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ORIGINAL ARTICLE

Protective Effect of Water Extracted *Spirulina maxima* on Glutamate-induced Neuronal Cell Death in Mouse Hippocampal HT22 Cell

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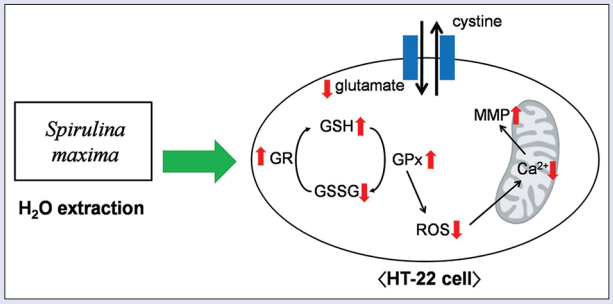
ABSTRACT

Introduction: *Spirulina maxima* was used as important nutritional source in the Aztec civilization because it is rich in proteins and vitamins. It contains various antioxidants such as phycocyanin and flavonoids. Based on abundant antioxidants, *S. maxima* is known to possess anti-inflammatory effect, especially on neuronal cells. **Materials and Methods:** *S. maxima* was extracted in water and contain of phycocyanin was identified by high-performance liquid chromatography. Cell viability test was performed with treatment of *S. maxima* extract. After, oxidative stress-related mechanisms were evaluated by detecting the accumulation of reactive oxygen species (ROS) and Ca^{2+} influx, and decrease of mitochondrial membrane potential (MMP) level. Then, the glutathione (GSH) related assays were conducted. **Results:** The water extracted *S. maxima* exerted the neuroprotective activity by attenuating the ROS and Ca^{2+} formation, maintaining the MMP level, and protecting the activity of the antioxidant enzymes by increasing reduced GSH against oxidative stress compared to control. **Conclusion:** The results suggested that water extracted *S. maxima* showed powerful neuroprotective effect through the mechanism related to antioxidant activity, able to preventing the radical-mediated cell death.

Key words: HT22 cell, neuroprotection, oxidative neurotoxicity, phycocyanin, *Spirulina maxima*

SUMMARY

- Water extracted *Spirulina maxima* contains C-phycocyanin
- Water extracted *Spirulina maxima* exerts neuroprotective effect on HT22 cell
- To investigate the protective mechanisms, reactive oxygen species, Ca^{2+}, mitochondrial membrane potential, Glutathione-related assays were performed.



Abbreviations used: ROS: Reactive oxygen species; MMP: Mitochondrial membrane potential; GSH: Glutathione; GSSG: Glutathione disulfide, oxidized glutathione; GPx: Glutathione peroxidase; GR: Glutathione reductase; DMEM: Dulbecco's modified Eagle's medium; FBS: Fetal bovine serum; DCF-DA: 2′,7′-dichlorofluorescein diacetate; PBS: Phosphate buffered serum; Rho 123: Rhodamine 123; NADPH: Nicotinamide adenine dinucleotide phosphate; DTNB: 5,5′-dithiobis-2-nitrobenzoic acid, Ellman's reagent; GSSG-R: Glutathione disulfide reductase; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DMSO: Dimethyl sulfoxide; HPLC: High-performance liquid chromatography.

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INTRODUCTION

Glutamate the endogenous neurotransmitter widely dispersed in the central nervous system. However, excessive glutamate is hazardous for neuronal cells.[1] Glutamate elicits numerous cytotoxic effects which led to pathological cell death.[2] Neuronal cell death is regarded as a major factor of neurological disorders such as Alzheimer's disease and Parkinson's disease. One main pathway that has been elucidates the glutamate toxicity is the inhibition of cystine/glutamate antiporter system caused by intracellular oxidative stress. In addition, decreased level of cystine led to the depletion of glutathione (GSH).[3]

Reactive oxygen species (ROS) becomes the main contributors of neuronal cell death and various pathological processes in our body.[4,5] In neurological aspect, elevated ROS brings about alterations in Ca^{2+} homeostasis which triggers the neurotoxicity.[6-8] Increased intracellular Ca^{2+} influx can give rise to mitochondrial dysfunction and also initiates the early apoptotic cell death.[9] After these processes,

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pores are formed at mitochondria, and the mitochondrial membrane potential (MMP) collapses.[10]

