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ORIGINAL ARTICLE

Curcumin revitalizes Amyloid beta (25–35)-induced and organophosphate pesticides pestered neurotoxicity in SH-SY5Y and IMR-32 cells via activation of APE1 and Nrf2

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Abstract Amyloid beta (Aβ) peptide deposition is the primary cause of neurodegeneration in Alzheimer's disease (AD) pathogenesis. Several reports point towards the role of pesticides in the AD pathogenesis, especially organophosphate pesticides (OPPs). Monocrotophos (MCP) and Chlorpyrifos (CP) are the most widely used OPPs. In this study, the role of MCP and CP in augmenting the Aβ-induced oxidative stress associated with the neurodegeneration in AD has been assessed in human neuroblastoma IMR-32 and SH-SY5Y cell lines. From the cell survival assay, it was observed that MCP and CP reduced cell survival both dose- and time-dependently. Nitro blue tetrazolium (NBT) based assay for determination of intracellular reactive oxygen species (ROS) demonstrated that Aβ(25–35), MCP or CP produce significant oxidative stress alone or synergistically in IMR-32 and SH-SY5Y cells, while pretreatment of curcumin reduced ROS levels significantly in all treatment combinations. In this study, we also demonstrate that treatment of Aβ(25–35) and MCP upregulated inducible nitric oxide synthase (iNOS/NOS2) whereas, no change was observed in neuronal nitric oxide synthase (nNOS/NOS1), but down-regulation of the nuclear factor erythroid 2-related factor 2 (Nrf2) level was observed. While curcumin pretreatment resulted in upregulation of iNOS and Nrf2 proteins. Also, the expression of key DNA repair enzymes APE1, DNA polymerase beta (Pol β), and PARP1 were found to be downregulated upon treatment with MCP, Aβ(25–35) and their combinations at 24 h and 48 h time points. In this study, pretreatment of curcumin to the SH-SY5Y cells enhanced the expression of DNA repair enzymes APE1, pol β, and PARP1 enzymes to counter the oxidative DNA base damage via base excision repair (BER) pathway, and also activated the antioxidant element (ARE) via Nrf2 upregulation. Furthermore, the immunofluorescent confocal imaging studies in SH-SY5Y and IMR-32 cells treated with Aβ(25–35) and MCP-mediated oxidative stress and their combinations at different time periods suggesting for cross-talk between the two proteins APE1 and Nrf2. The APE1's association with Nrf2 might be associated with the redox function of APE1 that might be directly regulating the ARE-mediated neuronal survival mechanisms.

Keywords Alzheimer's disease · Amyloid beta protein · Monocrotophos · Chlorpyrifos · APE1 · Nrf2 · Curcumin

Introduction

Many studies signify that pesticides have a causal relation to the development of neurological disorders, and one among them, Alzheimer's disease (AD) has been associated with exposure to the pesticides (Richardson et al. 2014). Organophosphate pesticides (OPPs) are mainly used in cotton, wheat, rice and other cash crops to enhance productivity. Monocrotophos (MCP) and Chlorpyrifos (CP) are the main two OPPs, which are most common in agricultural practices in this part of the world, due to its low cost and easy availability. These pesticides are neurotoxic in nature and act as Acetylcholinesterase inhibitors [AChEIs] (Khokhar and

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Tyndale 2012; Tripathi et al. 2014). The primary route of exposure to the pesticides is dietary ingestion, respiration, and dermally. MCP and CP induce apoptotic damage via caspase regulated cascade involving activation of Caspase-9 and Caspase-3 as studied in neuronal cells (Kashyap et al. 2011, 2010; Androutsopoulos et al. 2011; Lee et al. 2012). Exposure to 10 μM dose of MCP either alone or in combination with 10 μM resveratrol on rat neuronal PC-12 cells increased cell survival in the case of resveratrol and MCP combination treatments in comparison with MCP alone, but decreased as compared with that of only resveratrol treatment (Kumar et al. 2013). Treatment of MCP has been reported to induce a significant amount of ROS, lipid peroxidation, micronucleus, chromosomal aberration, and reduced glutathione (GSH) content and catalase inactivation (Mundhe and Pandit 2014). Exposure of MCP reported to enhance pro-apoptotic markers like caspase-3, caspase-9, Bax, and p53 while downregulate anti-apoptotic markers like Bcl2 (Kashyap et al. 2011). Different studies suggest that MCP exposure involved with the ROS-mediated oxidative stress, which subsequently triggers apoptotic pathways in both human neuronal and glial cell lines (Tripathi et al. 2014). In the human body, CP is converted to Chlorpyrifos-oxon (CPO) by the liver Cytochrome-450 enzymes, which binds and inhibits AChE and other serine active site esterases, lipases, and proteases (Cole et al. 2005). CP also reported to cause oxidative stress by overproduction of ROS in *Drosophila melanogaster,* and a significant positive correlation was observed among ROS generation, apoptosis and DNA damage (Gupta et al. 2010).

Neurodegenerative disorders, a group of diseases in which neuronal cells degenerate and finally results in neuronal cell death. AD is a progressive age-associated neurodegenerative disease, where people of old age are more vulnerable than younger aged people. Formation of intracellular neurofibrillary tangles (NFT) and Amyloid beta (Aβ) peptide deposition, imbalance in neurotransmitter levels, Ca^{2+} overload are some of the main hallmarks of AD (Francis et al. 1999; Hardy and Selkoe 2002; Hardy and Higgins 1992; Mattson et al. 1992). Age and genetics of the person are the other strongest risk factors for occurrence and development of AD. Reactive nitrogen species (RNS) and ROS are generated both exogenously as well as endogenously within the cell, and oxidative stress is the state of imbalance between ROS and RNS generation and the antioxidant defense machinery (Pham-Huy et al. 2008). Increased ROS and RNS production leads to the damage of DNA, RNA, proteins and lipids, and has been associated with several neurological diseases including AD (Chen et al. 2012). AD has been associated with exposure to pesticides (Hayden et al. 2010). The oxidative stress is counteracted by various internal response and repair enzymes. Apurinic/apyrimidinic endonuclease 1 (APE1) is the multifunctional protein which regulates many transcription factors (TFs) via its redox function, where it reductively activates various TFs by exchanging its sulphydryl groups of its active cysteine residues (Xanthoudakis et al. 1992). APE1 also plays a central role in base excision repair (BER)-pathway and is known as a multifunctional enzyme (Bhakat et al. 2009; Edwards et al. 1998; Mantha et al. 2014; Vascotto et al. 2009). The C-terminal domain contains amino acids required for its endonuclease activity in BER-pathway and the N-terminal domain contains nuclear localization signal (NLS), essential for transcriptional regulation through redox activity, and also known as redox effector factor-1 [Ref-1] (Xanthoudakis et al. 1992; Fishel et al. 2015; Bhakat et al. 2009). During oxidative stress, APE1 plays pivotal role and it has been shown that significant loss of cellular antioxidant capacity occurs in APE1-deficient or redox mutated A549 cells (Yang et al. 2010). The nuclear factor erythroid 2-related factor 2 (Nrf2) is a master regulator of cellular oxidation defense system (Yang et al. 2010). Nrf2 controls the basal and induced expression of an array of antioxidant response element (ARE) dependent genes to regulate the physiological and pathological outcomes of oxidant exposure. The cytoplasmic repression of Nrf2 activity depends on Keap1, which sequesters Nrf2 in the cytoplasm and controls its ubiquitin-dependent degradation (Sun et al. 2007). In response to oxidative stress, the Nrf2 translocate from the cytoplasm to the nucleus and transactivates expression of genes with antioxidant activity (Narasimhan et al. 2011). Loss of antioxidant capacity has been attributed either due to the downregulation of Nrf2 function in APE1-deficient/ redox mutated cells or via activation of nuclear localization of Nrf2 and transcribe downstream antioxidant genes (Shan et al. 2015). A study by Fishel and group demonstrated that repression of APE1 potently activates Nrf2 and its downstream targets in a dose-dependent fashion in pancreatic ductal adenocarcinoma (PDAC) MIA-PaCa-2 cell line (Fishel et al. 2015).

There are many natural plant based compounds with antioxidant capacity, and one of such compounds is curcumin. Curcumin is the principal active flavonoid derived from the rhizome of *Curcuma longa* [Zingiberaceae] (Kaur et al. 2016). Wu et al., have demonstrated that curcumin reduces brain damage in a rat model of brain focal ischemia, via the induction of Nrf2/HO-1-pathway (Wu et al. 2013). In an in vivo study, the efficacy of curcumin as a neuroprotective agent was tested, where a significant improvement in behavioral, neurochemical and immunohistochemical parameters were observed after curcumin administration (Yadav et al. 2009). Curcumin administration reduces the number of activated glial cells in PTZ treated animals, findings also suggest that curcumin is effective in attenuating glial cell activation and ameliorates cognitive deficits in chronic epilepsy cases (Kaur et al. 2015a). Curcumin dose-dependently inhibits fibrillary Aβ formation from the freshly soluble Aβ and destabilizes preformed fibrillary Aβ in vitro and Aβ plaque formation (Ono et al. 2004; Yang et al. 2005). In the present study, along with Aβ(25–35), OPPs have been used, and various

biochemical studies were performed to ascertain how this combination of oxidants pester toxicity in neuronal cells and pretreatment of curcumin for the revitalization against Aβ(25–35) and OPP induced neurotoxicity by activation of the key proteins APE1 and Nrf2, which are suggested to be associated with the control of cellular homeostasis during Aβ-induced oxidative stress in case of AD .

Materials and methods

Cell culture

Human neuroblastoma SH-SY5Y cells were generously gifted by Prof. Pankaj Seth, National Brain Research Centre (NBRC), Manesar, Haryana, India. The cells were cultured in complete medium containing Dulbecco's Modified Eagle's Medium (DMEM)/Ham's F-12 (1:1) supplemented with 10% fetal bovine serum (FBS)/ 10% horse serum (HS) and 1× penicillin-streptomycin (PS). SH-SY5Y cells were maintained in an incubator under 5% CO_2 at 37 °C (Mantha et al. 2012). The human neuroblastoma IMR-32 cells procured from the National Centre for Cell Sciences (NCCS), Pune, India were grown in DMEM containing 10% FBS and 1× PS and were maintained at 37 °C in a 5% CO_2 humidified incubator (New Brunswick, Galaxy® 170 S) as described above.

