

## Protective effects of curcumin against dopamine quinone-induced dopaminergic neurotoxicity in SH-SY5Y cells

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### Abstract

Auto-oxidation of 6-hydroxy dopamine (6-OHDA) has been known to generate free radicals and subsequent quinone proteins formation which is toxic to dopaminergic neurons. Curcumin, is a pure compound extracted from *Curcuma longa* which known to possess anti-oxidant, anti-inflammatory and anti-carcinogenic activities. In this study, we investigated the effects of curcumin on 6-OHDA-induced neurotoxicity in SH-SY5Y cells. It was found that curcumin significantly increased the viability of the cells. Along with the increase in cell survival the quinoprotein production was significantly decreased. These results indicate that curcumin has neuroprotective effects and may have a therapeutic potential for treatment of Parkinson disease.

**Keywords:** 6-OHDA, quinoprotein, *Curcuma longa*.

### Introduction

The formation of dopamine quinones has been known to exert toxic effects in dopaminergic cells (Asanuma et al., 2004). These quinones conjugate with the sulfhydryl group of the amino acid cysteine. The dopamine quinone modified proteins were called quinoproteins. Since the sulfhydryl group on cysteine is often found at the active site of functional proteins, quinoproteins inhibit protein function and cause cell death. Curcumin or diferuloylmethane is a purified natural compound extracted from *Curcuma longa* (turmeric). The previous study has reported that curcumin possesses multiple pharmacological properties such as anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic activities (Joe and Lokesh, 1994).

Therefore, we speculate that curcumin may prevent the degeneration of dopaminergic neurons. In this study, we investigated whether the anti-oxidant property of *C.longa* would prevent the neurotoxic effects of 6-OHDA in SH-SY5Y cells.

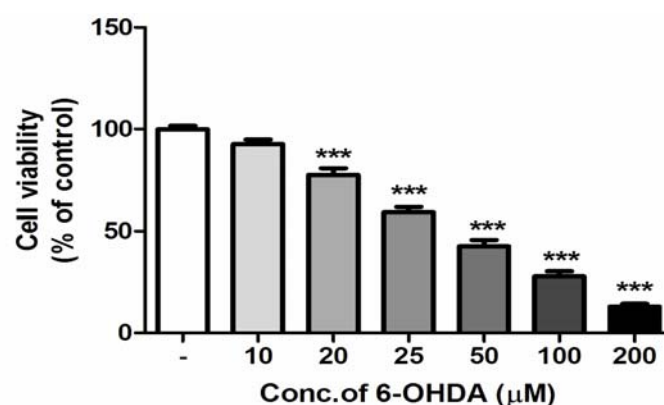
### Methods

To determine the effects of 6-OHDA and curcumin, on cell viability, SH-SY5Y cells were plated at a density of  $2 \times 10^4$  cells per well on 96-well culture plates. After treatment with various concentration of 6-OHDA ranging from 10  $\mu$ M to 200  $\mu$ M for 24 h, or pretreatment with different concentrations of curcumin for 30 min followed by 24 h of 6-OHDA treatment (25  $\mu$ M), the medium was removed and the cells were incubated with a solution of 1 mg/ml MTT for 4 h and then measuring absorbance at 570 nm. The protein-bound quinones (quinoprotein) were detected by the nitrobluetetrazolium (NBT)/glycinate colorimetric assay. Cells were plated at a density of  $1 \times 10^6$  cells/well in 6-well tissue culture plates and incubated for 24 h. After pretreatment with various concentrations of curcumin for 30 min follow by 24 h of 6-OHDA treatment (25  $\mu$ M). The cultures were lysated in 10%

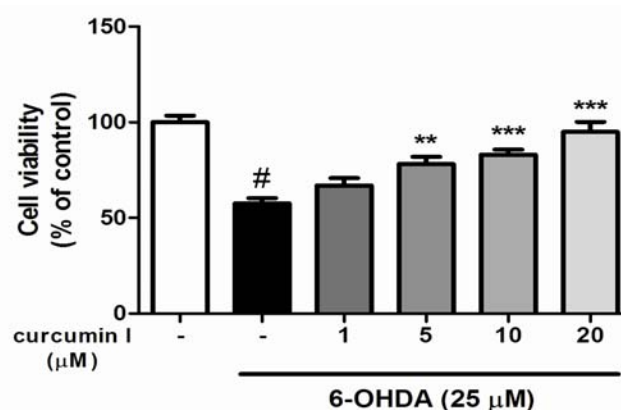
trichloroacetic acid (TCA) and NBT reagent was added (0.24 mM NBT in 2 M potassium glycinate, pH 10.0) followed by incubation the mixture in the dark for 2 h under constant shaking. The absorbance of blue-purple color developed in the reaction mixture was measured at 530 nm.

