N-trans-feruloyltyramine Protects Human Neuroblastoma SK-N-SH Cell Line Against H₂O₂-Induced Cytotoxicity

Rungtip Soi-ampornkul¹, Ei Ei Phyo Myint¹, Wipawan Thangnipon², Nutnicha Tantarungsee³, Chalermchai Mitrpant¹, Patoomratana Tuchinda³, Saksit Nobsathian⁴, and Chinnavuth Vatanashevanopakorn¹

Abstract
Excessive accumulation of reactive oxygen species (ROS) has been regarded as a major contributor of pathogenesis in neurodegenerative diseases. N-trans-feruloyltyramine (NTF), an alkaloid isolated from several plants, has demonstrated an ability to be a potent antioxidant. In this study, the antioxidative and anti-apoptotic properties of NTF extracted from the stems of Polyalthia suberosa were investigated in the human neuroblastoma cell line SK-N-SH. NTF at concentrations ranging from 10 µM to 500 µM were not toxic to cells and reduced intracellular ROS levels significantly. Furthermore, pre-treatment of NTF significantly decreased H₂O₂-mediated ROS generation and attenuated H₂O₂-mediated cytotoxicity. An increase in the expression of Bax and activated caspase-3 and reduction of Bcl-2 mediated by H₂O₂ was reversed by pre-treating the cells with 100 µM NTF. Likewise, NTF suppressed the increase of caspase-3 activity induced by H₂O₂. In conclusion, the findings reveal that NTF improves H₂O₂-induced intracellular ROS generation and decreases apoptosis. These protective effects of NTF could be useful for oxidative stress-related neurodegenerative conditions.

Keywords
oxidative stress, apoptosis, antioxidant, N-trans-feruloyltyramine, alkaloids

Received: December 29th, 2021; Accepted: July 14th, 2022.

Introduction
Reactive oxygen species (ROS) are a group of reactive molecules containing oxygen, generated as a byproduct of several cellular metabolisms. Although beneficial when maintained at low concentration, high levels of ROS have several detrimental effects, including lipid peroxidation, and protein and DNA damage. In order to prevent damage, cells utilize an antioxidant defensive system to scavenge ROS. However, in some conditions, production of ROS is much more pronounced, and can overwhelm cellular antioxidant capacity, leading to oxidative stress. Conditions known to be involved with oxidative stress include, but are not limited to, cardiovascular diseases, diabetes, cancer and neurodegenerative diseases. Among these, diseases of the nervous system are of interest since neurons exhibit characteristics that can cause them to become severely damaged by ROS, such as high proportion of unsaturated fatty acids within membranes, high oxygen consumption and scarce amount of antioxidants. Oxidative stress has been identified as a shared factor among neurodegenerative diseases such as Alzheimer’s disease, amyotrophic lateral sclerosis and Parkinson’s disease. Thus, finding a way to reduce oxidative stress may pave the way to prevent or ameliorate disease progression.

N-trans-feruloyltyramine (NTF, Figure 1A) is an alkaloid extracted from several plants, including the stems of Polyalthia suberosa, stems of Tinospora tuberculata, branches of Enicosanthum membranifolium, stems of Polygonum sachalinensis, leaves of Solanum sordidum, aerial parts of Triclisia sacleuxii, seeds of

¹Department of Biochemistry, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand
²Research Center for Neuroscience, Institute of Molecular Biosciences, Mahidol University, Salaya, Nakhonpathom, Thailand
³Department of Chemistry, Faculty of Science, Mahidol University, Bangkok, Thailand
⁴Nakhon Sawan Campus, Mahidol University, Phayahakiri, Nakhon Sawan, Thailand

Corresponding Author:
Chinnavuth Vatanashevanopakorn, Department of Biochemistry, Faculty of Medicine Siriraj Hospital, Mahidol University, 2 Wanglang Road, Bangkok, 10700, Thailand.
Email: chinnavuth.vat@mahidol.ac.th
Datura metel\textsuperscript{12} processed bulbs of Allium sativum\textsuperscript{13} and twigs of Celtis occidentalis\textsuperscript{14} Previous studies have reported that NTF is a potent antioxidant\textsuperscript{9, 10, 12, 13, 15, 16} and exhibits other pharmacological properties such as \( \alpha \)-glucosidase inhibition\textsuperscript{9} and anti-tumor activity.\textsuperscript{13} In this study, we investigated the protective effects of NTF isolated from the stems of Polyalthia suberosa against \( \mathrm{H}_2\mathrm{O}_2 \)-mediated cytotoxicity using an \textit{in vitro} model of human neuroblastoma cell line SK-N-SH. The effect of NTF on ROS levels, cell viability, expression of pro-apoptotic as well as anti-apoptotic proteins and apoptotic activity were evaluated.