Cell treatments

SH-SY5Y and IMR-32 cells were seeded onto 96-well plates at a density of 1 x 10^4 cells per well in complete media with 70–80% confluency as described elsewhere (Kaur et al. 2015b). The treatment with the oxidant Aβ(25–35) at 10 μM and the phytochemical Curcumin at 10 μM concentrations were used in this study.

Pesticide treatment

MCP is soluble in water (H_2O) as well as in organic solvents like DMSO or ethanol, while CP is insoluble in H_2O but is soluble in organic solvents. MCP (Sigma-Aldrich, PESTANAL®, Analytical Standard) was dissolved in deionized double distilled H_2O as 50 mM stock before the treatment. CP (Sigma-Aldrich, PESTANAL®, Analytical Standard) was dissolved in 100% DMSO as 50 mM stock before the treatment.

Preparation and treatment of Aβ(25–35)

Aβ (25–35) peptide (GenScript, Piscataway, NJ) stock solutions were freshly prepared before each treatment at 1 mM in double-distilled deionized water considered the soluble form (Mantha et al. 2012). The cells were then treated with Aβ (25–35) peptide in a range of 0–80 mM in serum-free medium containing 1× PS for 24 h. Then, the cells were incubated at 37 °C in a humidified and sterile atmosphere containing 5% CO_2 for 24 and 48 h of time periods.

Overnight grown IMR-32 and SH-SY5Y cells were treated with Aβ(25–35) peptide at a concentration of 10 μM in serum-free medium containing 1× PS (Kaur et al. 2015b). Then, the cells were incubated at 37 °C in a humidified and sterile atmosphere containing 5% CO_2 for 24 h and 48 h time points.

Curcumin preparation and treatment

Curcumin is yellow colored powder (Sigma-Aldrich, USA) and solutions of curcumin were prepared by using 100% DMSO as main stock solution of 1 M, working stock of 100 μM was prepared in 20% DMSO in 1 × PBS and finally 10 μM concentration was used on to the cells in serum free media (Thapa et al. 2013).

Cell survivability assay

Cell survival is widely determined by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay (Dhiman et al. 2012; Kaur et al. 2015b). IMR-32 and SH-SY5Y cells at a density of 1 × 10^4 cells per well were seeded in 96-well plates for overnight at 37 °C in a humidified CO_2 incubator. Then the cells were treated with different oxidants Aβ(25–35), MCP, CP, and their combinations in different set of cells. The phytochemical curcumin pretreated at 10 μM for 4 h then treated with 10 μM Aβ(25–35); MCP and CP at 10 μM each; and Aβ(25–35) and MCP/CP (10 μM + 10 μM) in combinations for various time points (24 h and 48 h). The different set of treated cells after 24 h and 48 h were incubated with 0.5 mg/ml MTT for 4 h. The purple formazan crystal formed was solubilized with acidified DMSO (200 μl per well), and, absorbance was measured at 570 nm using microplate reader (BioTek® Synergy H1).

Measurement of intracellular ROS

IMR-32 and SH-SY5Y cells were seeded in 96 well plates at a density of 1 × 10^4 cells per well for 24 h before the treatment. Nitroblue tetrazolium (NBT) is a colored substance, a fresh solution of NBT prepared in 1 × PBS for 1 mg/ml concentration, and the cells after the treatment incubated with 200 μL NBT in 96 well plate for 2 h (Choi et al. 2006). After the incubation, the NBT solution was removed from the wells and then washed with 1 × PBS, and then the cells were fixed with chilled Methanol, and air dried. The NBT deposited inside the cells was then dissolved, first by adding 120 μL of

2 M KOH to solubilize cell membranes and then by adding 140 μL of DMSO to dissolve the blue formazan with gentle shaking for 10 min at room temperature. The absorbance was read on a microplate reader at 620 nm (BioTek® Synergy H1).

Western blot analysis

SH-SY5Y cells were seeded onto 100 mm dishes at a density of 1×10^6 cells per dish, and after 24 h, cells were treated with control (vehicle control), 10 μM Aβ(25–35), MCP, and Aβ(25–35) + MCP (10 μM + 10 μM) in combinations to see the additive effect of oxidative stress. Pretreatment of 10 μM curcumin for 4 h followed by treatment with 10 μM Aβ(25–35), MCP, and Aβ(25–35) + MCP (10 μM + 10 μM) in combinations for 24 h and 48 h time points. This was followed by preparation of whole cell lysates using whole cell lysis buffer containing [20 mM Tris-HCl (pH 7.4), 150 mM NaCl, and 1 mM EDTA] (Mantha et al. 2012). The whole cell lysates were then subjected to SDS-PAGE for separation of proteins. After transferring the proteins onto PVDF membranes for overnight at 30 V, then the membranes were blocked for 2 h with 5% nonfat dry milk (NFDM) in 50 mM Tris-HCl (pH 7.4) containing 150 mM NaCl and 0.05% Tween 20 (TBST). All antibody dilutions were made in 5% NFDM-TBST. The membranes were incubated with the following primary antibodies (Santa Cruz Biotechnology at 1:1000 dilution) APE1, Nrf2, NOS1, NOS2, Pol β, PARP1 in 5% NFDM for overnight, then washed three times for 15 min in TBST. The membranes were further incubated with respective secondary horseradish peroxidase (HRP)-conjugated rabbit or mouse antibodies (1:5000) in the 5% NFDM for 1 h, then washed three times in TBST for 15 min each. Peroxidase activity was detected using the Bio-Rad enhanced chemiluminescence substrate (ECL-Plus) using either Protein Simple Fluor Chem HD2™ (or) Bio-Rad Chemi Doc™ MP gel documentation systems (Mantha et al. 2012).

Immunofluorescence and confocal laser scanning microscopy analysis

To study the expression and protein-protein interaction in-situ, the immunofluorescence and confocal laser scanning microscopy analyses were performed. IMR-32 and SH-SY5Y cells were grown on glass coverslips in 6-well plates, and these cells were treated with 10 μM Aβ(25–35) and MCP and the combination of Aβ(25–35) + MCP (10 μM + 10 μM) for 24 h and 48 h time periods. After completion of treatment, cells were fixed in 2% formaldehyde (PFA) and then washed thrice with 1 × PBS. For the cell permeabilization, cells were kept in 0.1% Triton X-100 for 5 min and then washed three times in 1 × PBS. Then cells were fixed in 10% FBS with 1 × PBS and followed by washing with 1 × PBS. Primary antibodies diluted at 1:100 in 1 × PBS containing 10% FBS. Then the cells were incubated overnight with the primary antibodies APE1 (1:100; mouse monoclonal antibodies; Santa Cruz Biotechnology) and Nrf2 (1:100; rabbit polyclonal Ab; Santa Cruz Biotechnology). The glass coverslips were washed in 1 × PBS and incubated at room temperature for 1 h with respective secondary antibodies, Alexa Fluor 488-conjugated anti-mouse IgG or Alexa Fluor 647-conjugated anti-rabbit IgG (Invitrogen, Grand Island, NY) at 1:100 dilution in 1 × PBS containing 10% FBS and after three times of washing. The cells on coverslips were incubated at room temperature for 10 min in 1% solution of Hoechst 33342. The glass coverslips were washed with 1 × PBS and mounted with a mounting solution as per the protocol described earlier (Zhao et al. 2009). Images of the cells were captured using OLYMPUS FV1200 Laser Scanning Microscope at Central Instrumentation Laboratory (CIL) facility of CUPB. The colocalization of the two interacting proteins APE1 and Nrf2 was analyzed by calculating Pearson's Correlation Coefficient (PCC) using OLYMPUS FLUOVIEW software. If PCC value of two interacting proteins is in the range of 0.5 and 1.0, then it attributing to perfect colocalization, and if PCC value is in the range of −1.0 and 0 then negatively correlated and attributing to no colocalization and if PCC values is in the range of 0 to 0.5 signifies less correlation (Dunn et al. 2011).

Statistical analysis

The data is presented as the mean value of standard deviation (SD) and standard error (SE). Results were analyzed by paired Student's t-test for statistical valuation of mean values for experimental and control samples. $P < 0.05$ and $P < 0.005$ was considered statistically significant across the treatment groups.

Results

(i) *Determination of OPPs Induced Cytotoxicity in Human Neuroblstoma Cell Lines*

To examine the neurotoxic effects of MCP and CP on human neuroblastoma (SH-SY5Y and IMR-32) cells and to determine the optimal concentration for further experimentation, neuroblastoma cells were treated with broad concentration range of MCP (0.01 μM −1 mM) and CP (0.01 μM −1 mM). Cytotoxicity was determined by cell viability (MTT) assay. The neuroblastoma IMR-32 cells were treated with various concentrations as indicated above. At 48 h time point the cell viability decreased significantly in 0.1 μM, 1 μM, 10 μM, 50 μM, 100 μM, 500 μM and 1 mM concentrations of MCP ($p < 0.05$), while 100 μM treatment has shown 42% decrease in cell viability as compared to the control IMR-32 cells (Fig. 1a).