## Results

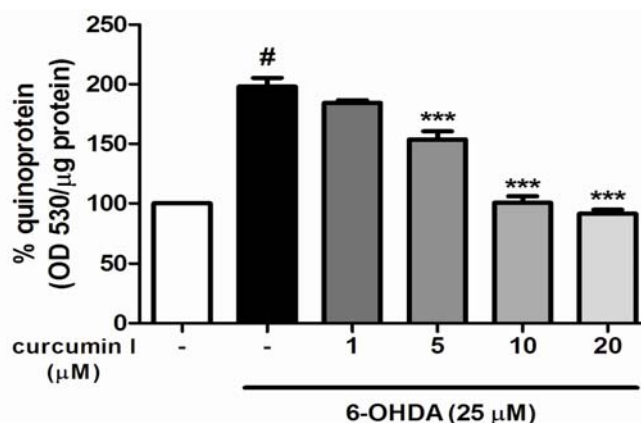
To evaluate the cytotoxic effects of 6-OHDA and curcumin, as shown in Fig.1, treatment with various concentration of 6-OHDA ranging from 10  $\mu$ M to 200  $\mu$ M for 24 h significantly decreased the cell viability in a dose -dependent manner. 6-OHDA at the concentration of 25  $\mu$ M, decreased the cell viability to 59 % of control, therefore 6-OHDA at this concentration was used to detect the neuroprotective effect of curcumin. Pretreatment the cells with curcumin at concentration of 1  $\mu$ M, 5  $\mu$ M, 10  $\mu$ M and 20  $\mu$ M for 30 min followed by treatment with 6-OHDA at the concentration of 25  $\mu$ M for 24 h significantly increased the cell viability (Fig.2). These results demonstrated the protective effect of curcumin on cell viability in 6-OHDA-induced cell death. To measure the levels of quinoprotein production, as shown in Fig. 3, after treatment the cells with 6-OHDA for 24 hr the level of quinoprotein production was significantly increased to  $198.01 \pm 7.38$  % compared with the control. Pretreatment the cells with curcumin significantly decreased the effect of 6-OHDA-induced quinoprotein production in a concentration-dependent manner. At the concentration of 1, 5, 10, and 20  $\mu$ M, curcumin reduced quinoprotein production to  $184.33 \pm 2.14$ ,  $153.56 \pm 6.87$ ,  $100.56 \pm 5.29$ , and  $91.27 \pm 3.42$  %, respectively, compared with the control. Data were represented as mean  $\pm$  SEM of three separate experiments.



**Figure 1.** Effects of the various concentrations of 6-OHDA on the viability of SH-SY5Y cells. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  as compared with control.



**Figure 2.** Effects of pretreatment with various concentration of curcumin on the viability of SH-SY5Y cells. # $p < 0.001$  as compared with the control; \*\* $p < 0.01$ , \*\*\* $p < 0.001$  as compared with 6-OHDA treated only.



**Figure 3.** Effects of various concentrations of curcumin on the levels of quinoprotein production in 6-OHDA-treated SH-SY5Y. Data were represented as mean  $\pm$  SEM of three separate experiments. # $p < 0.001$  as compared with the control; \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  as compared with 6-OHDA treated cells.

### Discussion

The results of our study demonstrated the protective effects of curcumin on 6-OHDA-induced neurotoxicity of SH-SY5Y cells. Previous study reported that 6-OHDA generated DAQ and quinoprotein (D. Blum et al., 2001). We demonstrated for the first time, that curcumin can decrease quinoprotein production in 6-OHDA-induced quinoprotein production.

### Conclusion

We demonstrated that curcumin significantly increased cell viability and decreased quinoprotein productions in 6-OHDA-induced neurotoxicity. This study suggests that curcumin possesses neuroprotective effects and may have a therapeutic potential for the treatment of neurodegenerative diseases caused by oxidative stress.

### Acknowledgements

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