Antioxidants are also required to reverse the actions of ROS. Mammalian cells possess defense systems against free radical detoxification. GSH plays a key role for essential cellular antioxidant. High contents of intracellular GSH protect cells from ROS offense by vicariously reacting with ROS. For example, it reacts with the hydrogen peroxide (H_2O_2) and then converts it to H_2O catalyzed by glutathione peroxidase (GPx). Then, GSH is oxidized to glutathione disulfide (GSSG). GPx derived GSSG is reduced to GSH catalyzed by the glutathione reductase (GR).[11,12]

Spirulina maxima is a free-floating filamentous blue-green-algae.[13] Conventionally, it have been used as important food source by the Aztec civilization.[14] It is nutritionally invaluable because of its high-protein content (about 70%).[15,16] In addition, it contains various antioxidants such as superoxide dismutase (SOD), provitamin-A (beta-carotene), Vitamin C, E, and phycocyanin, and flavonoids, so it decelerates the aging.[17] Currently, it gains more attention because of its various medicinal properties. It also shows anti-inflammatory effect, especially on neuronal cells.[18] *S. maxima* contains phycobilisomes which acts as the light-producing protein-pigment complexes.[19] Phycobilisomes are mainly consisted of polypeptides named phycobiliproteins. One of the important phycobiliproteins which is contained in this microalgae is phycocyanin. This phycocyanin seems to be related to its antioxidant and anti-inflammatory activity.[20,21]

As mentioned above, *S. maxima* and its component phycocyanin are already well-known to its antioxidant and anti-inflammatory properties.[22] Under oxidative stress and immoderate inflammatory responses, the nerve system is prone to be damaged and it can eventually cause neurotoxicity, so antioxidant and anti-inflammatory activities become major keywords for neuroprotection.[23] Based on this, it also demonstrated that *S. maxima* possesses neuroprotective activity on several cell lines.[17,24,25] However, there is just one previous study experimented with HT22 cell,[26] and in this paper, the extraction method is differed from it to give weigh on containing phycocyanin. Because phycocyanin is the water-soluble protein pigment, we hypothesized that it would be more abundant when extracted *S. maxima* with distilled water.

The immortalized mouse hippocampal HT22 cell, which lacks the functional ionotropic glutamate receptor fits perfectly to evaluate glutamate toxicity caused by oxidative stress.[19] Therefore, HT22 cell was used in this study to investigate the neuroprotective mechanisms of *S. maxima* against glutamate-induced injury. Because of the absence of glutamate receptor, cell death from excitotoxicity can be excluded from the study.[27] It also used as a tool for screening the novel neuroprotective reagents.

To assert the water extracted, *S. maxima* as potential candidate for prevention and/or treatment of the neurodegenerative diseases, we explored the possible mechanisms hypothesized that water extracted *S. maxima* reveals neuroprotective effects on glutamate-induced cytotoxicity in HT22 cell. First, cell viability test at HT22 cell was performed with the treatment of *S. maxima*. To discover the definite mechanisms of protection, the production of ROS, Ca^{2+} influx and mitochondrial disruption were evaluated. Furthermore, we also determined the contents of GSH- and other GSH-related antioxidant enzymes – GR and GPx.

MATERIALS AND METHODS

Plant material and sample preparation

The powder sample of *Spirulina maxima* was provided by Korea Institute of Ocean Science and Technology. For ultrasonic extraction, 50 g of *S. maxima* powder was put into 1 L of distilled water and treated for 6 h at a temperature of 25°C and 40 kHz using ultrasonic extractor (AUG-R3-900, ASIA ULTRASONIC, Gyeonggi-do, Korea). After centrifuged at 8000 rpm and evaporated, the *S. maxima* extract was obtained.

High-performance liquid chromatography analysis and component identification

To identify the phycocyanin from water extracted *S. maxima* sample, extracted *S. maxima* and purchased C-phycocyanin were analyzed by HPLC-DAD. C-phycocyanin from *Spirulina* sp. was purchased from Sigma–Aldrich (St. Louis, MO, USA, Cat # P2172). The high-performance liquid chromatography (HPLC) (Dionex) equipped with an LPG 3 × 00 pump, an ACC-3000 autosampler, a DAD-3000 (RS) diode array ultraviolet (UV)/visible detector, and a column oven. Both samples were separated on a Jupiter C_5 column (5 μm, 300 Å, 4.6 mm × 250 mm) at 25°C. The mobile phase consisted of 20% (v/v) aqueous acetonitrile (ACN) solution containing 0.1% (v/v) trifluoroacetic acid (TFA) and all reagents were purchased from J. T. Baker as HPLC grade. The flow rate was 1.0 mL/min and the concentration of *S. maxima* sample was 100 ppm and phycocyanin was 1000 ppm. The injection volume of each sample was 20 μL. The output signal of the detector was recorded using a Dionex Chromeleon ™ Chromatography Data System. The UV wavelength was 580 nm and 640 nm, respectively, and the chromatograms were acquired at 580 nm.