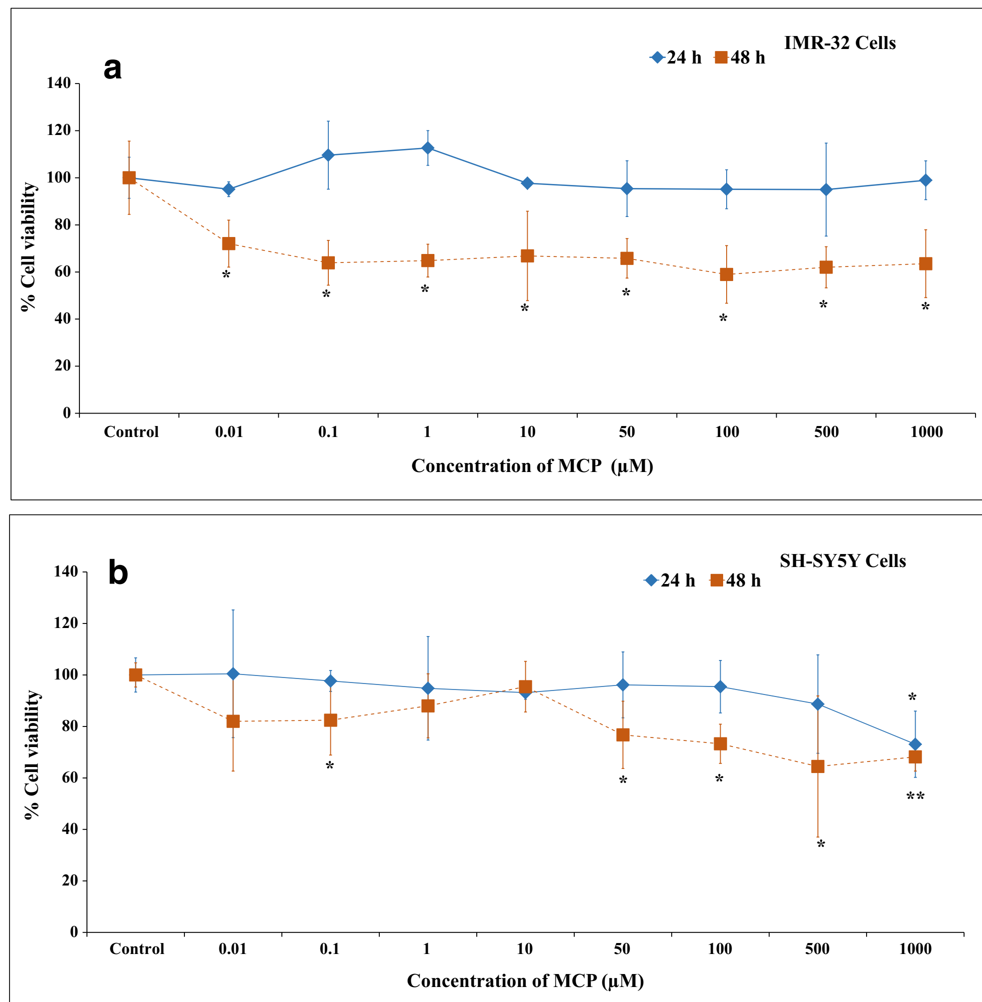

Fig. 1 The percent cell viability in human neuroblastoma (**a**) IMR-32 and (**b**) SH-SY5Y cells treated with various concentrations of Monocrotophos (MCP) for 24 h and 48 h time points. Data expressed as mean ± SD *(n = 3)*. *denotes a significance difference from the untreated respective control cells at * = $p < 0.05$ and ** = $p < 0.005$

In a similar treatment with MCP (0.01 μM −1 mM) in SH-SY5Y cells, the cell viability at 24 h in 1 mM concentration reduced by 27% as compared to the control cells, whereas no significant change in other concentrations was observed. At 48 h treatment the cell viability significantly decreased in 0.1 μM, 50 μM, 100 μM, 500 μM and 1 mM concentrations by 18%, 33%, 27%, 36% and 32%, respectively, as compared to the control SH-SY5Y cells ($p < 0.05$ and $p < 0.005$) (Fig. 1b).

In addition, IMR-32 and SH-SY5Y cells were also treated with another OPP, CP at a concentration range starting from 0.01 μM to 1 mM. In IMR-32 cells, the cell viability at 24 h treatment decreased upto 30% for 1 mM concentration of CP whereas no significant change was seen at lower concentrations as compared to the untreated control IMR-32 cells. At 48 h treatment, 10 μM, 25 μM and 50 μM concentrations were able to reduce the cell viability by 19%, 24%, and 37%, respectively, whereas, for other concentrations no significant change in cell viability was observed (Fig. 2a). In SH-SY5Y cells at 24 h treatment, concentrations such as 25 μM, and 50 μM; and higher concentrations 500 μM and 1 mM, the cell viability significantly decreased by 9% & 11%, and 13% & 12%, respectively. After 48 h treatment of CP at lower concentration 0.01 μM, the cell viability was decreased significantly by 41%, and the change in percent cell survival was moderate for the concentrations 0.1 μM to 5 μM. In addition, 48 h treatment of 100 μM, 500 μM, and 1 mM concentrations of CP to the SH-SY5Y cells, the cell viability was significantly reduced by 21%, 43%, and 51%, respectively, when compared with that of untreated control SH-SY5Y cells ($p < 0.05$ and $p < 0.005$) (Fig. 2b). From the results of CP and MCP treatment studies, the 10 μM concentration was found to be nontoxic, and literature also supports this optimal concentration for further experimentation (Tripathi et al. 2014).

(ii) *OPPs Augment Aβ(25–35) Induced Oxidative Stress in Human Neuroblastoma Cell Lines*

Oxidative stress responses synergistically induced by OPPs and Aβ(25–35) treatments were studied in SH-SY5Y and IMR-32 cells. NBT assay was performed for determination of internal ROS generated after exposure to MCP, CP and Aβ(25–35), along with the curcumin pretreatment. Aβ(25–35) below 10 μM concentration did not show any significant change in cell

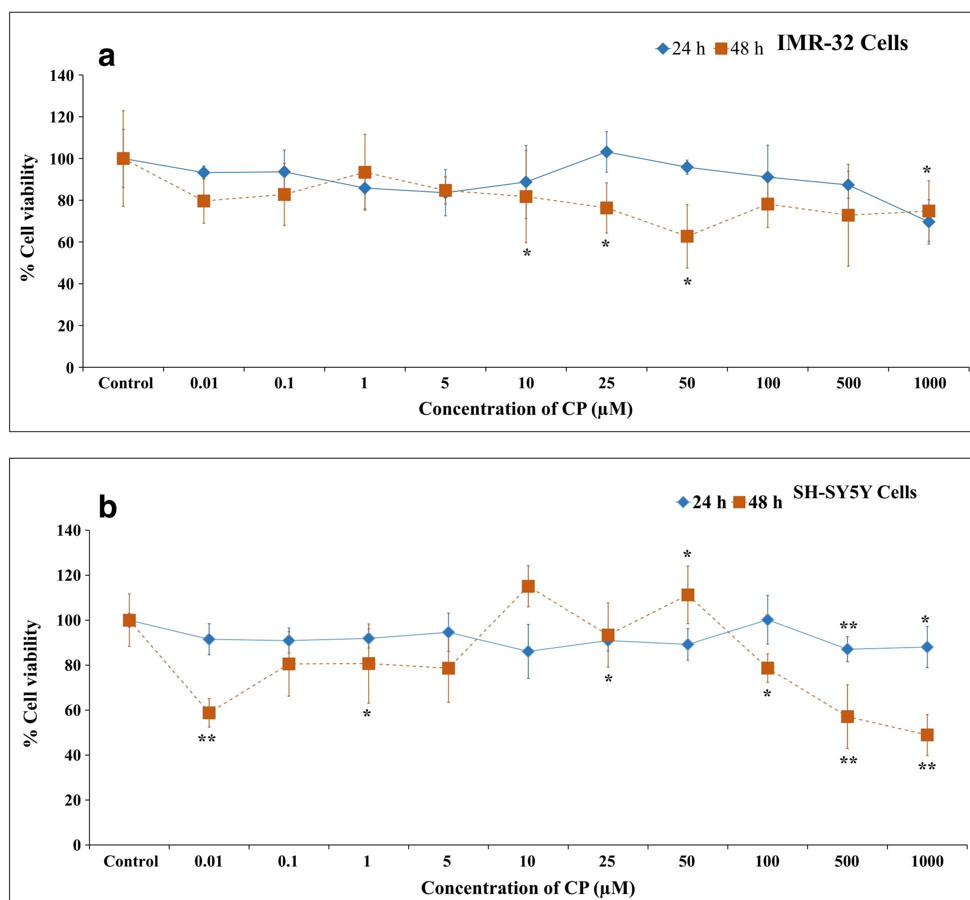

Fig. 2 The percent cell viability in human neuroblastoma (**a**) IMR-32 and (**b**) SH-SY5Y cells treated with various concentrations of Chlorpyrifos (CP) for 24 h and 48 h time points. Data expressed as mean ± SD of experiments *(n = 3)*. *denotes a significance difference from the untreated respective control cells at * = $p < 0.05$ and ** = $p < 0.005$

viability in either SH-SY5Y or IMR-32 cell lines (data not shown). From the literature, 10 μM Aβ(25–35) concentration was chosen as the optimal concentration for further experimentation (Kaur et al. 2015b; Mantha et al. 2012). The nontoxic concentration of 10 μM for both MCP and CP was used for treatment of IMR-32 and SH-SY5Y cells. After pretreatment with the curcumin at 10 μM for 4 h, individual treatments of 10 μM Aβ(25–35) and MCP/CP at 10 μM and combined treatment of Aβ(25–35) and MCP/CP at 10 μM was given for 24 h and 48 h time points.