### Results

**NTF was Not Toxic to SK-N-SH Cells and Exhibited Antioxidative Properties**

To assess whether NTF treatment would be detrimental to the SK-N-SH cells, varied concentrations of NTF were applied, ranging from 10 \( \mu \text{M} \) to 500 \( \mu \text{M} \). A cell viability analysis revealed that addition of NTF at indicated concentrations did not affect viability of the SK-N-SH cells while addition of 150 \( \mu \text{M} \) \( \mathrm{H}_2\mathrm{O}_2 \) significantly decreased the viability (Table 1 and Figure 1B, one-way ANOVA: \( F = 28.39, p < 0.0001 \); Dunnett’s multiple comparison test: \( p < 0.0001 \) for control vs. 150 \( \mu \text{M} \) \( \mathrm{H}_2\mathrm{O}_2 \), control vs. 10 \( \mu \text{M} \) NTF, control vs. 50 \( \mu \text{M} \) NTF, control vs. 100 \( \mu \text{M} \) NTF and control vs. 150 \( \mu \text{M} \) NTF; \( p = 0.0003 \) for control vs. 25 \( \mu \text{M} \) NTF and control vs. 250 \( \mu \text{M} \) NTF). A significant reduction in ROS levels was found in samples treated with NTF at all tested concentrations and in a sample treated with well-known antioxidant ascorbic acid (AA) (Figure 1C, one-way ANOVA: \( F = 40.65, p < 0.0001 \); Dunnett’s multiple comparison test: \( p < 0.0001 \) for control vs. 150 \( \mu \text{M} \) \( \mathrm{H}_2\mathrm{O}_2 \)). The findings demonstrate that NTF at concentrations between 1 \( \mu \text{M} \) to 500 \( \mu \text{M} \) is not toxic to the SK-N-SH cells and has antioxidative effects.

### Table 1

| NTF concentration (\( \mu \text{M} \)) | Cell viability (% of control) |
|---------------------------------------|-----------------------------|
| 10                                    | 99.43 ± 0.94                |
| 25                                    | 98.67 ± 1.90                |
| 50                                    | 94.27 ± 0.75                |
| 100                                   | 93.70 ± 1.99                |
| 150                                   | 94.72 ± 1.90                |
| 250                                   | 92.89 ± 2.86                |
| 500                                   | 96.34 ± 2.62                |

Values are the mean ± SEM of three independent experiments.

---

\( \textit{Datura metel} \),\textsuperscript{12} \( \textit{Allium sativum} \),\textsuperscript{13} and \( \textit{Celtis occidentalis} \).\textsuperscript{14} Previous studies have reported that NTF is a potent antioxidant\textsuperscript{9, 10, 12, 13, 15, 16} and exhibits other pharmacological properties such as \( \alpha \)-glucosidase inhibition\textsuperscript{9} and anti-tumor activity.\textsuperscript{13} In this study, we investigated the protective effects of NTF isolated from the stems of Polyalthia suberosa against \( \mathrm{H}_2\mathrm{O}_2 \)-mediated cytotoxicity using an \textit{in vitro} model of human neuroblastoma cell line SK-N-SH. The effect of NTF on ROS levels, cell viability, expression of pro-apoptotic as well as anti-apoptotic proteins and apoptotic activity were evaluated.
NTF Ameliorated H2O2-Induced Cytotoxicity Through Inhibition of H2O2-Mediated ROS Induction

