

## Original article

## Appraisal of a neuroprotective consequence of *Curcuma zedoaria* Roxb. rhizomes on memory malfunction in oxidative tension convinced C6 glioma cells of rats

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### Abstract

Perennial herbaceous plant of the ginger family Zingiberaceae, *Curcuma longa* L. is competent in anticancer and neuroprotective development. The neuroprotective event of plant *Curcuma zedoaria* Roxb. from the same family has not yet documented. *C. zedoaria* rhizomes powder extracted by 70% ethanol. The protective effects of *C. zedoaria* rhizomes on oxidative tension convinced cell destruction by tert-butyl hydroperoxide and hydrogen peroxide were examined by MTT test in C6 cell lines. Pretreatment with ethanolic extract of rhizomes of *C. zedoaria* effectively guarded the cell destruction convinced by oxidative tension in C6 cells with 71% maximum cell viability. *C. zedoaria* pretreatment could alleviate oxidative tension in glioma C6 cells. In such a way ethanolic extract of rhizomes of *C. zedoaria* might be advantageous for forbidding the deterioration of learning and memory.

**Key words:** Memory malfunction, C6 glioma cells, Alzheimer's disease, *Curcuma zedoaria* Roxb, MTT test

### 1. Introduction

Alzheimer's disease (AD) is the largest universal neurodegenerative disorder in elderly people and is the main origin of almost two of three cases of dementia (Pathan and Alshahrani, 2018). The therapeutic target for the management has been determined which consist of several signaling molecules. The chemical constituents obtained from many traditional plants are considered beneficial in neurotransmitter receptors. Among traditional medicine, several plants have been reported to treat cognitive disorders. Plant-derived herbs are safe and without side effects used in traditional practice such as Chinese, Ayurvedic and Unani medicines (Kumar and Khanum, 2012; Pathan and Alshahrani, 2018). Glial cells perform the imperative duty to support and protect the neurons and also domination competence for learning and memory. Free radical provocation consequence into neuronal cell destruction (Cerbai *et al.*, 2012; Liu and Hong, 2013). For the hunting contemporary drug radicals, rat glioma C6 and Microglial BV2 cell models are considerable in neurological studies (Shen *et al.*, 2005; Jung *et al.*, 2007). *Curcuma zedoaria* Roxb. belongs to family Zingiberaceae and universally in known as turmeric. In India, it is found, most of the states mainly Madhya Pradesh, West Bengal, and Uttar Pradesh. It grows better in the moist deciduous forest region. Rhizomes of the plant are used in

the manufacturing of cosmetics. The scientist discovered the benefits of turmeric since a long ago in blood disorders, leukoderma laxative, anthelmintic, and vulnerary hence, it has a high economic concern. Perennial herbaceous plant of the ginger family Zingiberaceae, *C. longa* is competent in anticancer and neuroprotective development. The neuroprotective event of plant *C. zedoaria* from the same family has not yet documented (Pathan *et al.*, 2015; Pathan *et al.*, 2016).

The instant investigation, therefore, directed to acknowledge the capability of *C. zedoaria* as neuroprotective development for the learning and memory.

### 2. Materials and Methods

#### 2.1 Chemicals and reagents

Fetal bovine serum (FBS), Dulbecco's modified eagle medium (DMEM), and other cell culture reagents were obtained from Gibco BRL (Grand Island, NY). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), DMSO, tert-butyl hydroperoxide, hydrogen peroxide was purchased from Sigma Chemical Co. (St. Louis, MO).

#### 2.2 Plant material

The fresh rhizomes of *Curcuma zedoaria* Roxb. were collected from the farm area and authenticated by the Botanist of the Institution and sample specimen were stored for further reference (Specimen Number: AMR 1520). Rhizomes were dried in shades and powdered. Dried powder of *C. zedoaria* was extracted with 70% ethanol by using soxhlet extractor. The extract was evaporated with rotary

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vacuum evaporator and dried to form a powder. The dried powder was used for further experiment, (Institutional Ethical Committee Approval Number: SUCOM/LIRB/2019-08).

