103Q-htt in the presence of trehalose was confirmed by immunoblotting (Figures 1d and S2b).

The intensity of the band for the monomer, that is, 103Q-htt, was found to be ∼1.5-fold higher as compared to cells which were not exposed to trehalose. Being an osmolyte, trehalose stabilizes the protein in its native conformation which leads to decreased aggregation.\textsuperscript{41,42}

The effect of trehalose on aggregation of 103Q-htt was further confirmed by filter retardation assay using a cellulose acetate membrane which retains aggregates.\textsuperscript{46} Densitometric analysis of dots showed that in the presence of trehalose, the amount of aggregates was reduced by ∼1.2-fold as compared to untreated cells (Figure 1e). Aggregation of proteins is related to increased ROS levels within the cell.\textsuperscript{47,48} High levels of ROS in the cell cause modification of functional groups of amino acids which leads to protein aggregation. Addition of trehalose had no effect on the basal level of ROS in cells expressing 25Q-htt (Figure S3a). A significant increase (>3.5-fold) in the fluorescence intensity of 2-hydroxyethidine (2-EOH) was observed in cells expressing 103Q-htt as compared to those expressing wild-type 25Q-htt (Figure 1f), which matched reports in the literature showing a positive correlation where it was negligible (Figure 1b). Native PAGE analysis showed a significant increase in the fraction of soluble 103Q-htt when cells were grown in the presence of trehalose (Figures 1c and S2a). Solubilization of 103Q-htt in the presence of trehalose was confirmed by immunoblotting (Figures 1d and S2b).

Aggregation of the mutant huntingtin fragment has been linked to lower survival of yeast cells.\textsuperscript{37} Addition of trehalose had no effect on the viability of yeast cells expressing 25Q-htt (Figure S3b). The viability of yeast cells expressing 103Q-htt was significantly higher (∼2-fold) when grown in the presence of trehalose than in its absence (Figure 1g). This correlates well with higher solubilization (Figure 1c,d) and reduced aggregation (Figure 1e) of 103Q-htt observed in the presence of trehalose.

**Presence of Harmine Reduces Aggregation of 103Q-htt.** Harmine is a phyto-antioxidant and decreases the cellular oxidative stress level by scavenging ROS.\textsuperscript{23–26} Addition of harmine had no effect on the expression of 25Q-htt in yeast cells as seen by fluorescence microscopy (Figure S4a), native PAGE (Figure S4b), and western blot analysis (Figure S4c).

Aggregation of 103Q-htt was monitored in yeast cells grown in the presence of different concentrations of harmine, at a fixed concentration (4% w v\textsuperscript{−1}) of trehalose. As mentioned above (Figure 1b−d) and in previous reports,\textsuperscript{43} at this concentration, trehalose has an attenuating effect on aggregation of 103Q-htt in yeast cells. Increasing solubilization of 103Q-htt was observed in yeast cells with increasing concentration of harmine along with trehalose as compared to untreated cells or cells grown in the presence of trehalose alone (Figure 2a).

At the highest concentration of harmine (25 μg mL\textsuperscript{−1}) with trehalose, approximately 55% of total cells showed diffusible fluorescence because of EGFP indicating expression of soluble 103Q-htt as compared to untreated cells, where it is negligible (Figure 2b). Thus, in addition to the disaccharide, the antioxidant was able to solubilize 103Q-htt. Interestingly, at the highest concentration of harmine (25 μg mL\textsuperscript{−1}), the extent of solubilization of 103Q-htt was the same, irrespective of the presence of trehalose. Increased solubilization of 103Q-htt was also seen by native PAGE. The intensity of the band for EGFP fused to 103Q-htt increased with increasing concentration of harmine in the presence of trehalose (Figures 2c and S5a). As mentioned before, at the highest concentration of the antioxidant, solubilization of 103Q-htt was independent of the presence of trehalose. A similar pattern was also observed when solubilization of 103Q-htt was monitored by immunoblotting. Cells which were grown in the presence of the maximum concentration of harmine (25 μg mL\textsuperscript{−1}) along with trehalose showed 3-fold increase in the intensity of the band for soluble 103Q-htt as compared to untreated cells, while this increase in intensity jumped to ∼8-fold for cells grown in the presence of harmine (25 μg mL\textsuperscript{−1}) without trehalose (Figures 2d and S5b).