As a ROS, H2O2 causes reduction of cell viability in a concentration-dependent manner (Table 2, one-way ANOVA: F = 56.61, p < 0.0001; Dunnett’s post-hoc test: p = 0.0253 for 37.5 μM H2O2 vs. control; p = 0.0031 for 75 μM H2O2 vs. control; p < 0.0001 for 150, 300, 600, 1200 and 2400 μM H2O2 vs. control). An H2O2 concentration that caused 50% reduction in viability of the cells (CC50) was 157 μM (Figure 2A). Thus, to test whether NTF would counteract H2O2-mediated reduction in cell viability, we pre-treated the cells with various concentrations (10, 25, 50, 100, 150, 250 and 500 μM) of NTF for three hours prior to the induction of cytotoxicity with H2O2. By using a phase contrast inverted microscope, we noticed morphological changes of the cells treated with H2O2 which unlike untreated cells (Figure 2B), shrunk and became detached, resembling dead cells (Figure 2C). Interestingly, pre-treatment with NTF prior to H2O2 addition lessened the number of detached cells (Figure 2D). A cell viability analysis consistently revealed a sharp decrease in viability of H2O2-treated cells (Figure 2E, one-way ANOVA: F = 49.46, p < 0.0001; Dunnett’s test: p < 0.0001 for control vs. H2O2-treated group). Nevertheless, similar to pre-treatment with AA, the addition of NTF was able to help protect the cells against H2O2-induced toxicity as determined by a significantly higher percentage of viability in cells pre-treated with NTF at all tested concentrations compared to the H2O2-treated group (Figure 2E, Dunnett’s test: p < 0.0001 for H2O2-treated group vs. pre-treated AA and H2O2-treated group vs. pre-treated NTF at all concentrations). To further assess whether NTF-mediated protection against H2O2 is a consequence of a decrease in ROS levels induced by H2O2, the SK-N-SH cells were pre-treated with NTF at the indicated concentrations. As expected, H2O2 treatment caused a sharp rise in ROS levels (Figure 2F, one-way ANOVA: F = 17.69, p < 0.0001; Dunnett’s test: p < 0.0001 for control vs. H2O2-treated group). Pre-treatment of NTF at all tested concentrations significantly improved an H2O2-mediated increase in ROS levels (Figure 2F, Dunnett’s test: p < 0.0001 for H2O2-treated group vs. pre-treated AA and H2O2-treated group vs. pre-treated NTF at all concentrations).

**Table 2. H2O2 Cytotoxicity in SK-N-SH Cells.**

| H2O2 concentration (μM) | Cell viability (% of control) |
|-------------------------|-------------------------------|
| 37.5                    | 82.80 ± 1.14**               |
| 75                      | 77.32 ± 1.14***              |
| 150                     | 56.26 ± 5.55****             |
| 300                     | 46.52 ± 5.08****             |
| 600                     | 35.34 ± 5.36****             |
| 1200                    | 27.90 ± 2.77****             |
| 2400                    | 23.33 ± 3.89****             |

*p < 0.05, **p < 0.01, ****p < 0.0001 versus H2O2-treated control. Values are the mean ± SEM of three independent experiments.

NTF Inhibited H2O2-Mediated Apoptosis in SK-N-SH Cells

It is an established fact that apoptosis can be triggered by H2O2. Hence, a decline in observed cell viability of H2O2-treated SK-N-SH cells is the result of apoptosis activation. A Western blot analysis demonstrated that levels of the pro-apoptotic protein Bax were elevated in H2O2-treated cells (Figure 3A, one-way ANOVA: F = 9.747, p = 0.0018; Dunnett’s test: p = 0.0017 for control vs. H2O2-treated group). The H2O2-induced Bax expression was abolished when cells were pre-treated with 100 μM NTF (Figure 3A Dunnett’s test: p = 0.0089 for H2O2-treated group vs. pre-treated NTF at 100 μM). Interestingly, levels of the anti-apoptotic protein Bel-2, which was down-regulated in the cells treated with H2O2 (Figure 3B, one-way ANOVA: F = 8.405, p = 0.0031; Dunnett’s test: p = 0.0236 for control vs. H2O2-treated group) was significantly elevated when the cells were pre-treated with NTF at 50 μM and 100 μM (Figure 3B, Dunnett’s test: p = 0.0031, and p = 0.0012, respectively). To further determine anti-apoptotic effects of NTF, quantitation of activated caspase-3 levels as well as measurement of its activity was performed. In the presence of H2O2, up-regulation of activated caspase-3 was found (Figure 3C, one-way ANOVA: F = 9.361, p = 0.0021; Dunnett’s test: p = 0.0137 for control vs. H2O2-treated group). This caused a significant increase in caspase-3 activity (Figure 3D, one-way ANOVA: F = 17.04, p = 0.0002; Dunnett’s test: p < 0.0001 for control vs. H2O2-treated group). Consistent with Bax levels, pre-treatment of 100 μM NTF significantly decreased activated caspase-3 levels (Figure 3C, Dunnett’s test: p = 0.0017 for H2O2-treated group vs. pre-treated NTF at 100 μM). Additionally, caspase-3 activity was significantly lower in cells pre-treated with NTF at all tested concentrations (Figure 3D, Dunnett’s test: p = 0.0007 for H2O2-treated group vs. pre-treated NTF at 25 and 100 μM; p = 0.0016 for H2O2-treated group vs. pre-treated NTF at 50 μM). These findings indicate the effects of NTF against H2O2-mediated apoptosis.

