Neuroprotective Effect of the n-Hexane Extracts of *Laurus nobilis* L. in Models of Parkinson’s Disease

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**Abstract**

Free radical scavenging and antioxidants have attracted attention as a way to prevent the progression of Parkinson’s disease (PD). This study was carried out to investigate the effects of n-hexane fraction from *Laurus nobilis* L. (Lauraceae) leaves (HFL) on dopamine (DA)-induced intracellular reactive oxygen species (ROS) production and apoptosis in human neuroblastsoma SH-SY5Y cells. Compared with apomorphine (APO, IC₅₀=18.1 μM) as a positive control, the HFL IC₅₀ value for DA-induced apoptosis was 3.0 μg/ml, and two major compounds from HFL, costunolide and dehydrocostus lactone, were 7.3 μM and 3.6 μM, respectively. HFL and these major compounds significantly inhibited ROS generation in DA-induced SH-SY5Y cells. A rodent 6-hydroxodopamine (6-OHDA) model of PD was employed to investigate the potential neuroprotective effects of HFL in vivo. 6-OHDA was injected into the substantia nigra of young adult rats and an immunohistochemical analysis was conducted to quantitate the tyrosine hydroxylase (TH)-positive neurons. HFL significantly inhibited 6-OHDA-induced TH-positive cell loss in the substantia nigra and also reduced DA induced α-synuclein (SYN) formation in SH-SY5Y cells. These results indicate that HFL may have neuroprotective effects against DA-induced in vitro and in vivo models of PD.

**Key Words:** *Laurus nobilis* L, Dopamine, Parkinson’s disease, Neuroprotective, α-synuclein

**INTRODUCTION**

Parkinson’s disease (PD) is one of the most common progressive neurodegenerative disorders, affecting about 2% of the population over the age of 60 (Lo Bianco *et al.*, 2004). PD is characterized by severe motor deficits, including uncontrollable tremors, postural imbalance, slowness of movement, and rigidity. These symptoms are caused by neuronal cell death common to many neurodegenerative disorders, such as PD and Alzheimer’s disease (Golde, 2009). Many studies suggest that an imbalance between cytoplasmic and vesicular dopamine (DA) may cause cell degeneration (Barzilai *et al.*, 2001), and that this imbalance may underlie the dopaminergic degeneration observed in PD. Additionally, exogenous DA has been described as a neurotoxic factor in both in vivo and in vitro studies using primary cultures and several cell lines (Gomez-Santos *et al.*, 2003). Hence, in the course of PD studies, 6-OHDA is frequently used to generate experimental models of PD for in vivo studies and for the induction of neuronal cell death in vitro. Although the etiology of idiopathic PD remains unknown, evidence points to the involvement of a broad range of factors, including oxidative stress, mitochondrial dysfunction, and environmental toxins (Mattson, 2000). However, treatment of PD remains unsatisfactory (Chen and Obering, 2005).

Histologically, PD is distinguished by the selective loss of DA-producing neurons in the substantia nigra (Dauer and Przedborski, 2003). In addition, SYN the major component of Lewy bodies, is one of the neuropathological hallmarks of PD (Dedov *et al.*, 2001). Diffused accumulation of SYN protein occurs with aging in the substantia nigra pars compacta (Chu and Kordower, 2007) and prior to frank inclusion formation in PD patients (Chu *et al.*, 2006). These observations indicate that a simple increase in the levels of SYN expression is suf-
sufficient to cause neurodegeneration and that such an increase may underlie the pathogenesis of PD. Thus, we used SYN as a degenerative indicator of PD to screen plant extracts that inhibit SYN expression in SH-SY5Y cells. From these studies, we selected the leaves of *Laurus nobilis* as an active plant extract against PD.

Bay Laurel (*Laurus nobilis*, Lauraceae) is natively distributed in the Mediterranean Basin and has been used as a fixture in European and North American cuisines and also as a flavor in many classic French dishes. It has been reported that *Laurus nobilis* has analgesic, anti-inflammatory, anticonvulsant (Sayyah et al., 2002; Sayyah et al., 2003), and antioxidant activities (Conforti et al., 2006). However, the neuroprotective effect of *Laurus nobilis* against PD has not been reported. In this study, the biological activity of the *n*-hexane fraction from *Laurus nobilis* (Lauraceae) leaves (HFL) and its two major compounds, costunolide and dehydrocostus lactone, were examined in vitro and in vivo to evaluate the therapeutic potential of HFL against PD.

### MATERIALS AND METHODS

#### Materials

Propidium iodide (PI), Dulbecco’s modified Eagle’s medium, fetal bovine serum, penicillin, and streptomycin were purchased from Meiji Seika (Tokyo, Japan). DA, 6-OHDA, and sodium dodecylsulfate were from Sigma-Aldrich Co. (St. Louis, MO, USA). Hybond-polyvinylidene difluoride membrane was obtained from Amersham Pharmacia Biotechnology (Piscataway, NJ, USA).

#### Extraction and analysis of HFL

The leaves of *Laurus nobilis* were imported from Turkey by Orege Forest Agricultural & Food Products Foreign Trade, Ltd. The plant was identified by professor Youngbae Shu. Voucher specimens (NPRI-Q003) have been deposited in the Natural Products Research Institute herbarium, Seoul National University, Korea. The dried leaves of *Laurus nobilis* (40.0 kg) were extracted with methanol (3×20 L, 24 hr each) at room temperature and concentrated under vacuum at 40°C to yield a brown residue (2.4 kg). The residue was partitioned between

### Table 1. IC_{50} value represents the concentration (μM) required for 50% neuroprotective effects of the compounds against H_{2}O_{2}- or DA-induced apoptotic cell death in SH-SY5Y cells. CC_{50} represents the concentration of compound required for 50% toxic effects.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Units</th>
<th>IC_{50} mean ± S.D. H_{2}O_{2} induced apoptosis</th>
<th>Dopamine-induced apoptosis</th>
<th>50% cytotoxic concentration (CC_{50}) mean ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HFL</td>
<td>(μg/ml)</td>
<td>17.21 ± 1.2</td>
<td>3.02 ± 0.22</td>
<td>47.1</td>
</tr>
<tr>
<td>Costunolide</td>
<td>(μM)</td>
<td>3.7 ± 1.1</td>
<td>7.3 ± 0.90</td>
<td>23.3 ± 0.50</td>
</tr>
<tr>
<td>Dehydrocostus lactone</td>
<td>(μM)</td>
<td>1.9 ± 0.41</td>
<td>3.6 ± 0.82</td>
<td>18.8 ± 1.1</td>
</tr>
</tbody>
</table>

### Fig. 1. (A) HPLC chromatogram of HFL. (B) The relative constituents of HFL and the major compounds, costunolide (26.2%) and dehydrocostus lactone (14.6%).