The effect of harmine on aggregation of 103Q-htt was confirmed by filter retardation assay using a cellulose acetate membrane (Figure 2e). This membrane filters the proteins on the basis of size, allowing only aggregates to be retained on the membrane.\textsuperscript{46} Densitometric analysis of dots showed that the amount of 103Q-htt aggregates formed in the presence of harmine and trehalose in treated cells decreased continuously as compared to untreated cells, with the maximum reduction (>2-fold) seen with the highest concentration of harmine in the absence of trehalose (Figure 2e).

**Harmine Decreases Oxidative Stress and Increases Cell Viability.** Aggregation of proteins is associated with increased oxidative stress in the cell. The mechanism of aggregation-lowering ability of harmine was followed by measuring the generation of ROS in the cell. Addition of harmine in the media had no effect on the basal level of ROS in yeast cells expressing 25Q-htt (Figure S4d). This shows that the activity of harmine as an anti-oxidant is seen only when the level of ROS exceeds a threshold value. Aggregation of 103Q-htt led to increased generation of ROS (Figure 1e) which was reduced when cells were grown in the presence of harmine, in the absence or presence of trehalose (Figure 2f). Reduced aggregation of 103Q-htt and concomitant decrease in oxidative stress resulted in increased survival of yeast cells expressing 103Q-htt in the presence of harmine (Figure 2g). This increase was ∼2-fold at the highest concentration of harmine (25 μg mL\textsuperscript{−1}) in the absence of trehalose. Addition of trehalose and harmine did not show any effect on the viability of yeast cells expressing 25Q-htt (Figure S4e). Thus, in the presence of the antioxidant harmine, aggregation of 103Q-htt was reduced,
corresponding with reduced oxidative stress and increased cell viability.

The presence of prions, specifically [RNQ1⁺], seems to be an essential condition for aggregation-induced proteotoxicity in yeast cells.⁵⁷ Hence, the aggregation status of Rnq1 was monitored in yeast cells in the presence of harmine. Yeast cells transformed with pYES2-Rnq1-EGFP were induced to express Rnq1-EGFP in the absence and presence of harmine. Native PAGE analysis of the cell lysate showed that solubilization of Rnq1 increased with increasing concentration of harmine (Figures 2h and S6). Prion formation ([RNQ1⁺]) was monitored by filter retardation assay. Analysis of the blot showed that in the presence of harmine, the extent of aggregation of Rnq1 was significantly reduced as compared to untreated cells (Figure 2i). Attenuated prion formation ([RNQ1⁺]) also correlated with reduced intracellular oxidative stress (Figure 2j). Increased prion formation, that is, [RNQ1⁺], has been directly correlated with aggregation of 103Q-htt in yeast cells due to the “seeding” activity of the prion protein.⁵⁷ Decreased formation of prion in the presence of the antioxidant harmine may be responsible for the inhibition of aggregation of 103Q-htt observed in this case.

**Figure 3.** Harmine has no effect on aggregation of 103Q-htt in vitro. (a) Purification of GST-51Q-htt was carried out by affinity chromatography and followed by SDS-PAGE. Lane 1: molecular weight marker (bovine serum albumin, 65 kDa), lane 2: uninduced cell lysate, lane 3: induced cell lysate, lane 4: flowthrough; lanes 5 and 6: washings, lanes 7 and 8: eluates, and lane 9: dialyzed protein. Protein load was 10 μg in each case. The gel was Coomassie stained. (b) Western blot analysis of purification of GST-51Q-htt; lane 1: cell lysate, lane 2: flowthrough, lanes 2 and 3: washings, lanes 5 and 6: eluted GST-51Q-htt, and lane 7: dialyzed protein. Protein load was 20 μg each lane. The membrane was probed with a polyglutamine antibody followed by an FITC-conjugated antimouse antibody. The Ponceau S-stained membrane is shown in Figure S7. (c) GST-51Q-htt (1 mg mL⁻¹) was incubated at 37 °C. Time-dependent formation of aggregates was monitored by Thioflavin T fluorometry (λₚ, 440 nm, λₑ, 484 nm). The final concentrations of the protein and the fluorophore were 1.5 and 50 μM, respectively. (d) GST-51Q-htt (1 mg mL⁻¹, 40 mM Tris-HCl buffer, pH 8.0 containing 150 mM NaCl) was incubated at 37 °C at different time intervals. Filter retardation assay using a cellulose acetate membrane (0.2 μm) was carried out at different time intervals, and the amount of aggregates retained was probed with the polyglutamine antibody. The intensity of the dot at the last point of analysis was assigned an arbitrary value of 100%. Triplicate dots are shown for each time point in Figure S8a. (e) GST-51Q-htt (1 mg mL⁻¹, 40 mM Tris-HCl buffer, pH 8.0 containing 150 mM NaCl) was incubated at 37 °C at different time intervals. The incubated protein was filtered through the nitrocellulose membrane and analyzed with the oliogomer-specific A1 antibody using a dot-blot assay. The intensity of the dot for the protein incubated alone till 75 h was assigned a value of 100%. Triplicate dots are shown for each time point in Figure S8b. Values shown are mean ± sem of three independent experiments.