Discussion

NTF is an alkaloid found in various types of plants, such as tropical shrubs (Polyalthia suberosa), herbaceous plants (Tinospora tuberculata, Polygonum sachalinensis) and edible plants (Allium sativum). NTF is known for its broad ranges of biological effects such as being an antioxidant, anti-inflammation, anti-melanogenesis, α-glucosidase inhibition, anti-cancer, and anti-microbial. Its availability in many plant species, together with its broad pharmacological effects, make NTF an attractive molecule for drug development. Antioxidative effects of NTF have been demonstrated in a number of studies. In a rat cortical neuronal culture, NTF at 25–250 μM ameliorated amyloid-β peptide (Aβ)-mediated ROS generation. Likewise, reduction of ROS levels was observed in human hepatocyte cell line L02 treated with
In this study, we evaluated possible antiradical effects of NTF in the SK-N-SH cell lines. In conditions where the oxidative status was within normal limits, treatment of NTF ranging in concentration from 10 $\mu$M to 500 $\mu$M significantly decreased ROS levels (Figure 1C) without compromising cell viability (Figure 1B), demonstrating the antioxidative property of NTF.

More importantly, in conditions where oxidative stress is induced by 150 $\mu$M H$_2$O$_2$, pre-treatment of NTF at all tested concentrations also significantly enhanced the oxidant scavenging capacity of the cells (Figure 2F). In addition to a ROS reduction effect in H$_2$O$_2$-treated cells, we also observed an increase in cell viability in cells pre-treated with NTF (Figure 2E).

It is common knowledge that H$_2$O$_2$ can induce apoptosis in various cell types via ROS-mediated activation of several mediators such as p53, p38 mitogen-activated protein kinase (MAPK), extracellular signal-regulated protein kinase 1/2 (Erk1/2) and c-Jun N-terminal kinase (JNK), which causes stimulation of pro-apoptotic proteins including Bax and inhibition of anti-apoptotic proteins such as Bcl-XL and Bcl-2. Consequently, a release of cytochrome c triggers the intrinsic pathway of apoptosis via activation of caspase-3. Thus, it is highly likely that a decline in cell viability in H$_2$O$_2$-treated SK-N-SH cells (Figure 2E) would be a result of apoptosis. Although H$_2$O$_2$ can also induce other types of cell death such as necroptosis, this usually occurs when high dosage of H$_2$O$_2$ is used. The use of antioxidant N-acetyl-cysteine (NAC) has been shown to reverse H$_2$O$_2$-mediated neuronal apoptosis by impeding JNK, Erk1/2 and p38 activation in the differentiated neuroblastoma cell line SH-SY5Y. Likewise, pre-treatment of an iridoid glycoside loganin alleviated H$_2$O$_2$-induced phosphorylation of JNK, Erk1/2 and p38, augments the Bcl-2/Bax ratio, leading to decreased activated caspase-3 levels. Consistent with previous studies, our data also showed evidence of increased apoptosis as determined by increased levels of Bax (Figure 3A), a decrease in Bcl-2 levels and activity of activated caspase-3 (Figure 3C and D) in cells treated with H$_2$O$_2$. Collectively, a rise in cell viability in samples pre-treated with NTF prior to induction of cytotoxicity by H$_2$O$_2$ (Figure 2E) would result in reduced ROS levels, which in turn prevent apoptosis as evidenced by a restoration of Bax, Bcl-2 and activated caspase-3 levels (Figure 3A-C) as well as caspase-3 activity (Figure 3D). The anti-apoptotic effects of NTF reported in this study correlates well with other studies. NTF has demonstrated an ability to mitigate Aβ$_{1-42}$-induced apoptosis in primary rodent neuronal cell culture. A protective effect of NTF against H$_2$O$_2$-induce cytotoxicity in fetal hepatocyte cell line L02 has also been reported.