In IMR-32 cells ROS level was increased after 24 h treatment of Aβ(25–35) and Aβ(25–35) + MCP by 36% and 32%, respectively, as compared to control cells. Whereas, ROS level was increased by 51% and 28% in Aβ(25–35) and MCP treatment alone, while ≈1.4 fold increase was observed in Aβ(25–35) + MCP treatment as compared to control cells after 48 h treatment. Curcumin has been reported to have anti-oxidative, anti-inflammatory as well as neuroprotective properties (Mishra and Palanivelu 2008). Curcumin has been used in many studies where it showed the ameliorative effect against Aβ induced oxidative stress (Lim et al. 2001; Ono et al. 2004). In the present study we have used 10 μM concentration of curcumin to check its neuroprotective efficiency in both Aβ and OPPs induced oxidative stress, which is supported by the literature (Daval et al. 2010; Fan et al. 2017). Pretreatment with curcumin alone decreased the ROS levels in IMR-32 cells by 10% as compared to control cells after 24 h treatment; similarly curcumin pretreatment followed by MCP treatment also reduced ROS by 10% as compared to control IMR-32 cells (Fig. 3a). In SH-SY5Y cells, Aβ(25–35), MCP, and Aβ(25–35) + MCP treatment for 24 h induced ROS level by 16%, 17% and 38%, while curcumin pretreatment reduced ROS level by 18%. For 48 h, curcumin pretreatment followed by Aβ(25–35) and MCP treatment resulted in ROS level to be reduced by 24% and 18%, respectively, as compared to the control SH-SY5Y cells (Fig. 3b).

In IMR-32 cells, Aβ(25–35) + CP treatment at 10 μM each enhanced ROS levels by 25% as compared to control cells (Fig. 4a). While in SH-SY5Y cells, Aβ(25–35) treatment in both 24 h and 48 h time points increased ROS level by 23% and 22%, respectively. The Aβ(25–35) + CP treatment after 24 h as well as 48 h enhanced ROS levels significantly by 32% and 36%, respectively, as compared to control cells. While curcumin pretreatment alone after 48 h of treatment reduced ROS level significantly by 16% as compared to control cells (Fig. 4b). These results indicate that curcumin relieves neuroblastoma cells from the oxidative burden caused by OPPs and Aβ(25–35)-induced oxidative stress.

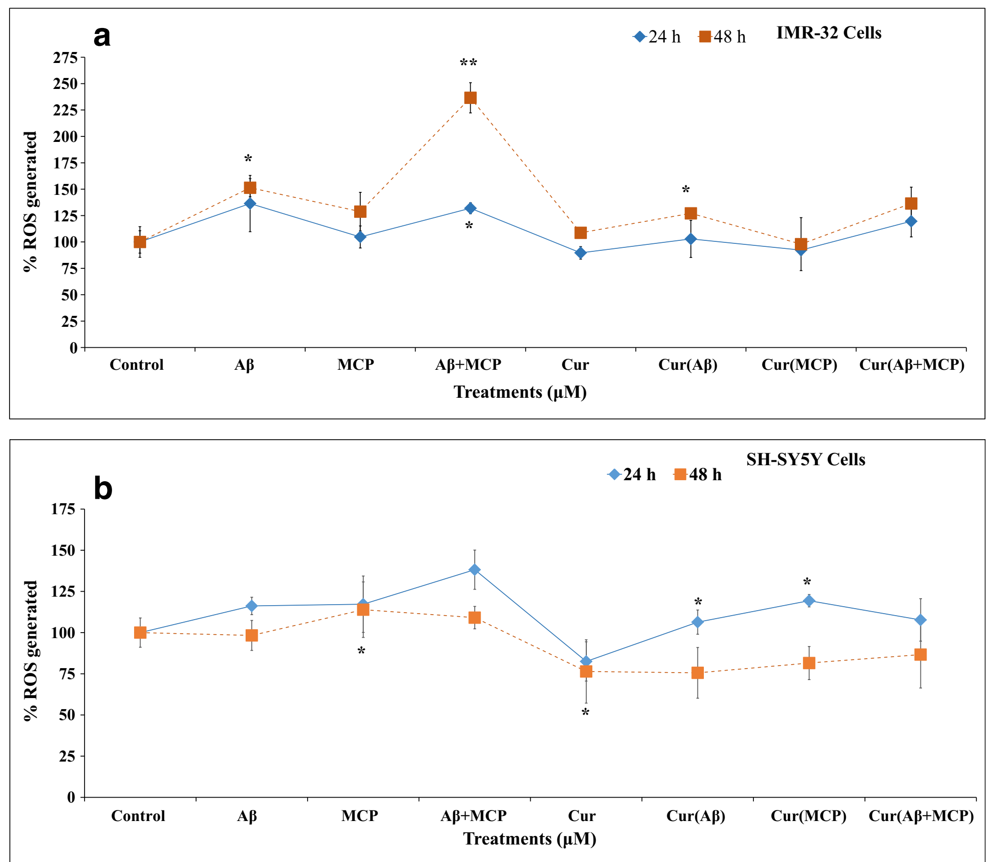

Fig. 3 Analysis of intracellular ROS generated upon treatment with MCP in (**a**) IMR-32, and (**b**) SH-SY5Y cells by NBT assay for 24 h and 48 h time points. Cells were treated with Aβ(25–35) at a concentration of 10 μM and MCP at a concentration of 10 μM and also in combination of these two oxidants Aβ(25–35) and MCP (10 μM + 10 μM). Phytochemical pretreatment with curcumin (10 μM) and followed by the treatment with Aβ(25–35), MCP and their combinations displayed the ameliorating effect of curcumin against Aβ(25–35) and MCP neurotoxicity. Data expressed as mean ± SD of experiments *(n = 3)*. *denotes a significance difference from the untreated respective control cells at * = $p < 0.05$ and ** = $p < 0.005$

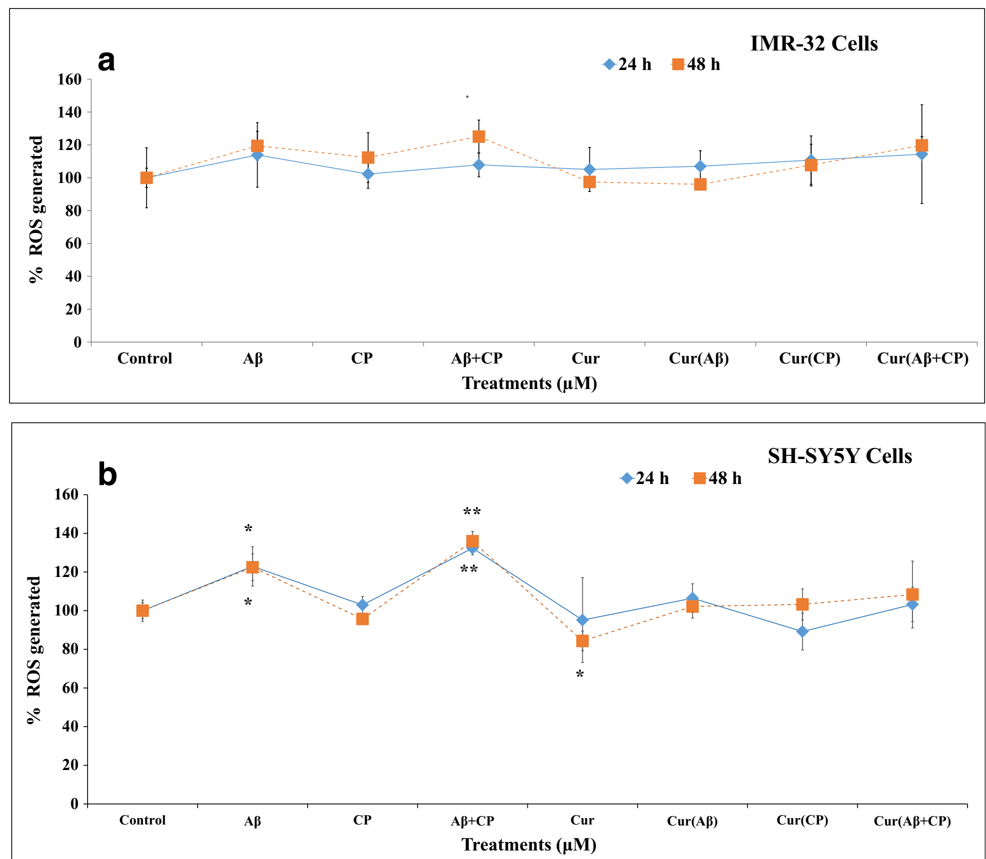

Fig. 4 Analysis of intracellular ROS generated upon treatment with CP in (**a**) IMR-32, and (**b**) SH-SY5Y cells by NBT assay for 24 h and 48 h time points. Cells were treated with Aβ(25–35) at a concentration of 10 μM and CP at a concentration of 10 μM and also in combination of these two oxidants Aβ(25–35) and CP (10 μM + 10 μM). Phytochemical pretreatment with curcumin (10 μM) and followed by the treatment with Aβ(25–35), CP and their combinations displayed the ameliorating effect of curcumin against Aβ(25–35) and CP neurotoxicity. Data expressed as mean ± SD of experiments *(n = 3)*. *denotes a significance difference from the untreated respective control cells at * = $p < 0.05$ and ** = $p < 0.005$

(iii) *Analysis of Protein Expression upon Aβ(25–35) and OPPs Induced Neurotoxicity and the Phytochemical Modulation by Curcumin Pretreatment*

To know more about the role of OPPs and Aβ(25–35) induced oxidative stress at the molecular level, we analyzed the expression of regulatory proteins: (i) neuronal nitric oxide synthase (nNOS/NOS1); (ii) inducible nitric oxide synthase (iNOS/NOS2); and (iii) Nuclear factor erythroid factor 2-related factor 2 (Nrf2); and also the key DNA repair proteins: (iv) PARP1; (v) DNA pol β; and (vi) APE1 in human neuroblastoma SH-SY5Y cells.

a) *Expression of Regulatory Proteins*

Expression of different proteins like nNOS and iNOS has been reported to play significant role during the oxidative stress in AD pathology. In SH-SY5Y cells the NOS1 expression level decreased in Aβ(25–35), MCP + Aβ(25–35), and MCP treatments. Pretreatment of curcumin alone restored the basal level of nNOS as comapred to the control cells. While pretreatment of curcumin followed by Aβ(25–35), MCP and MCP + Aβ(25–35) treatments further decreased NOS1 expression after 24 h and 48 h time points as compare to control SH-SY5Y cells (Fig. 5a and b).

iNOS expression was found to be increased significantly in Aβ(25–35), MCP, and Aβ(25–35) + MCP treatments at 48 h time point, while no change was observed in 24 h treatment time point. In 48 h Aβ(25–35) + MCP treatment, iNOS expression increased by 2.5 fold as compared to control SH-SY5Y cells. Curcumin pretreatment alone reduced the iNOS expression significantly after 24 h treatment time point. In curcumin pretreatment, followed by Aβ(25–35), MCP and Aβ(25–35) + MCP treatments, the NOS2 level decreased at 24 h time point (Fig. 5a and c).

