# Antioxidative and Neuroprotective Constituents Isolated from *Opuntia ficus-indica* var. *saboten*

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**Abstract** - *Opuntia ficus-indica* var. *saboten* (Cactaceae) is widely cultivated in Jeju Island (South Korea) for use in the manufacture of health foods such as tea, jam and juice. Its fruit and stem have been traditionally used as oriental folk medicine to treat diabetes, hypertension, asthma, burns, edema and indigestion. During the search for naturally occurring antioxidative neuroprotective agents, it was found that the ethyl acetate fraction of the stems and fruits of *Opuntia ficus-indica* var. *saboten* exhibited potent antioxidant effect. Using a chromatographic fractionation method, several constituents were isolated from this fraction. Among isolates, KYS50182 showed the most potent *in vitro* and *in vivo* neuroprotective activities, suggesting that the flavonoid can serve as a lead structure for the development of neuroprotective agents by providing neuroprotection against oxidative and focal ischemic neuronal injuries.

## Introduction

Ischemic stroke is caused by blockage in an artery that supplies blood to the brain, resulting in a deficiency in blood flow. During ischemic stroke, diminished blood flow initiates a series of events that may result in additional, delayed damage to brain cells. Recurrent stroke is also frequent; about 25 percent of people who recover from their first stroke will have another stroke within 5 years. Recurrent stroke is a major contributor to stroke disability and death, with the risk of severe disability or death from stroke increasing with each stroke recurrence.

Early treatment can help minimize damage to brain tissue and improve the prognosis (outcome). Acute treatment for ischemic stroke involves removing the blockage and restoring blood flow. Tissue plasminogen activator (t-PA) is only a medication at the present time that can break up blood clots and restore blood flow when administered within 3 hours of the event. However, this medication carries a risk for increased intracranial hemorrhage and is not used for hemorrhagic stroke.

Neuroprotectants are medications that protect the brain from secondary injury caused by stroke. There are several different classes of neuroprotectants that show promise for future therapy, including calcium channel antagonists and glutamate

receptor antagonists. These agents modulate neuronal receptors to reduce release of excitatory neurotransmitters. However, these agents often produce severe toxic or side effects probably due to the contribution to early stage of neuronal signal transduction cascades.

On the other hand, brain ischemia initiates a complex cascade of metabolic events, several of which involve the generation of nitrogen and oxygen free radicals. These free radicals and related reactive chemical species mediate much of damage due to their higher reactivity that occurs after transient brain ischemia, and in the penumbral region of infarcts caused by permanent ischemia. Therefore, the use of antioxidant compounds, for example, 21-amino steroids, has shown neuroprotection in animal models.

*Opuntia ficus-indica* var. *saboten* is a tropical or subtropical plant that has been widely used as folk medicine for the treatment of asthma, burn, edema and gastritis in Korea.[1] During the search program for biologically active compounds, the ethyl acetate extract of *O. ficus-indica* var. *saboten* was shown to possess potent antioxidant activities on DPPH radical and superoxide anion radicals scavenging assay systems. Phytochemical studies have led to isolation of several flavonoids that have potent antioxidative activities in several assay systems.[2] Based on these results, we examined neuroprotective effects of some flavonoids isolated from *O. ficus-indica* var. *saboten* in

a rat model of transient focal cerebral ischemia as an attempt to develop neuroprotective agents for the treatment of ischemic stroke.

## Methods

### 1. Extraction and isolation

The extraction and isolation of active principles from the stems of *O. ficus-indica* var. *saboten* were conducted according to our previous report.[2] In brief the stem of *O. ficus-indica* var. *saboten* was extracted with methanol and partitioned with $CH_2Cl_2$, EtOAc, and *n*-butanol. The most antioxidative EtOAc-soluble fraction from *O. ficus-indica* var. *saboten* yielded nine compounds including quercetin (KYS50043), kaempferol (KYS50179), (+)-dihydroquercetin (KYS50181), and quercetin 3-methyl ether (KYS50182) as the anti-oxidative principles after chromatography in silica gel and LiChroprep® RP-18 (Merck). The structure of the compounds was determined by its physico-chemical and spectral data.

### 2. Evaluation of brain injury induced by 2-hr transient focal cerebral ischemia

Rats were sacrificed by decapitation 1 day after occlusion of middle cerebral artery (MCA). After removing brains, seven serial coronal slices of 2-mm thickness were made starting at 1 mm from the frontal pole, incubated in 2% solution of 2,3,5-triphenyltetrazolium chloride (TTC) in normal saline at 37℃ for 60 min for vital staining, and fixed in 10% phosphate-buffered formalin for photography. The areas of the uncorrected infarct area in the cortex and striatum, and the total areas of both hemisphere were measured for each slice using a computerized image analysis system (Optimas). Areas not stained red with TTC were considered infarcted. The uncorrected infarct volume was calculated by multiplying the area by the slice thickness and summing the volumes. The corrected infarct area in a slice was calculated to compensate for the effect of brain edema by subtracting the area of normal tissue in the ipsilateral hemisphere from the total area of the contralateral hemisphere. Total corrected infarct volume was then calculated by multiplying the area by the slice thickness and summing the volumes. Hemispheric swelling representing tissue edema was expressed as the percent increase in the size of the ipsilateral hemisphere compared with the contralateral hemisphere (% edema).

**Results and Discussion**

In the preliminary study, some flavonoids isolated from *O. ficus-indica* var. *saboten* showed potent protective effects against oxidative neuronal cell injuries caused by $H_2O_2$ and superoxide anion radicals.[3] Among isolates, KYS50182 was shown to produce the most potent protective effects in the neuronal cell culture system. Thus, KYS50182 was selected and further examined for the neuroprotective effects in a rat model of transient focal cerebral ischemia.

Neuroprotective effects of flavonoids at a dose of 10 mg/kg (i.p.) on ischemic brain damage were examined in a rat model of 2-hr transient focal cerebral ischemia. The extent of brain damage was measured 1 day after MCA occlusion. KYS50182, which produce the most potent protective effects in the neuronal cell culture system, showed significant neuroprotective effect reducing infarct volume and edema by 45.6% and 54.7%, respectively, compared with the vehicle-treated control group with behavioral recovery effect. KYS50034 and quercetin, also produced some degree of neuroprotective effect. However, KYS50190, a racemic mixture form of KYS50181 had no protective effect.

Dose-related neuroprotective effects of KYS50182 on infarct volumes, edema and neurological score, were also examined by administrating KYS50182 intravenously 30 min after induction of 2-hr transient ischemia at the doses of 1, 3, 10, 30 and 50 mg/kg. It showed a "U"-shaped dose-response curve. At the doses of 10 and 30 mg/kg significant neuroprotective effects were observed.

**References**


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