Although our study demonstrated protective effects of NTF against ROS-induced apoptosis in a cell line with neuronal phenotype, these effects would need to be tested in vivo.
bioavailability study conducted in a rat model revealed that, after oral administration of NTF at a dose of 20 mg/kg, its metabolites could be detected in plasma and urine. Interestingly, the hydroxy groups at 4-position of benzene rings, which is important for ROS scavenging, were well preserved in these metabolites. This supports a possibility of using NTF as an antioxidative agent via oral administration. Nevertheless, recent evidence has shown that when using a parallel artificial membrane permeability assay (PAMPA), NTF is unable to passively cross the blood-brain barrier. However,

Figure 3. Anti-apoptotic effects of NTF. (A and C) Pre-treatment of NTF at 100 μM significantly reduced Bax (A) and activated caspase-3 (C) levels in H2O2-treated SK-N-SH cells. (B) Cells pre-treated with 50 and 100 μM NTF prior to H2O2 addition showed significantly higher levels of Bcl-2. (D) Down-regulation of activated caspase-3 caused a decrease in caspase-3 activity in cells pre-treated with NTF prior to H2O2 treatment. #p < 0.05, ###p < 0.01, ####p < 0.0001 versus untreated control. **p < 0.01, ***p < 0.001 versus H2O2-treated control. Values are the mean ± SEM of three independent experiments.
with the aid of novel strategies such as nanotechnology-based delivery, molecules could be engineered to cross the blood-brain barrier. This would offer an option to determine the usefulness of NTF in protecting ROS-mediated neurodegeneration in vivo.

**Conclusion**

To summarize, our findings demonstrate the antioxidative effects of NTF in an in vitro SK-N-SH model. NTF treatment for up to 500 μM was not toxic to the SK-N-SH cells. Moreover, pre-treatment of NTF successfully prevented H2O2-induced ROS generation and resulted in inhibition of H2O2-mediated apoptosis. Given its antioxidative property, NTF may be useful in preventing and/or ameliorating severity of conditions where oxidative stress is involved in the pathophysiology.

**Experimental**

**Chemicals**

N-trans-feruloyltyramine (NTF) was isolated from a stem of Polyalthia longifolia, grown in Kalasin, Thailand, by chromatography using hot acetone extract. Hydrogen peroxide was purchased from Merek. L-ascorbic acid, 2′,7′-dichlorofluorescin diacetate (DCFH-DA), dimethyl sulfoxide (DMSO) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma.

**Cell Culture and NTF Treatment**

The SK-N-SH cell line, originating from human neuroblastoma, was obtained from ATCC. The cells were maintained in MEM (Gibco) supplemented with 10% (v/v) fetal bovine serum (FBS), 100 units/ml penicillin and 100 μg/ml streptomycin (all from Gibco) at 37 °C with 5% CO2. For each experiment, cells were dissociated with 0.25% trypsin-EDTA, counted, plated at 2 × 10⁵ cells/ml density and maintained at 37 °C with 5% CO2 for 24 h. The cells were treated with NTF at indicated concentrations (10, 25, 50, 100, 150, 250 and 500 μM) for three hours before 150 μM H2O2 was added. Cells treated with 150 μg/ml ascorbic acid (AA) was used as a positive control. Analyses were performed 24 h after the addition of H2O2.

**Cell Viability Analysis**

Spectrophotometric analysis of MTT was used as described previously to determine the viability of SK-N-SH cells. After being treated with H2O2 for 24 h, 20 μl of 5 mg/ml MTT was added to the cells in each well of a 96-well plate. The cells were kept at 37 °C with 5% CO2 for four hours. The medium was then removed and DMSO was added (150 μl for each well) to solubilize the formazan crystals. The absorbance was measured at 570 nm with a microplate reader (BioTek). Cell viability was presented as a percentage of untreated cells.