Nrf2 expression was downregulated in Aβ(25–35) treatment in both the time points. While in MCP and Aβ(25–35) + MCP treatments, Nrf2 expression reduced in 24 h time point. In curcumin treated cells, Nrf2 protein expression was found to be upregulated by 1.4 folds as compared to the control cells at 48 h. In curcumin pretreatment followed by Aβ(25–35) treatment, Nrf2 expression decreased in 24 h treatment and increased its expression after 48 h of treatment time point. In curcumin pretreatment followed by MCP and Aβ(25–35) + MCP treatments, the Nrf2 expression was found to be downregulated (Fig. 5a and d). It can be said that curcumin pre-treatment may activate the Nrf2 protein and translocate to the nucleus to activate ARE-pathway. Further to check this, we have also performed the immunofluorescence confocal laser scanning microscopy studies of Nrf2 and APE1 proteins in Aβ(25–35) and curcumin treated SH-SY5Ycells.

b) *Expression of DNA Repair Proteins*

BER-pathway plays a pivotal role in many disease conditions and especially in neuronal cells which are differentiated cells. Thus alteration, dysfunction, and any other change would directly associate with the efficacy of the BER pathway and is correlated with neuronal cell survival.

In the present study the expression of PARP1 was found to be changed moderately after Aβ(25–35) treatment for 24 h and 48 h time points as compared to control SH-SY5Y cells. At 24 h MCP treatment, PARP1 expression upregulated significantly, while decrease in expression was observed for 48 h treatment. In Aβ(25–35) + MCP treatment, PARP1 expression was downregulated significantly for 48 h time point. Curcumin pretreatment upregulated the PARP1 expression for 24 h treatment, while in curcumin pretreatment followed by Aβ(25–35) treatment, the protein expression was found to be upregulated only at 24 h time point. In curcumin pretreatment followed by MCP, and Aβ(25–35) + MCP, PARP1 expression decreased after 48 h as compared to untreated control SH-SY5Y cells (Fig. 6a and b).

DNA pol β protein expression was reduced after 24 h treatment of Aβ(25–35) and MCP, while in 48 h treatment, the expression of pol β was found to be increased. In Aβ(25–35) + MCP treatment for 24 h and 48 h time points, the protein level increased as compared to control SH-SY5Y cells. Curcumin pretreatment increased pol β level in 24 h treatment, but decreased in 48 h treatment. Similarly pol β level was found to be increased after 24 h treatment but decreased in 48 h in pretreatment of curcumin followed by Aβ(25–35) treatment of SH-SY5Y cells. Curcumin pretreatment followed by MCP treatment for 24 h and 48 h time points, the pol β expression was found to be increased significantly. In curcumin pretreatment followed by Aβ(25–35) + MCP treatment the pol β level downregulated moderately in both the time points studied in SH-SY5Y cells as compared to untreated control cells (Fig. 6a and c).

DNA repair protein APE1 expression was found to be downregulated in Aβ(25–35), MCP and Aβ(25–35) + MCP treatments in both 24 h and 48 h time points in SH-SY5Y cells. While curcumin pretreatment upregulated APE1's expression after 48 h treatment. In curcumin pretreatment followed by Aβ(25–35) treatment, APE1 expression upregulated in 24 h and 48 h time points. Similarly, curcumin pretreatment followed by MCP, and Aβ(25–35) + MCP treatments, APE1 protein expression was found to be downregulated after 24 h treatment; whereas at 48 h curcumin pretreatment followed by MCP treatment, APE1 expression upregulated significantly as compared to the control SH-SY5Y cells.

(iv) *Nuclear Translocation of Nrf2 and APE1 in Response to Aβ(25–35) Induced Neurotoxicity and Treatment of Phytochemical Curcumin*

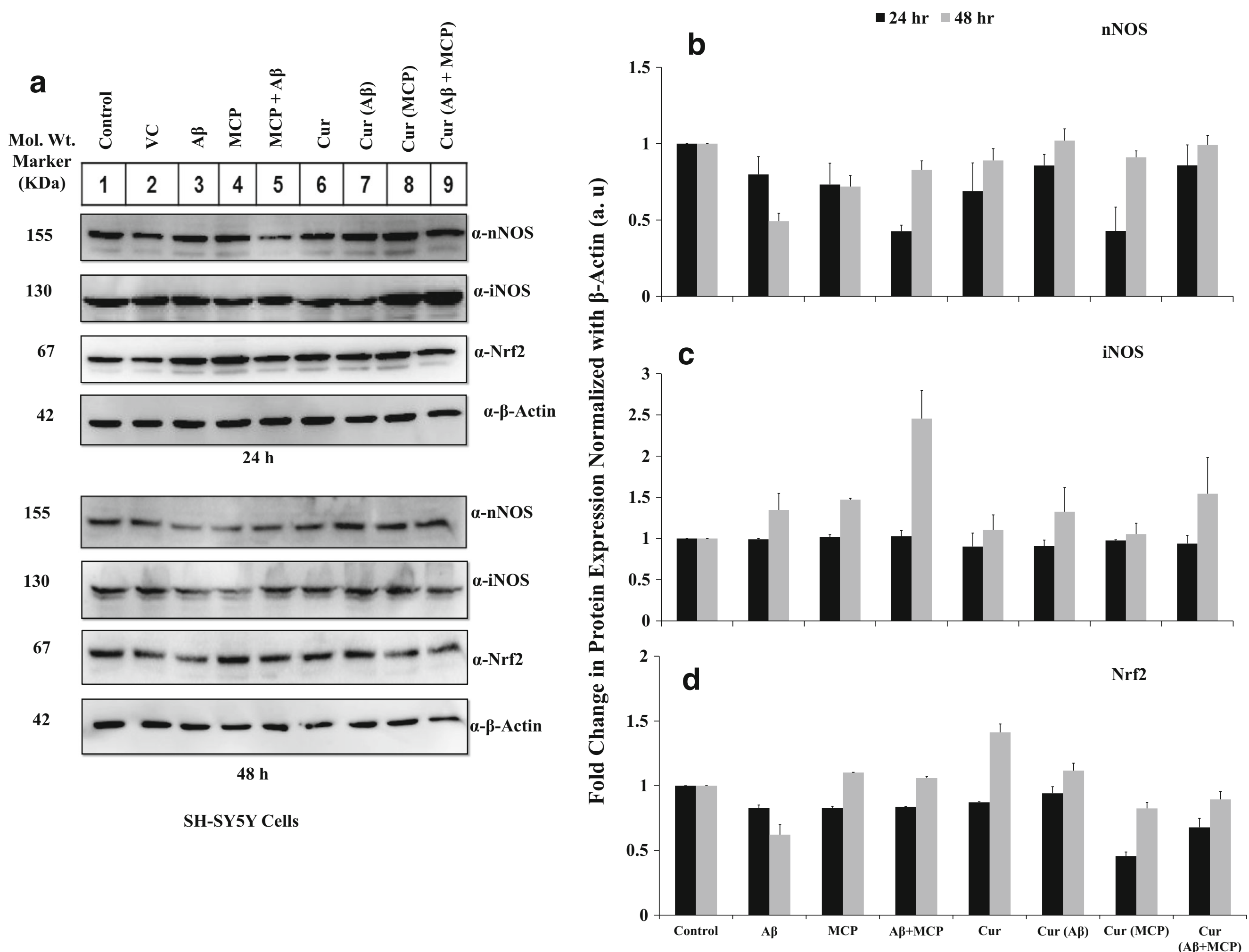

Fig. 5 Western blot analysis of iNOS, nNOS, and Nrf2 proteins expression in SH-SY5Y cells after 24 h and 48 h time points. (**a**) Western blot images showing levels of iNOS, nNOS, and Nrf2 in control, vehicle control (VC), 10 μM Aβ(25–35), 10 μM MCP, Aβ(25–35) + MCP (10 μM each), curcumin 10 μM, Cur + Aβ(25–35), Cur + MCP and Cur + MCP + Aβ(25–35) treated SH-SY5Y cells. Densitometric analysis of protein expression as compared to β-Actin of iNOS, nNOS, and Nrf2 are presented as **b**, **c**, and **d**, respectively. The histograms represent the mean values with standard error for two independent experiments

Nrf2 and APE1 expression and their nuclear translocation (subcellular distribution) upon oxidative stress induced by the treatment of 10 μM Aβ(25–35) and modulation by the treatment of 10 μM curcumin on neuroblastoma IMR-32 and SH-SY5Y cells was determined using immunoreactivity using specific antibodies by confocal laser scanning microscopy. The treatment of Aβ(25–35) at 10 μM concentration for 24 h and 48 h time points in both human neuroblastoma IMR-32 and SH-SY5Y cell lines showed increased expression of APE1 (in green color) in the nuclear compartment as compared to untreated control cells. APE1 also showed foci formation in the nuclear compartment as compared to the untreated control IMR-32 and SH-SY5Y cells at both the time (24 h and 48 h) points studied. The distribution of Nrf2 was predominantly to the cytoplasmic compartment in the control IMR-32 and SH-SY5Y cells, upon Aβ(25–35) induced oxidative stress, both the cell lines showed Nrf2 activation/upregulation in the cytosolic as well as in the nuclear compartments, predominant effect was observed after 48 h time point (Figs. 7a, b, 8a and b). In addition, the treatment of curcumin significantly induced nuclear localization of APE1 (in green color) and Nrf2 (in red color) as analyzed by immunofluorescent stained IMR-32 cells at 24 h and 48 h time points (Fig. 7a and b). In a similar experiment on SH-SY5Y cells, treatment with curcumin induced the nuclear migration of Nrf2 and APE1 in the SH-SY5Y cells as compared to untreated control cells (Fig. 8a and b).