### 2.3 Cell culture

C6 rat glioma cells were obtained from the Korean Cell Line Bank (KCLB, Seoul, Korea) and cultured in DMEM (Gibco BRL, Grand Island, NY) with 10% FBS (Gibco BRL, Grand Island, NY) and 1% streptomycin/penicillin in a 37°C humidified incubator in an atmosphere of 5% CO<sub>2</sub> in air (Santos *et al.*, 2010).

### 2.4 Cell viability

Cell viabilities were resolved by the MTT test. C6 cells (5 × 10<sup>4</sup> cells/well) were implanted in a 96-well plate and pretreated with many concentrations of *C. zedoaria* for one day. The cells were incubated with tert-butyl hydroperoxide (1 mM) for one-hour and hydrogen peroxide (2 mM) for 30-min to induce oxidative tension. After treatment, 10<sup>-1</sup> of an MTT solution (5 mg/ml in phosphate-buffered saline) was added to each well and additionally incubated for 4 h at 37°C. Afterward, 100<sup>-1</sup> of dimethyl sulfoxide (DMSO) was combined to each well to solubilize any deposited formazon. The optical density of each well was confined at 550 nm with a microplate reader (Molecular Devices, Spectra max 340PC, USA) (Santos *et al.*, 2010; Cheong *et al.*, 2017).

### 2.5 Statistical analysis

Investigations were achieved somewhat in a set of three. Inputs were articulated as the mean ± standard error of the mean (SEM) or standard deviation (SD). The meaningful discrepancy from the respective controls for each test condition was analyzed using the Student's t-test for each paired experiment. Statistical significance was set at *p*<0.05.

## 3. Results

There was 71% cell viability seen in the first assay of tert-butyl hydroperoxide (1 mM) for one hour mediated glioma C6 cell destruction and 64% cell viability seen in second assay of hydrogen peroxide treatment (2 mM) for 30 min mediated glioma C6 cell destruction.

## 4. Discussion

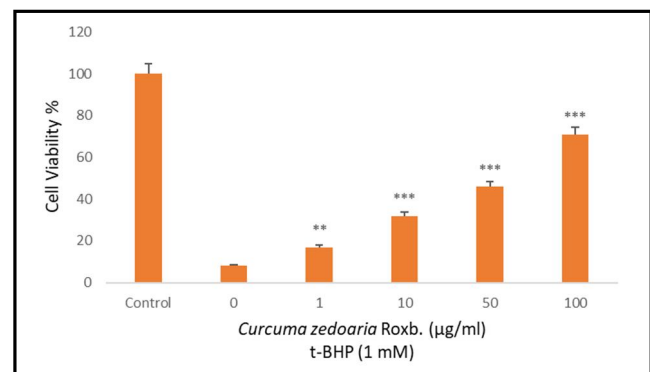
### 4.1 Neuroprotective development of *C. zedoaria* in tert-butyl hydroperoxide and H<sub>2</sub>O<sub>2</sub> treated C6 cells

To explore the neuroprotective impact of *C. zedoaria* across oxidative tension *in vitro*, its inhibitory effects on tert-butyl hydroperoxide and hydrogen peroxide medicated cell destruction were calculated in C6 glial cells. *C. zedoaria* never demonstrated cytotoxicity in C6 cells by MTT test (*p*>0.05). The tert-butyl hydroperoxide (1 mM) for 1 h mediated glioma C6 cell destruction was obviated by pretreatment with *C. zedoaria* (ethanolic extract of rhizomes) with 71% cell viability in a dose-dependent aspect (Figure 1, *p*<0.001). Moreover, hydrogen peroxide treatment (2 mM) for 30 min mediated glioma C6 cell destruction was obviated by pretreatment with *C. zedoaria* (ethanolic extract of rhizomes) with 64% cell viability in a dose-dependent aspect (Figure 2, *p*<0.05). The neuronal cell destruction caused by free radical

provocation such as hydrogen peroxide and tert-butyl hydroperoxide *in vitro* (Santos *et al.*, 2010; Forman, 2007; Cheong *et al.*, 2017).