**Measurement of ROS Production**

Intracellular ROS levels were measured using DCFH-DA. After adding H2O2 for 24 h, 10 μM DCFH-DA was applied to the cells within each well of a 96-well plate before incubating at 37 °C with 5% CO2 for 30 min. Qntification of fluorescence from DCF was done using an excitation wavelength at 485 nm and emission wavelength at 530 nm with a Multi-Detection microplate reader (BioTek). ROS levels were presented as a percentage of untreated cells.

**Western Blotting Analysis**

Western blotting was performed as described previously with some modifications. The SK-N-SH cells were cultured at a density of 1 × 10⁶ cells/cm². The cells were treated with NTF and H2O2 as described in the previous section. Twenty-four hours after being treated with H2O2, the cells were lysed using lysis buffer. The lysis was incubated on ice for 30 min before being centrifuged. The supernatant was obtained and the concentration of protein was determined using a nanophotometer (Implen). Samples were electrophoresed on 10% SDS-PAGE before being transferred to a nitrocellulose membrane (Millipore). The membranes were blocked with buffer comprising 5% bovine serum albumin (BSA) for 1 h at room temperature. The membranes were applied with primary antibodies (mouse anti-human ß-actin antibody, Cell signaling; mouse anti-human Bax antibody, Santa Cruz; rabbit anti-human Bel-2 antibody, Cell signaling; rabbit anti-human activated caspase-3 antibody, Cell signaling) at 1:1000 dilution overnight at 4 °C. Then, the membrane was treated with appropriate HRP-conjugated secondary antibodies (goat anti-mouse IgG and goat anti-rabbit IgG, Santa Cruz) at 1:1000 dilution for two hours at room temperature. The membranes had enhanced chemiluminescence substrate solution (Biorad) applied to them and were visualized on x-ray film. The band density was analyzed using densitometric quantification by ImageJ software (National Institutes of Health).

**Determination of Caspase-3 Activity**

After being treated with H2O2 for 24 h, the cells were harvested and lysed. Approximately 50 μg of protein from the lystate of SK-N-SH cells were used to measure caspase-3 activity with the CaspACE™ assay system (Promega). Briefly, 2 μl of the DEVD-pNA substrate was added into the lystate before incubating it at 37 °C for four hours. Absorbance of pNA, a product cleaved by activated caspase-3, was measured at 405 nm with a microplate reader (BioTek). Caspase-3 activity was presented as a percentage of controls.
**Statistical Analyses**

All experiments were conducted in three independent replicates. Results are presented as the mean ± SEM. Statistical differences were determined by one-way ANOVA with the Dunnett’s multiple comparison test done to analyze differences between the H$_2$O$_2$-treated group and NTF-pre-treated groups using Prism version 9 (GraphPad Software). Statistical significance was accepted when a p-value was less than 0.05.

**Acknowledgements**

EPM is supported by Siriraj Graduate Scholarship, Faculty of Medicine Siriraj Hospital, Mahidol University. CV is supported by the Chalermprakiat Grant, Faculty of Medicine Siriraj Hospital, Mahidol University.

**Author Contributions**

RS provided conceptual input, designed and performed experiments, analyzed data and drafted the manuscript. EPM and NT designed and performed experiments. WT provided conceptual input, analyzed data and edited the manuscript. CM designed experiments and analyzed data. PT & SN provided conceptual input. CV provided conceptual input, designed and performed experiments. RS provided conceptual input, designed and performed experiments, and analyzed data. All authors approved the manuscript.

**Declaration of Conflicting Interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Ethical Approval**

Ethical approval is not applicable for this article.

**Funding**

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Faculty of Medicine Siriraj Hospital, Mahidol University, (grant number R016032001, R016333045).

**Trial Registration**

Trial registration is not applicable for this article.

**ORCID iD**

Chinnavuth Vatanashevanopakorn https://orcid.org/0000-0002-4911-9227

**Statement of Informed Consent**

There are no human subjects in this article and informed consent is not applicable.

**Statement of Human and Animal Rights**

This article does not contain any studies with human or animal subjects.