**Harmine Has No Effect on Aggregation of the Mutant Huntingtin Fragment in Vitro.** The increased ROS level in cells causes oxidative damage to proteins.⁴⁹,⁵⁰ Conversely, the presence of misfolded and aggregated proteins enhances the level of ROS in the cell, primarily by mitochondrial dysfunction.¹⁵,⁵¹–⁵⁴ Reduction in intracellular oxidative stress observed above could be due to decrease in protein aggregation in the presence of harmine. On the other hand, reduced oxidative stress with consequent reduction in oxidative damage to proteins could result in decreased aggregation of 103Q-htt in the presence of harmine. Whether oxidative damage precedes or follows protein aggregation and the step at which harmine intervenes in this process remain to be determined. Hence, we decided to investigate whether harmine has any effect on aggregation of expanded polyglutamine in an extracellular milieu. Aggregation of the polyglutamine tract (in the pathogenic range) occurs in a length-dependent manner. The pattern of aggregation remains unaltered. A change is seen in aggregation kinetics, with longer polyQ stretches exhibiting faster rates of aggregation⁴⁶ and higher toxicity.³⁷,⁵⁸–⁵⁹ Because for lengths >72Q, aggregation occurs quite fast⁴⁶ and measurement of difference in rates becomes difficult, we
selected the well-validated elongated polyQ-containing 51Q-htt system to monitor differences in rates of aggregation of polyQ in the presence of harmine in vitro. The mutant huntingtin fragment (GST-51Q-htt) was purified by affinity chromatography, as described before. Purification of the protein was followed by SDS-PAGE which showed a band at the expected position (~50 kDa) (Figure 3a). Immoblotting with the polyglutamine antibody showed a tail (Figures 3b and S7) because of truncated polyglutamylic tracts. In vitro aggregation of 51Q-htt was monitored by Thioflavin T fluorescence assay. Aggregation kinetics showed distinct nucleation, growth (fibrillation), and equilibrium (saturation) stages (Figure 3c) and confirmed that the aggregates formed were of cross β-sheet nature. No significant difference in the pattern of aggregation of 51Q-htt was seen when the protein was incubated in the presence of harmine (6.25 or 25 μg mL⁻¹) than in its absence.

The effect of harmine on aggregation of 51Q-htt was further studied by filter retardation assay using the polyglutamine antibody as the probe (Figures 3d and S8a). Unlike Thioflavin T which is an amyloid-specific dye and quantifies the fibrillar aggregates formed (Figure 3c), filter retardation assay quantifies the total amount of aggregates. Comparison of the two curves suggests formation of amorphous aggregates at initial stages followed by fibrillar aggregates. The formation of oligomers during incubation was detected with an oligomer-specific A11 antibody (Figures 3e and S8b). Almost similar patterns were observed in the absence and presence of harmine when the membrane was probed with the A11 antibody. Analysis of both curves suggests that harmine had no effect on either oligomer formation or aggregation of 51Q-htt in vitro.