**Table 1:** Guarding consequence of *C. zedoaria* across oxidative tension in C6 cells. Event on the cell destruction mediated by tert-butyl hydroperoxide was calculated by the MTT test. Cells were employed with tert-butyl hydroperoxide (1 mM) for 1 h after the incubated with *C. zedoaria* inputs represent means ± SEM of three separate analyses. \* *p*<0.05 \*\* *p*<0.01 as compared with the tert-butyl hydroperoxide treated group

Test extract dose (µg/ml)	Cell viability
Control	100
0	8
1	17
10	32
50	46
100	71



**Figure 1:** Guarding consequence of *C. zedoaria* across oxidative tension in C6 cells. Event on the cell destruction mediated by tert-butyl hydroperoxide was calculated by the MTT test. Cells were employed with tert-butyl hydroperoxide (1 mM) for one hour after the incubated with *C. zedoaria* inputs represent means ± SEM of three separate analyses. \* *p*<0.05 \*\* *p*<0.01 as compared with the tert-butyl hydroperoxide treated group.

## 5. Conclusion

Outcomes of the investigation validate that ethanolic extract of rhizomes of *C. zedoaria* guarded the cell destruction mediated by tert-butyl hydroperoxide and hydrogen peroxide in glioma C6 cell models. *C. zedoaria* pretreatment could alleviate oxidative tension in glioma C6 cells. In such a way, ethanolic extract of rhizomes of *C. zedoaria* might be advantageous for forbidding the deterioration of learning and memory. The additional probe is warranted.

### Acknowledgements

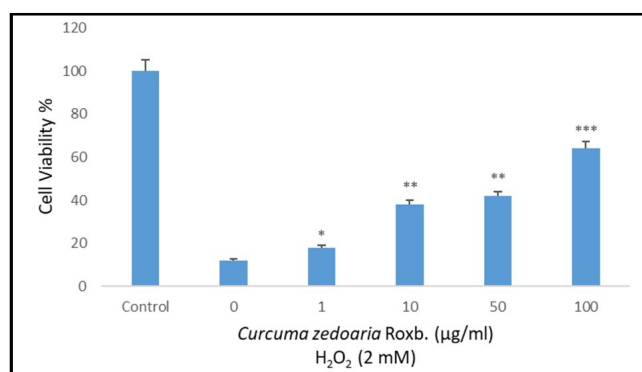
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### Conflict of interest

The author declared that there is no conflicts of interest in the course of conducting the research. The author has final decision regarding the manuscript and decision to submit the findings for publication.

**Table 2:** Guarding consequence of *C. zedoaria* across oxidative tension in C6 cells. Event on the cell destruction mediated by hydrogen peroxide was calculated by the MTT test. Cells were employed with hydrogen peroxide (2 mM) for 30 min after the incubated with *C. zedoaria* inputs represent means  $\pm$  SEM of three separate analyses. \*  $p < 0.05$  \*\*  $p < 0.01$  as compared with the hydrogen peroxide treated group

Test Extract Dose ( $\mu\text{g/ml}$ )	Cell viability
Control	100
0	12
1	18
10	38
50	42
100	64



**Figure 2:** Guarding consequence of *C. zedoaria* across oxidative tension in C6 cells. Event on the cell destruction mediated by hydrogen peroxide was calculated by the MTT test. Cells were employed with hydrogen peroxide (2 mM) for 30 min after the incubated with *C. zedoaria* inputs represent means  $\pm$  SEM of three separate analyses. \*  $p < 0.05$  \*\*  $p < 0.01$  as compared with the hydrogen peroxide treated group.

### Abbreviations

AchE	: Acetylcholinesterase
AD	: Alzheimer's disease
BDNF	: Brain-derived neurotropic factor
COX-2	: Cyclooxygenase-2
CREB	: cAMP response element-binding protein
DMEM	: Dulbecco's modified eagle medium
DMSO	: dimethyl sulfoxide
H <sub>2</sub> O <sub>2</sub>	: Hydrogen peroxide
iNOS	: Inducible NO synthase
MTT	: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
t-BHP	: Tert-butyl hydroperoxide

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