**References**

1. Pizzino G, Irrera N, Cucinotta M, et al. Oxidative stress: harms and benefits for human health. *Oxid Med Cell Longev*. 2017;2017:8416763. doi:10.1155/2017/8416763
2. Liu Z, Zhou T, Ziegler AC, Dimitrion P, Zuo L. Oxidative stress in neurodegenerative diseases: from molecular mechanisms to clinical applications. *Oxid Med Cell Longev*. 2017;2017:2525967. doi:10.1155/2017/2525967
3. Patel M. Targeting oxidative stress in central nervous system disorders. *Trends Pharmacol Sci*. 2016;37(9):768–778. doi:10.1016/j.tips.2016.06.007
4. Barnham KJ, Masters CL, Bush AI. Neurodegenerative diseases and oxidative stress. *Nat Rev Drug Discov*. 2004;3(3):205–214. doi:10.1038/nrd1330
5. Kim GH, Kim JE, Rhee SJ, Yoon S. The role of oxidative stress in neurodegenerative diseases. *Exp Neurol*. 2015;24(4):325–340. doi:10.5607/en.2015.24.4.325
6. Tuchinda P, Pohmakot M, Manuyoo B, Reatrakul V, Santisuk T. An azaanthracene alkaloid from Polyalthia suberosa. *Phytochemistry*. 2000;53(8):1079–1082. doi:10.1016/s0031-9422(99)00535-x
7. Yokozawa T, Tanaka T, Kimura T. Examination of the nitric oxide production-suppressing component in Tinospora cordifolia. *Biol Pharm Bull*. 2001;24(10):1153–1156. doi:10.1248/bpb.2001.24.10.1153
8. Efthi M, Ohguchi K, Akao Y, Nozawa Y, Koketsu M, Ishihara H. N-trans-feruloyltyramine as a melanin biosynthesis inhibitor. *Biol Pharm Bull*. 2007;30(10):1972–1974. doi:10.1248/bpb.2007.30.1972
9. Fan P, Terrier L, Hay AE, Marston A, Hostettmann K. Antioxidant and enzyme inhibition activities and chemical profiles of Polygonum sachalinense F.Schmidt ex maxim (Polygonaceae). *Fitoterapia*. 2010;81(2):124–131. doi:10.1016/j. Fitot.2009.08.019
10. Kanada RM, Simionato JI, Arruda R, Santin S, Souza M, Silva C. Isolation of a new beta-carboline alkaloid from aerial parts of Tridiclis sacleuxii and its antibacterial and cytotoxicity effects. *Nat Prod Res*. 2017;31(5):529–536. doi:10.1080/14786419.2016.1201666
11. Xu S, Liu Y, Xiang L, et al. Metabolites identification of bioactive compounds daturataturin A, daturametelin I, N-trans-feruloyltyramine, and cannabisin F from the seeds of Datura metel in rats. *Front Pharmacol*. 2012;22(3):502–506.
12. Samita F, Ochieng CO, Owuor PO, Manguro LO, Midwo JO. Isolation of a new beta-carbolene alkaloid from aerial parts of Tridiclis sacleuxii and its antibacterial and cytotoxicity effects. *Nat Prod Res*. 2017;31(5):529–536. doi:10.1080/14786419.2016.1201666
13. Gao X, Wang C, Chen Z, et al. Effects of N-trans-feruloyltyramine isolated from laba garlic on antioxidant, cytotoxic activities and H$_2$O$_2$-induced oxidative damage in HepG2 and L02 cells. *Food Chem Toxicol*. 2019;130:130–141. doi:10.1016/j.fct.2019.05.021
14. Ayanlowo AG, Garadi Z, Boldizsar I, et al. UHPLC-DPPH method reveals antioxidant tyramine and octopamine derivatives in Celtis occidentalis. *J Pharm Biomed Anal*. 2020;191:113612. doi:10.1016/j.phnar.2020.113612
15. Thangrnon W, Suwanna N, Kittayanant N, et al. Protective role of N-trans-feruloyltyramine against beta-amyloid peptide-induced neurotoxicity in rat cultured cortical neurons. *Neurosci Lett*. 2012;513(2):229–232. doi:10.1016/j.neulet.2012.02.047
16. Yang Y, Song ZG, Liu ZQ. Synthesis and antioxidant capacities of hydroxyl derivatives of cinnamoylphenethylamine in protecting DNA and scavenging radicals. *Free Radic Res*. 2011;45(4):445-453. doi:10.3109/10715762.2010.540576

17. Soi-ampornkul R, Ghimire S, Thangnipon W, Suwanna N, Vatanashevanopakorn C. Curcumin attenuates hydrogen peroxide-induced cytotoxicity in human neuroblastoma SK-N-SH cells. *Srinj Med J*. 2018;70(3):184-190.