Thus, it is clear that harmine had no direct effect on aggregation of the mutant huntingtin fragment. Instead, inhibition of aggregation of the mutant huntingtin fragment observed in yeast cells resulted from antioxidation activity of harmine. Harmine scavenges ROS which lowers oxidative damage to the mutant huntingtin fragment. As damage to proteins due to oxidative stress is responsible for protein aggregation in many protein misfolding disorders, the presence of the antioxidant reduces aggregation of the mutant huntingtin fragment by attenuating oxidative damage and ameliorating aggregation-induced cytotoxicity.

Misfolding and aggregation of proteins have been linked to the development and progression of a number of neurodegenerative and other disorders. A number of epidemiological studies have established correlations between lifestyle choices and progression of disease conditions. For example, coffee drinking and cigarette smoking have been shown to have a negative correlation with disease progression in Parkinson’s disease. We have shown that caffeine and nicotine alter the rate of aggregation of α-synuclein and increase the survival of yeast cells expressing α-synuclein, thus providing a mechanistic explanation for the observations. In the present case, the antioxidant harmine is seen to be as good a protein stabilizer under intracellular conditions as the disaccharide trehalose, a known protein stabilizer, although it follows a different mode of action. The level of inhibition of aggregation seen in the presence of harmine and trehalose was marginally lower than that in the presence of the highest concentration of harmine alone. The decreasing level of aggregation of 103Q-htt with increasing concentration of harmine and fixed concentration of trehalose also reflects the importance of the antioxidant in slowing down protein aggregation inside the cell. The presence of harmine lowers the oxidative stress and hence aggregation of oxidatively damaged proteins. Under these conditions, the presence of trehalose does not provide any additional benefit to the cell.

### EXPERIMENTAL SECTION

**Materials.** *S. cerevisiae* BY4742 [MATa, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0, (RNQ1⁺)] is a product of Open BioSystems and was purchased from SAF Labs Pvt. Ltd., Mumbai, India. Harmine, glutathione-agarose matrix, thioflavin T, dihydroethidium (DHE), and the antimonique FITC conjugated antibody were purchased from Sigma-Aldrich, Bengaluru, India. Mouse anti-polyglutamine (polyglutamine expansion disease marker monoclonal antibody, MAB1574) was a product of Chemicon International and was purchased from Millipore (India) Pvt. Ltd., New Delhi, India. A goat anti-Rnaq1 antibody was purchased from Santa Cruz, California, USA. The oligomer-specific A11 polyclonal antibody was purchased from Invitrogen Corporation, California, USA. The nitrocellulose membrane (0.2 μm) was purchased from Advanced Microdevices Pvt. Ltd. Ambala Cantt, India. The cellulose acetate membrane was purchased from Sartorius Stedim Biotech, Goettingen, Germany. All other reagents and chemicals used were of analytical grade or higher.

**Methods.** **Expression of 25Q-htt and 103Q-htt in Yeast Cells.** *S. cerevisiae* BY4742 strain was transformed separately with pYES2-25Q-htt-EGFP or pYES2-103Q-htt-EGFP by the lithium acetate–polyethylene glycol (PEG) method. Transformed cells were grown in SC-URA media containing 2% (w/v) dextrose at 30 °C, 200 rpm till OD₆₀₀ nm, 0.6–0.8. Expression of proteins was induced by changing the media to SC-URA media containing 2% (w/v) galactose, with and without 4% (w/v) trehalose and different concentrations of harmine for 10 h. Expression of 25Q-htt and 103Q-htt was monitored by fluorescence microscopy (E600 Eclipse microscope, Nikon, Japan) as the proteins were tagged with EGFP. Yeast cells were disrupted using acid-treated glass beads and lysed using lysis buffer (0.05 M Tris, 0.15 M NaCl, 0.002 M DTT, pH 7.5 supplemented with 1 mM PMSF). The lysate was centrifuged at 800 g for 10 min, and the supernatant obtained was analyzed for the presence of aggregates using filter retardation assay. This supernatant was further centrifuged at 12,000g for 45 min, and the presence of soluble protein was confirmed by native PAGE and immunoblotting using the polyglutamine-specific antibody. The respective gel and blot were scanned using an image scanner (Typhoon Trio, GE Healthcare). Estimation of the protein content in different samples was carried out by the dye binding method, using bovine serum albumin as the standard protein.