(v) *Analysis of Protein-protein Interaction between APE1 and Nrf2*

To check APE1-Nrf2 interaction endogenously as well as during oxidative stress, IMR-32 and SH-SY5Y cells were treated with 10 μM Aβ(25–35) and 10 μM MCP alone, and Aβ(25–35) and MCP 10 μM each, and pretreatment of 10 μM

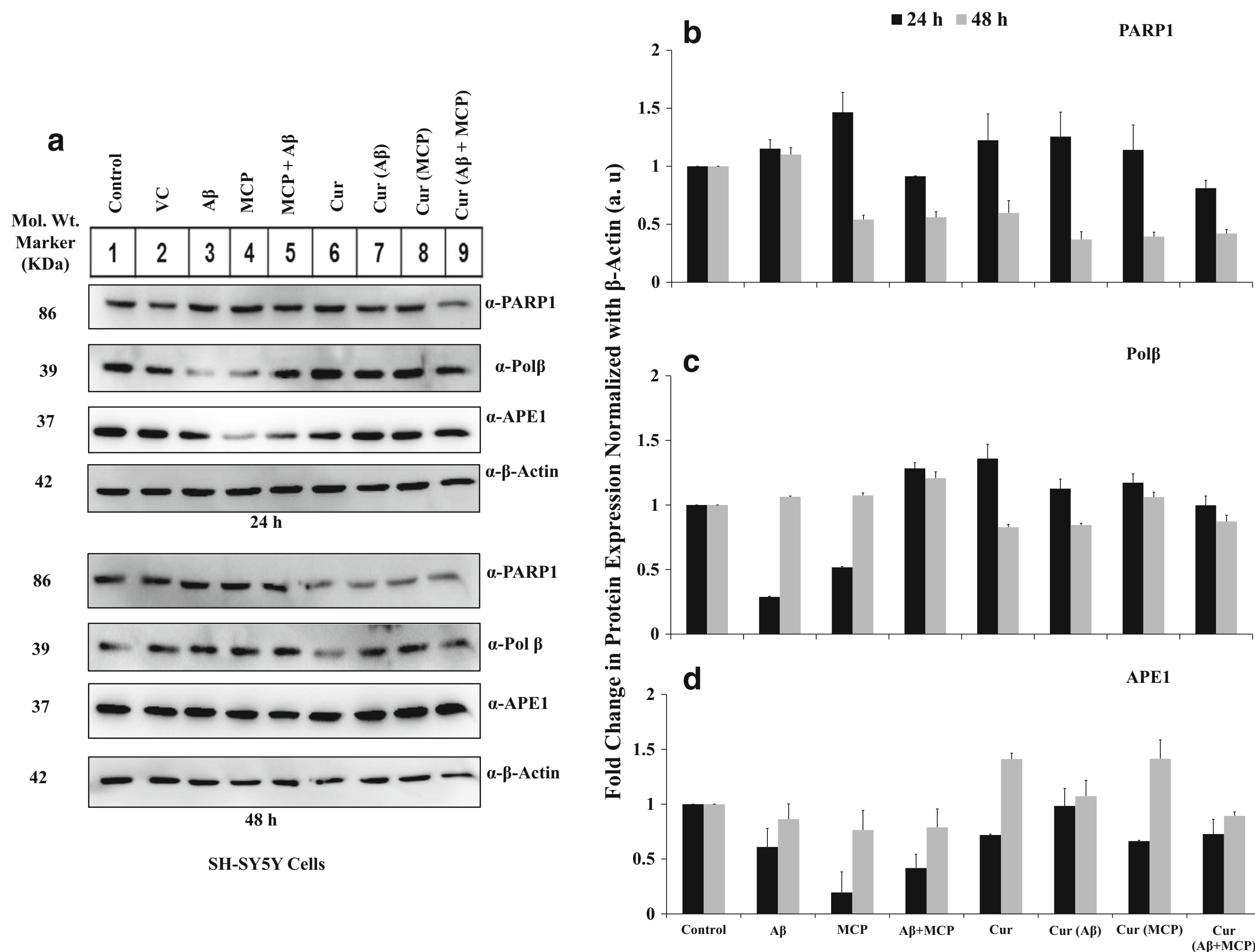

Fig. 6 Western blot analysis of PARP1, Pol β, and APE1 proteins expression in SH-SY5Y cells after 24 h and 48 h time points. (**a**) Western blot images showing PARP1, Pol β, and APE1 in control, vehicle control (VC), 10 μM Aβ(25–35), 10 μM MCP, Aβ(25–35) + MCP (10 μM each), curcumin 10 μM, Cur + Aβ(25–35), Cur + MCP and Cur + MCP + Aβ(25–35) treated SH-SY5Y cells. Densitometric analysis of protein expression as compared to β-Actin of PARP1, Pol β, and APE1 are presented as **b**, **c**, and **d**, respectively. The histograms represent the mean values with standard error for two independent experiments

curcumin for 4 h followed by the treatment of Aβ(25–35), MCP and Aβ(25–35) + MCP. After treatment for 24 h and 48 h, APE1 was tagged with Alexa Fluor 488 with green color, Nrf2 was tagged with Alexa Fluor 647 with red color and nucleus was stained Hoechst 33342 with blue color. In this experiment, we found Nrf2 to be localized predominantly in the cytoplasm in control IMR-32 cells. Stimulation of cells by oxidants Aβ(25–35), MCP, and Aβ(25–35) + MCP resulted in its nuclear localization and association with APE1. The PCC values are 0.114, 0.240, 0.127, and 0.231, respectively, in 24 h treatment point and −0.114, 0.34, and −0.22, respectively, in 48 h treatment point (Fig. 9(i) a, b, c, d, & e). Nuclear migration and association of Nrf2 with APE1 was observed with the curcumin treatment (PCC values being 0.263 and 0.401 for 24 h and 48 h time points, respectively). The colocalization of APE1 and Nrf2 was seen to be predominant in curcumin treatment, and also after the pretreatment of curcumin followed by the Aβ(25–35) and MCP treatments with observed PCC values being 0.192 and 0.28 for 24 h, and 0.383 and 0.398 for 48 h (Fig. 9(i) f, g, h, & i).

In an another experiment, using SH-SY5Y cells, the Nrf2 and APE1 expression was seen in the cytoplasm in control cells with PCC values being 0.015 and 0.153 for 24 h and 48 h time points. The pretreatment of curcumin resulted in colocalization of APE1 and Nrf2 proteins with PCC values being 0.115 and 0.253, respectively, for 24 h and 48 h time points (Fig. 9(ii) f). The curcumin pretreatment followed by Aβ(25–35) and MCP treatment in SH-SY5Y cells also resulted in colocalization of APE1 and Nrf2 proteins in the nucleus after 24 h and 48 h curcumin pretreatment followed by Aβ(25–35) + MCP treatment after 48 h time points (Fig. 9(ii) f, g, h, & i). In both the experiments one can observe that APE1 and Nrf2 gets localized to the nucleus after the curcumin treatment (Fig. 9(i) & (ii)). Due to the oxidative stress, Nrf2 and APE1 levels increased in

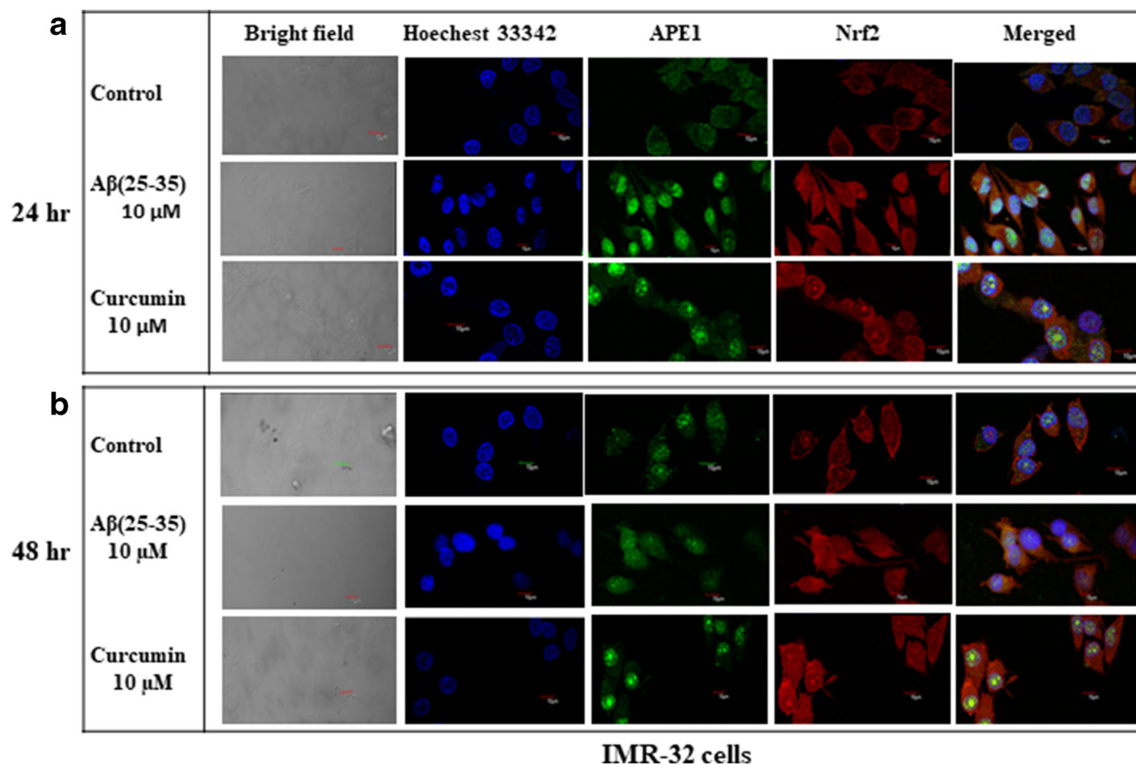

Fig. 7 Immunofluorescence study of APE1 and Nrf2 proteins and their colocalization in IMR-32 cells after the treatment of Aβ(25–35) and curcumin. The human neuroblastoma IMR-32 cells were treated with 10 µM Aβ(25–35) and 10 µM curcumin for (**a**) 24 h and (**b**) 48 h time points. In all the images first panel contains bright field images, then counterstaining with Hoechst 33,342, then followed by the immunofluorescence staining of APE1 and Nrf2, and finally merged the images

