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Antioxidant and Cholinesterase Inhibitory Activities of Aqueous Extract from Rainbow Trout *Oncorhynchus mykiss*

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We investigated the antioxidant and cholinesterase inhibitory activities of the aqueous extract of rainbow trout *Oncorhynchus mykiss*. The antioxidant activity of *O. mykiss* aqueous extract was determined by *in vitro* peroxynitrite scavenging activity and reducing power assays. The aqueous extract of *O. mykiss* showed potent peroxynitrite radical scavenging activity (IC_{50}=0.12±0.001 mg/mL) and reducing power (absorbance=0.47±0.001) at the concentration of 1 mg/mL. The *in vitro* cholinesterase inhibitory activity of *O. mykiss* aqueous extract was examined using spectrophotometric analyses of acetyl- and butyrylcholinesterase. The aqueous extract of *O. mykiss* showed acetylcholinesterase inhibitory activity (IC_{50}=1.61±0.13 mg/mL), but did not exhibit inhibitory activity against butyrylcholinesterase. These results suggest that *O. mykiss* possesses antioxidant and acetylcholinesterase inhibitory activities and provide scientific evidence for the health benefits of *O. mykiss* aqueous extract.

Key words: *Oncorhynchus mykiss*, Rainbow trout, Antioxidant, Cholinesterase

Introduction

The overproduction of free radical species causes oxidative stress that contributes to cellular aging and neuronal damage, and it is associated with chronic degenerative diseases, including Alzheimer's disease (AD) (Sastre et al., 2000). AD, a progressive neurodegenerative disorder that is commonly seen in elderly individuals, is characterized by memory and cognitive ability loss, behavior disturbances, and personality changes (Bachman et al., 1992). The etiology of AD remains unclear; one of the most convincing theories is the cholinergic hypothesis, which implicates a deficiency in neurotransmitters, such as acetylcholine (ACh) and butyrylcholine (BCh) (Schneider, 2001). Efforts to prevent AD have focused on the activation of cholinergic functions through the inhibition of cholinesterase (ChE), which hydrolyzes the cholinergic neuromediators and reduces oxidative stress (Schneider, 2001).

Oncorhynchus mykiss belongs to the Salmonidae family of North American cold-water fish (Behnke, 1992). *O. mykiss* extracts have been reported to have potential health benefits, such as 3-hydroxy-3-methylglutaryl coenzyme A reductase expression reduction and antimutagenic effects (Oh et al., 2008, 2009).

In the present study, the antioxidant and ChE inhibitory activities of *O. mykiss* aqueous extract were investigated *in vitro* by evaluating its ability to scavenge peroxynitrite anions ($ONOO^-$), its reducing power, and its inhibitory activity against acetyl- (AChE) and butyrylcholinesterase (BChE).

Materials and Methods

Materials

O. mykiss (average total length: 40-50 cm) were collected in May 2010 at Sangju City, Gyeongbuk, South Korea. Samples were prepared by removing the heads, fins, bones, and internal organs of the fish and obtaining extract in five times the volume of distilled water at 100°C for 3 h. After evaporating,

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the extract was freeze-dried.

Reducing power assay

Reducing power was evaluated by the method of Oyaizu (1986). Various concentrations of samples (2.5 mL) were mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. After incubation at 50°C for 20 min, 2.5 mL of 10% trichloroacetic acid (w/v) was added. The mixture was centrifuged at 2,000 *g* for 10 min, and 5 mL of the upper layer was mixed with deionized water and 1 mL of 0.1% ferric chloride. Absorbance was measured at 700 nm using a spectrophotometer (Powerwave XS; Bio-Tex, Inc., Houston, TX, USA). L-ascorbic acid was used as a positive control.

Peroxynitrite scavenging activity

$ONOO^-$ scavenging activity was measured by the method of Kooy et al. (1994) with minor modification. Each sample was mixed with rhodamine buffer (pH 7.4) and 5 mM dihydrorhodamine (DHR) 123. After incubation for 5 min, the sample was treated with authentic $ONOO^-$. The fluorescence intensity of the oxidized DHR 123 was measured with a microplate fluorescence reader (Wallac 1420; PerkinElmer, Waltham, MA, USA) at excitation and emission wavelengths of 485 nm and 530 nm, respectively. L-ascorbic acid was used as a positive control.

Cholinesterase inhibitory activity assay

Inhibitory activity against ChE was measured using the spectrophotometric method developed by Ellman et al. (1961). The reaction mixture contained 140 mL of sodium phosphate buffer (pH 8.0), 20 μL of sample, and 20 μL of either AChE (0.36 U/mL) or BChE (0.36 U/mL). The solution was placed in a 96-well microplate, mixed, and incubated at room temperature for 15 min. After incubation, 10 μL of Ellman's reagent and 10 μL of ACh or BCh were added. The absorbance of all reactions was measured using a spectrophotometer (Powerwave XS; Bio-Tex, Inc.). Eserine was used as a positive control.

Proximate composition analysis

The proximate composition analysis was performed using the methods described by the Association of Official Analytical Chemists (1995).

Results and Discussion

Antioxidant activity

Antioxidant properties are related to radical scavenging and reduction capacities. $ONOO^-$ is a strongly oxidizing and nitrating species that induces lipid peroxidation, thiol oxidation, and amino acid modification and causes DNA damage. $ONOO^-$ overproduction may cause several neurological diseases, such as AD and Parkinson's disease (Squadrito and Pryor, 1998). Reduction is related to the donation of hydrogen atoms to ferric complexes and can disturb reactions between radicals (Singh and Rajini, 2004).