**Filter Retardation Assay.** The supernatants (obtained after centrifugation of yeast cell lysates at 800 g for 10 min) were filtered through a cellulose acetate membrane (0.2 μm pore size) using a dot blot apparatus (Whatman Schleicher & Schuell, UK). For in vitro analysis of the aggregation pattern, affinity purified 51Q-htt was incubated at 37 °C for 95 h. Aliquots (50 μg protein each) were withdrawn at regular intervals and vacuum-filtered through a prewetted cellulose acetate membrane. The membranes were probed with the polyglutamine-specific antibody for detection of aggregates. Aliquots were also filtered through a nitrocellulose membrane and probed with the oligomer-specific A11 polyclonal antibody for detection of oligomers. The membranes were scanned on an image scanner (Typhoon Trio, GE Healthcare). The
where $y_i + mx_i$ is the initial line, $y_f + mx_f$ is the final line, and $x_0$ is the midpoint of the maximum signal.

**Measurement of Oxidative Stress.** Intracellular ROS levels were quantified using DHE (dihydroethidium) dye. After the end of the induction period, yeast cells were washed with phosphate-buffered saline, pH 7.4 (PBS), and counted using Neubauer’s chamber. Cells (1 × 10⁷) were aliquoted into a microcentrifuge tube, and DHE (0.01 M, in PBS) was added at a final concentration of 10 μM. The final reaction mixture was made up to 1 mL with PBS and incubated at 37 °C with shaking at 200 rpm for 20 min. The emission intensity of ethidium was recorded at $\lambda_{em}$ 635 nm using $\lambda_{ex}$ 535 nm.

**Cell Viability Assay.** Postinduction yeast cells were pelleted down, resuspended in 1 mL autoclaved water, and counted using Neubauer’s chamber. Cells (1 × 10⁷) were plated on SCURA containing 2% dextrose plates, and growth of colonies was observed at 30 °C for 3 days.

**Measurement of Uptake of Harmine by Yeast Cells.** The amount of harmine taken up by yeast cells was determined by HPLC (SCL-10A VP, Shimadzu, Japan). Yeast cell pellets were thawed on ice, resuspended in 500 μL of 0.5 M trichloroacetic acid, and incubated at room temperature for 1 h. The cell lysates were centrifuged at 12,000 rpm for 30 min at room temperature. The supernatants were collected, and the pellets were resuspended in 500 μL of 0.5 M trichloroacetic acid and incubated at room temperature for 1 h. The suspensions were centrifuged as mentioned above, and the supernatants were pooled and filtered through a 0.2 μm syringe filter. Samples were injected into a C18 Zorbax analysis column (Agilent Technologies, USA), and the eluate was monitored using a photodiode array detector (UV 10A, Shimadzu, Japan) at a flow rate of 1 mL min⁻¹. The mobile phase used was isopropyl alcohol/acetonitrile/water/formic acid in the ratio 100:100:300:0.3 (v/v/v/v), pH 8.6 [adjusted with triethylamine (99%)].

**Aggregation of the Mutant Huntingtin Protein Fragment in Vitro.** Competent *Escherichia coli* BL21 (DE3) cells were transformed with plasmid pGEX-SXI-HDex1-CAG51 and grown at 37 °C in Luria−Bertani media. Protein expression was induced with 1 mM IPTG for 5 h at 37 °C. The mutant huntingtin protein fragment (GST-51Q-htt) was purified by affinity chromatography, as described earlier. Purification of 51Q-htt protein was confirmed by SDS-PAGE.

Purified 51Q-htt (1 mg mL⁻¹) was incubated with and without harmine (6.25 and 25 μg mL⁻¹) at 37 °C. Aliquots (25 μg each) were withdrawn at regular time intervals and the aggregation pattern of 51Q-htt was monitored by thioflavin T fluorescence assay. Fluorescence intensity of the dye was measured using a spectrofluorimeter (RF-S301PC, Shimadzu) with $\lambda_{ex}$ 440 nm and $\lambda_{em}$ 484 nm.

### ASSOCIATED CONTENT

1. Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.9b02375.

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Coomassie-stained native gels, Ponceau S-staining of immunoblots, microscopy images, native gels, and immunoblots of control (25Q- htt) (PDF)

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**Notes**

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