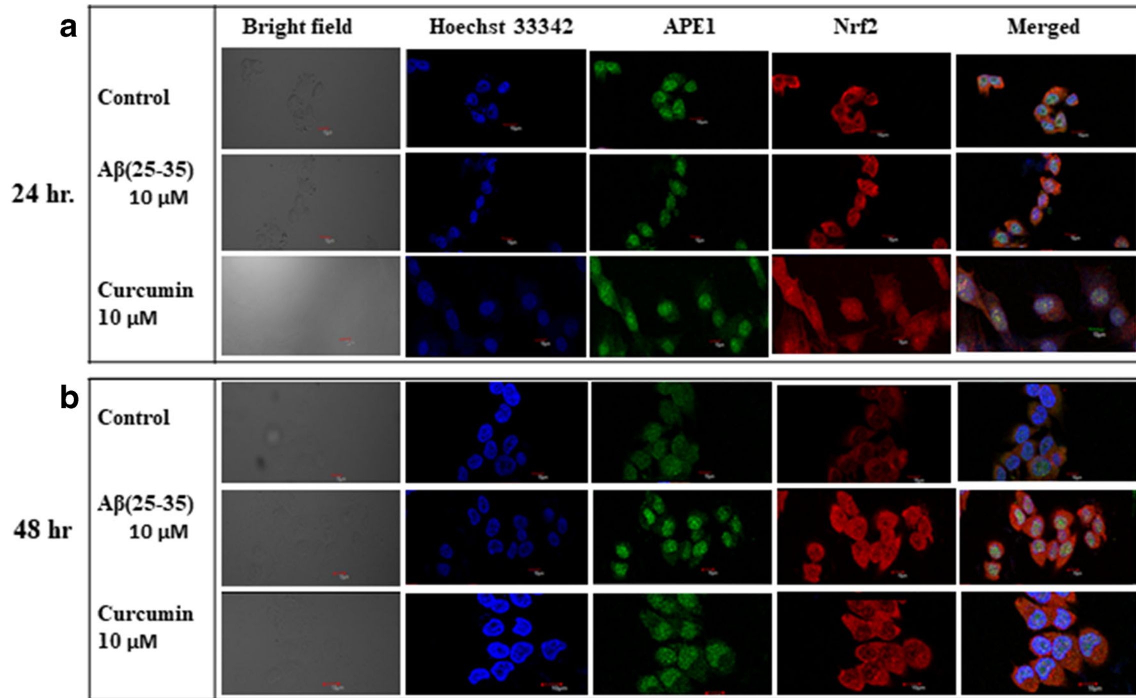

Fig. 8 Immunofluorescence study of APE1 and Nrf2 proteins and their colocalization in SH-SY5Y cells after the treatment of Aβ(25–35) and curcumin. The human neuroblastoma SH-SY5Y cells were treated with 10 µM Aβ(25–35) and 10 µM curcumin for (**a**) 24 h and (**b**) 48 h time points. In the entire images first panel contains bright field images, then counterstaining with Hoechst 33,342, followed by the immunofluorescence staining of APE1 and Nrf2, and finally merged the images

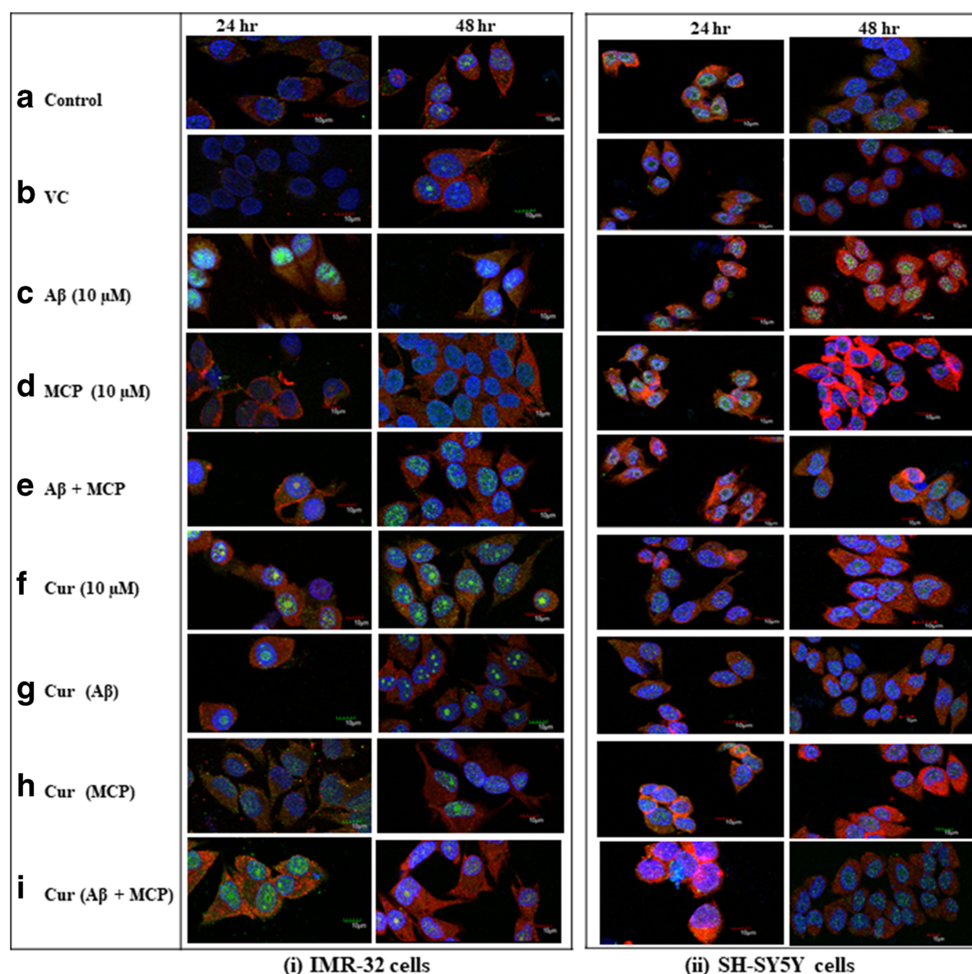

Fig. 9 A comprehensive immunofluorescence study of APE1 and Nrf2 proteins and their colocalization after the treatment of Aβ(25–35), MCP and pretreatment of curcumin followed by Aβ(25–35) and MCP treatment for 24 h and 48 h time points. (i) IMR-32 cells and (ii) SH-SY5Y cells were treated with Aβ(25–35) at 10 μM and MCP at 10 μM and Aβ(25–35) + MCP (10 μM + 10 μM) in combination to see the synergistic effect. Curcumin pretreated IMR-32 cells at 10 μM for 4 h followed by 10 μM Aβ(25–35) for different (24 h and 48 h) time points, and 10 μM MCP and Aβ(25–35) and MCP (10 μM + 10 μM) in combinations were studied for their immunoreactivity to see the colocalization of Nrf2 and APE1 proteins

MCP, and Aβ(25–35) treated cells, but their nuclear migration started with the pretreatment of curcumin, which signifies for the anti-oxidative potential of curcumin against oxidative stress (Fig. 9(i) & (ii)).

Discussion

The role of Aβ deposition in AD is a well-known fact, but the real molecular events which are triggering the Aβ accumulation and disease progression are not exactly apparent so far. The Aβ accumulation triggers severe oxidative stress to the neuronal cells (Butterfield et al. 2013). Environmental factors play a pivotal role in the development and progression of many diseases, one of such environmental factors is pesticides (Kamel and Hoppin 2004). In AD, pesticides play a significant role in disease development and progression (Richardson et al. 2014). In the present study, the synergistic role of Aβ and OPPs in AD pathology using neuroblastoma cell lines has been investigated. The soluble oligomeric form of Aβ is more toxic than non-soluble fibrillar form and play main cause for AD (Sengupta et al. 2016). In the present study treatment of freshly prepared Aβ(25–35) caused severe oxidative stress in both SH-SY5Y and IMR-32 neuroblastoma cells. In a study by Wang et al., it was shown that 25 μM Aβ(25–35) treatment induced significant ROS generation in SH-SY5Y cells (Wang et al. 2016). MCP and CP are neurotoxic and also reported to induce oxidative stress by elevating ROS levels (Janssens and Stoks 2017; Kumar et al. 2013). In the present study, ROS levels increased significantly when cells were treated with Aβ(25–35) in combination with MCP or CP, this increase in ROS signifies synergistic activity of Aβ and pro-oxidants MCP or CP (Garcia-Matas et al. 2010). Curcumin is a potent scavenger of ROS and neuroprotective in nature, and pretreatment of curcumin prevents ROS production. In the present study, ROS generation was checked by pretreatment of curcumin for 4 h, followed by Aβ(25–35) and MCP/CP treatments which reduced the production of ROS. A study by Ramkumar and group reported that pretreatment of demethoxycurcumin, a derivative of curcumin significantly decreased the levels of ROS (Ramkumar et al. 2017). Curcumin at lower concentrations also inhibits Aβ fibril formation, as well as oligomerization (Yang et al. 2005). Pretreatment of 20 μM curcumin for 0, 1, 3, 6 and 12 h, followed by 24 h co-

incubation of 10 μM Aβ improved cell survivability of PC-12 cells as compared to non-treated cells (Fan et al. 2017). Even though many beneficial effects of curcumin have been reported, its therapeutic value is limited due to its poor absorption, bio distribution, metabolism, and bioavailability (Prasad et al. 2014; Yang et al. 2007). Phase 1, phase 2 and phase 2a trials have shown rapid clearance of curcumin from the body and very limited serum concentrations (Carroll et al. 2011; Cheng et al. 2001; Dhillon et al. 2008). To increase the bioavailability of different curcumin formulations have been made. Curcumin encapsulated solid lipid nanoparticles (CSLNs) have shown improved oral bioavailability, and it showed neuroprotective efficacy against 3-NP-induced neurotoxicity in case of experimental model of Huntington's disease, HD (Sandhir et al. 2014). In the present study, 10 μM curcumin +10 μM Aβ(25–35) treatment reduced the ROS production as compared to control cells. Further, pretreatment of curcumin + MCP/CP treatment, and combinational treatment of curcumin + MCP/CP + Aβ(25–35) also reduced the production of ROS after 24 h and 48 h time points studied.