18. Hoekenbery DM, Oltvai ZN, Yin XM, Milliman CL, Korsmeyer SJ. Bcl-2 functions in an antioxidant pathway to prevent apoptosis. *Cell*. 1993;75(2):241-251. doi:10.1016/0092-8674(93)80066-n

19. Singh M, Sharma H, Singh N. Hydrogen peroxide induces apoptosis in HeLa cells through mitochondrial pathway. *Mitochondrion*. 2007;7(6):367-373. doi:10.1016/j.mito.2007.07.003

20. Jiang Y, Yu L, Wang MH. N-trans-feruloyltyramine inhibits LPS-induced NO and PGE2 production in RAW 264.7 macrophages: involvement of AP-1 and MAP kinase signalling pathways. *Chem Biodivers*. 2015;235:56-62. doi:10.1016/j.chembi.2015.03.029

21. Sadeghi M, Zolfaghari B, Senatore M, Lanzotti V. Spirostane, furostane and cholestane saponins from Persian leek with antifungal activity. *Food Chem*. 2013;141(2):1512-1521. doi:10.1016/j.foodchem.2013.04.009

22. Dhanasekaran DN, Reddy EP. JNK Signaling in apoptosis. *Oncogene*. 2008;27(48):6245-6251. doi:10.1038/onc.2008.301

23. Lee JE, Sohn J, Lee JH, Lee KC, Son CS, Toelgto YC. Regulation of bel-2 family in hydrogen peroxide-induced apoptosis in human leukemia HL-60 cells. *Exp Mol Med*. 2000;32(1):42-46. doi:10.1038/emmm.2000.8

24. Redza-Dutordoir M, Averill-Bates DA. Activation of apoptosis signalling pathways by reactive oxygen species. *Biochim Biophys Acta*. 2016;1863(12):2977-2992. doi:10.1016/j.bbamer.2016.09.012

25. Nagata S. Apoptosis and clearance of apoptotic cells. *Annu Rev Immunol*. 2018;36:489-517. doi:10.1146/annurev-immunol-042617-053010

26. Xiang J, Wan C, Guo R, Guo D. Is hydrogen peroxide a suitable apoptosis inducer for all cell types? *BioMed Res Int*. 2016;2016:7343965. doi:10.1155/2016/7343965

27. Troiano A, Sancho P, Fernandez C, de Blas E, Bernardi P, Aller P. The selection between apoptosis and necrosis is differentially regulated in hydrogen peroxide-treated and glutathione-depleted human promonocytic cells. *Cell Death Differ*. 2003;10(8):889-898. doi:10.1038/sj.cdd.4401249

28. Chen L, Liu L, Yin J, Luo Y, Huang S. Hydrogen peroxide-induced neuronal apoptosis is associated with inhibition of protein phosphatase 2A and 5, leading to activation of MAPK pathway. *Int J Biochem Cell Biol*. 2009;41(6):1284-1295. doi:10.1016/j.biocel.2008.10.029

29. Kwon SH, Kim JA, Hong SI, et al. Loganin protects against hydrogen peroxide-induced apoptosis by inhibiting phosphorylation of JNK, p38, and ERK 1/2 MAPKs in SH-SY5Y cells. *Neurochem Int*. 2011;58(4):533-541. doi:10.1016/j.neuint.2011.01.012

30. Park JB. Identification and quantification of a major anti-oxidant and anti-inflammatory phenolic compound found in basil, lemon thyme, mint, oregano, rosemary, sage, and thyme. *Int J Food Sci Nutr*. 2011;62(6):577-584. doi:10.3109/09637486.2011.562882

31. Naqvi S, Panghal A, Flora SJS. Nanotechnology: a promising approach for delivery of neuroprotective drugs. *Front Neurol*. 2020;11:494. doi:10.3389/fneur.2020.00494

32. Telecanu DM, Chirov C, Grunezescu AM, Volceanov A, Telecanu RI. Blood-brain delivery methods using nanotechnology. *Pharmaceutics*. 2018;10(4):209. 10.3390/pharmaceutics10040209