The antioxidant activity of *O. mykiss* aqueous extract was determined by evaluating $ONOO^-$ scavenging activity and reducing power. Table 1 and Fig. 1 show the results of these analyses. *O. mykiss* aqueous extract exhibited potent $ONOO^-$ scavenging activity (IC_{50}=0.12±0.001 mg/mL) and considerable reducing power that increased in a dose-dependent manner (absorbance=0.19±0.003, 0.26±0.0001, 0.30±0.004, and 0.47±0.001 at concentrations of 0.1, 0.2, 0.5, and 1 mg/mL, respectively). However, the $ONOO^-$ radical scavenging activity and reducing power of *O. mykiss* were lower than those of L-ascorbic acid, which was used as a positive control.

According to Atif et al. (2006) and Oh et al. (2008), fish in the Salmonidae family contain small quantities

Table 1. The peroxynitrite ($ONOO^-$) scavenging and cholinesterase inhibitory activity of the aqueous extract of *Oncorhynchus mykiss*

Samples	IC_{50} (mg/mL)		
	$ONOO^-$	AChE	BChE
O. mykiss	0.12 ± 0.001	1.61 ± 0.13	NE
L-ascorbic acid	0.00013 ± 0.00007		
Eserine		0.0025 ± 0.0002	0.0076 ± 0.0003

The values of $ONOO^-$ scavenging and cholinesterase (ChEs) inhibitory activities were expressed as the means±SD of 50% inhibitory concentrations of three experiments. L-ascorbic acid and eserine were used as positive controls of $ONOO^-$ scavenging and cholinesterase inhibitory activity assays, respectively.
AChE, acetylcholinesterase; BChE, butyrylcholinesterase; NE, no effect.

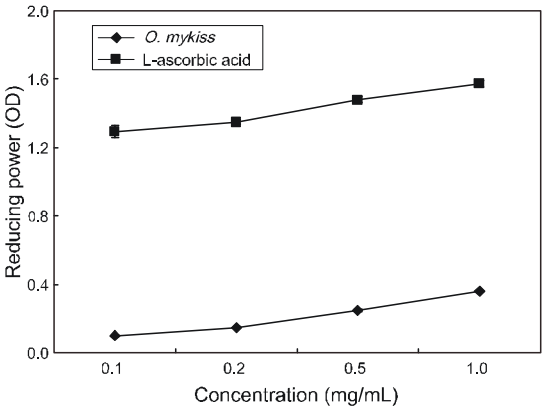

Fig. 1. The reducing power of the aqueous extract of *Oncorhynchus mykiss*.

of several bioactive constituents, including phenolic ingredients and metallothioneins, which have shown positive correlations with antioxidant activity. The antioxidant activities of *O. mykiss* aqueous extract may be due to the presence of these constituents.

Cholinesterase inhibitory activities

ChE inhibitors are recognized as the most promising therapeutic agents for AD, and have been shown to affect cognitive and behavioral symptoms in clinical studies (Giacobini, 2004). AChE inhibitors increase the endogenous levels of ACh and cholinergic neurotransmission. BChE inhibitors decrease the accumulation of neural plaques in senile brains. Thus, the achievement of a balance between AChE and BChE inhibitors may increase the efficacy of treatment (Yu et al., 1999).

The ChE inhibitory activity of *O. mykiss* aqueous extract was evaluated by AChE and BChE inhibitory activity assays. Table 1 presents the results of these analyses. *O. mykiss* aqueous extract exhibited AChE inhibitory activity (IC_{50}=1.61±0.13 mg/mL) but showed no BChE inhibitory activity. This selective AChE inhibitory activity may be due to the binding properties between the enzyme and substrate (Silman and Sussman, 2005). AChE is a substrate-specific enzyme that exists in nerve synapses and can degrade only ACh, whereas BChE is a nonspecific enzyme located in plasma and tissues (Silman and Sussman, 2005). As with antioxidant activities, the AChE inhibitory activity of *O. mykiss* aqueous extract was lower than that of eserine, which was used as a positive control.

Proximate composition analysis

The proximate composition of *O. mykiss* meat is shown in Table 2. The moisture, crude protein, crude lipid, and ash contents of *O. mykiss* meat were determined to be 71.9±1.04, 20.8±0.78, 4.5±0.28, and 1.7±0.09% per 100 g of edible meat, respectively. The lipid components of *O. mykiss* meat contain bioactive constituents, including carotenoids and unsaturated fatty acids (Matsuno et al., 1980; Kim and Choi, 1993, Choi et al., 2010). Carotenoids, such as astaxanthin, zeaxanthin, canthaxanthin, diatoxanthin, and cryptoxanthin, and polyunsaturated fatty acids, including eicosapentaenoic acid and docosahexaenoic acid, are abundant in the integument and muscle of *O. mykiss*. These constituents have been reported to exhibit several beneficial bioactivities, including neuroprotective effects against neurotoxicity (Matsuno et al., 1980; Kim and Choi, 1993; Lim et al., 2005).

Table 2. The proximate composition of *Oncorhynchus mykiss* meat

Components	*O. mykiss* meat (%)
Moisture	71.9 ± 1.04
Crude protein	20.8 ± 0.78
Crude lipid	4.5 ± 0.28
Ash	1.7 ± 0.09

The values were expressed as the mean±SD (n=3).

In conclusion, *O. mykiss* aqueous extract showed potent AChE inhibitory activity that may be related to its antioxidant properties. Further detailed investigations are necessary to isolate and identify the active constituents in the extract and to clarify the mechanism of their activity.

Acknowledgments

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