The cellular system during oxidative stress regulates expression of various proteins, like activation of APE1 and Nrf2 proteins and translocation of these proteins to the nucleus for further activation of other proteins (Antoniali et al. 2014; Zhang et al. 2015). The Nrf2 protein acts as switch module of the antioxidant system, and during oxidative stress, it activates the antioxidant system via ARE-pathway (Zhang 2006). Nrf2 down-regulation was well documented, and it was implicated in the progression of many diseases. Nrf2 is a cytoplasmic protein and makes a complex with Kelch-like ECH-associated protein 1 (Keap-1), but upon oxidative stress, it gets translocated to the nucleus to activate ARE-dependent gene expression (Ma 2013; Zhang 2006). APE1 is a multifunctional protein, which is mainly responsible for oxidized DNA base damage repair, and it also regulates various proteins by its redox activity, as reviewed extensively else where [AP-1, NF-κB, p53 and Nrf2] (Fishel et al. 2015; Hegde et al. 2012; Mantha et al. 2014). In recent years APE1 and Nrf2 relationship has been studied by many researchers (Fishel et al. 2015; Shan et al. 2015). The present study also highlights APE1-Nrf2 interaction during oxidative stress. The Aβ(25–35) treatment significantly suppressed the Nrf2 expression. Furthermore, curcumin treatment upregulated Nrf2 protein expression, while curcumin pretreatment followed by Aβ(25–35) treatment also restored Nrf2 protein level to basal expression in SH-SY5Y cells. The previous study by Liu and group demonstrated similar results, where Aβ(25–35) treatment suppressed Nrf2 expression while lutein maintained the Nrf2 expression level and also induced its nuclear translocation (Liu et al. 2017). A study by Wang et al., showed that using 20 μM pinocembrin enhanced nuclear Nrf2 level and provides knowledge how phytochemicals relive cells from the oxidative stress via Nrf2 mediated pathway (Wang et al. 2016). The nuclear translocation of Nrf2 was seen in brain capillary endothelial (bEND.3) cells after the treatment of phytochemical lutein. A similar result was observed in our study upon curcumin treatment in SH-SY5Y as well as IMR-32 cells (Liu et al. 2017). APE1 plays a central role in the cellular responses to oxidative stress, and many attempts have been made to link increased levels of APE1 with protection in neuronal cells. Earlier it was found that APE1 level was reduced in $AlCl_3$ treated rats at both mRNA level and protein level, while phytochemical resveratrol pretreatment restored the level of APE1 in adult Wistar rats (Zaky et al. 2013). In the present study APE1 level was found to be reduced in Aβ(25–35), MCP, and Aβ(25–35) + MCP-treated SH-SY5Y cells, while in curcumin pretreated SH-SY5Y cells, APE1 protein level upregulated significantly as determined by Western blot analysis at various time points studied. Our observation is in accord with previous study done by Tan et al., which showed that 5 μM concentration of Aβ(1–42) caused a reduction in APE1 expression leading to neuronal cell death, whereas lower concentrations (0.5–2.5 μM) of Aβ(1–42) transiently induced APE1 expression and associated with neuronal survival (Tan et al. 2009). In a study by Kaur et al., the mitochondrial APE1 level was found to be reduced after 20 μM Aβ(25–35) treatment in SH-SY5Y cells, whereas phytochemical Ginkgolide B (GB) pretreatment restored APE1 level to a normal state as compared to control cells and additionally it was also demonstrated that, overexpression of APE1 also protects the SH-SY5Y cells from Aβ-induced oxidative stress (Kaur et al. 2015b).

The relationship between AD and nitric oxide (NO) has been widely reported, remarking on the neurotoxic effects of NO as induced by extracellular Aβ species (Balez and Ooi 2016; Law et al. 2001). The Aβ deposition does not contribute to NOS mediated NO release and subsequent release of superoxide radicals in between wild-type and transgenic mice expressing mutant forms of APP associated with familial AD and exhibiting high production of Aβ (Lahiri et al. 2003). Another study reported that Aβ(25–35) treatment enhanced expression of inducible NOS/NOS2 mRNA in C6 cells (Venturini et al. 2002). Aβ treatment induced NOS2 level in Sprague-Dawley rats, which was normalized by resveratrol treatment (Huang et al. 2011). In our study, the NOS2 level was upregulated in Aβ(25–35) + MCP treatment, while curcumin pretreatment normalized the NOS2 level in SH-SY5Y cells. We also found that, there is a reduction in neuronal nitric oxide synthase (NOS1) expression after Aβ(25–35), MCP, and Aβ(25–35) + MCP treatments. The pretreatment of curcumin in neuroblastoma cells upregulated the NOS1 expression (not to a significant level). A study on retinal neuronal culture showed that both Aβ and glutamate decreased NO production in the absence of neuronal death. These findings also suggest that NO overproduction does not play a significant role in the Aβ-induced death of retinal neurons (Oliveira

et al. 2011). Thus NO plays a dual role in AD, on the one hand, it acts as a neuroprotective agent while on the contrary, it act as neurotoxic agent.

BER-pathway plays the central role in neuronal DNA damage repair process, with APE1 being one of the key enzymes in BER-pathway, the DNA repair system. Oxidative base damage to DNA and other macromolecules has been reported to be increased in the brain of patients with AD (Huang et al. 2010; Love et al. 1999). Studies indicate that overexpression of the DNA repair protein PARP1 in response to oxidative damage to DNA can cause cell death due to depletion of NAD^+ (Alano et al. 2010). A study by Bayrakdar et al., showed that Aβ(1–42) treatment elevated PARP1 level, while nicotinamide (NAD) treatment normalizes the PARP1 level (Bayrakdar et al. 2014). In the present study, Aβ(25–35) treatment elevated PARP1 at 24 h and 48 h time points, while MCP treatment after 24 h enhanced PARP1 levels and curcumin treatment lowered the PARP1 expression. Huang et al., showed that overexpression of phosphorylated $APE1^{T232E}$ does not protect neurons against Aβ toxicity. The increased phosphorylated APE1 was observed in postmortem brain samples of patients with Parkinson's disease (PD) and AD, suggesting a link between APE1 phosphorylation and the pathogenesis of neurodegenerative disease (Huang et al. 2010). In AD patients, pol β is the rate limiting enzyme for DNA repair via BER-pathway (Sykora et al. 2015; Weissman et al. 2007). A study by Sykora et al., showed that 3xTgAD mice there is a 50% reduction in pol β expression, these haploinsufficient mice, which showed deficits in memory retention and also DNA repair ability (Sykora et al. 2015). Levels of Aβ accumulation in the olfactory bulb were similar in 3xTgAD and 3xTgAD/pol β+/− mice, and the cultured pol β deficient neurons exhibited increased vulnerability to Aβ-induced death (Misiak et al. 2017). In the present study, the pol β expression was found to be reduced at all-time points in Aβ(25–35) and MCP treatments in SH-SY5Y cells. While pretreatment of curcumin upregulated the expression of pol β after the pretreatment of curcumin followed by Aβ(25–35) and MCP treatments.

Nrf2 and APE1 are the two key proteins which cross-talk with each other during the oxidative stress. APE1 reductively activates nuclear-localized Nrf2 to transcribe downstream antioxidant genes (Shan et al. 2015). From the literature and the present study, it is suggestive that APE1 regulates various proteins via its redox activity, and APE1 controls transcriptional activity of Nrf2 via its redox activity. APE1 and Nrf2 expression and their association were analyzed by immunofluorescent confocal laser scanning microscopic technique, and the PCC values were determined, to compute colocalization of these two proteins (Dunn et al. 2011). In all images, control cells were seen to have less nuclear localization of Nrf2 and APE1 in both the SH-SY5Y and IMR-32 cells with PCC values less than zero. Curcumin pretreatment translocated Nrf2 and APE1 proteins to the nucleus with PCC values close to ≈ 0.5, which signifies for the colocalization of Nrf2 and APE1 proteins for reductive transcriptional activation of Nrf2 by APE1 (Shan et al. 2015). It was also observed that APE1 and Nrf2 mostly translocate to the nucleus after curcumin pretreatment. Curcumin is a potent antioxidant and can be attributed that it may activate both APE1 and Nrf2 proteins and translocate them into the nucleus to activate cell survival pathways during Aβ-induced oxidative stress in AD.

Summary and conclusion

In conclusion, one can say that Nrf2 and APE1 proteins are the two vital proteins, which play a pivotal role in regulation of oxidative stress. APE1, the "hub protein" has significant correlation with Nrf2 during Aβ-induced oxidative stress. This study also establishes that soluble (oligomeric) form of Aβ causes severe damage to the neuronal cells, and the environmental pollutants, OPPs might trigger this damage via DNA damage to the neuronal cells. While curcumin pretreatment revitalizes the neuronal cells from Aβ stress by the help of Nrf2 and APE1 protein-mediated pathways.

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Compliance with ethical standards

Conflict of interest Authors declare that no conflict of interest exists.

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