Cell viability test

The mouse hippocampal HT22 cells were obtained from Seoul National University, Korea. Cells were cultured in Dulbecco's modified Eagle's medium containing 10% (v/v) fetal bovine serum (FBS), 1% penicillin-streptomycin, 2 mg/mL of $NaHCO_3$, and 15 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) at 37°C with 5% CO_2. The cultured cells were seeded in a 48-well plate at a density of 1.9×10^4 cells/well. After 23 h incubation, cells were pretreated with 50 μM of trolox as a positive control and different concentrations of *S. maxima* (1, 10, or 100 μg/mL) for 1 h, followed by treatment with glutamate (2.5 mM) for 24 h and except nontreated control. The cell viability of *S. maxima* was assessed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)-based colorimetric assay. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide solution) (150 μL of 1 mg/mL) was added to each well. After the 3 h incubation at 37°C, the supernatant was removed, and produced MTT formazan crystals were solubilized in 200 mL dimethyl sulfoxide (DMSO). The absorbance was read at 540 nm using microplate reader. FBS was obtained from Gibco BRL. Co. and other materials were purchased from Sigma-Aldrich (USA).

Measurement of intracellular reactive oxygen species

To evaluate the intracellular ROS formation, the cells were stained with 2',7'-dichlorofluorescein diacetate (H_2-DCF-DA) (Sigma-Aldrich, USA), an indicator of oxidative stress. 10 μM of DCF-DA was melted in Hanks' balanced salt solution. After the pretreatment and treatment as same as cell viability test and 8 h incubation, the cells were then 30 min more harvested and washed with phosphate buffered serum (PBS). The washed cells were suspended in 1% Triton X-100 (Sigma-Aldrich, USA). Fluorescence intensity was checked at an excitation wavelength of 485 nm and an emission wavelength of 528 nm using fluorometer.

Measurement of intracellular Ca^{2+}

For the measurement of intracellular free Ca^{2+}, HT22 cells were cultured with 20 μM of Fura-2 AM (Dojindo, USA), which is the indicator of membrane-permeable Ca^{2+} after pretreatment with *S. maxima* and trolox. After an hour, the cells were loaded with 3.8 mM of glutamate and 2 h more incubated at 37°C. Subsequently, cells were washed with PBS and melted by 1% Triton X-100. Ca^{2+} dependent fluorescence intensity was measured at 340 nm of excitation wavelength and 380 nm of emission wavelength using fluorometer.

Measurement of mitochondrial membrane potential

Briefly, after treatment and 24 h incubation, cells were stained with rhodamine 123 (Rho 123) (Sigma-Aldrich, USA). Rho 123 was used to evaluate the MMP. 10 μM of Rho 123 was added to whole well and incubated for 30 min. The cells were washed by PBS and suspended in 1% Triton X-100. The fluorescence intensity was analyzed by fluorometer at an excitation wavelength and emission wavelength of each 485 nm and 528 nm.

Determination of glutathione content

The cultured cells were seeded onto 6-well plate at a density of 3.4×10^4 cells/well. After overnight incubation, cells were treated with sample to be tested for an hour followed by 2.5 mM glutamate insult. Treated cells were then incubated one more overnight and centrifuged for 30 min at 3000 g, 4°C. The supernatant was collected and used for the measurement of antioxidant enzymes– total GSH (GSH + GSSG), GR, and GPx.

Total GSH (GSH + GSSG) in the supernatant was detected using the enzymatic cycling method by Tietze.[28] The supernatant was mixed together with 0.3 mM NADPH, 0.6 mM DTNB (5,5'-dithiobis-2-nitrobenzoic acid, Ellman's reagent) and 5 unit/mL of GSH disulfide reductase (GSSG-R). After 30 s incubation at 37°C for reaction, the absorbance was read at 312 nm using microplate reader.

GR activity was investigated followed by the method of Carlberg and Mannervik,[29] which based on the diminution of GSH in the presence of NADPH. Supernatant, 0.1 mM NADPH and 1 mM oxidized glutathione (GSSG) were mixed together and reacted for 2 min at 37°C. The absorbance of the assay was immediately measured under 340 nm by microplate reader.

The activity of GPx was evaluated by quantifying the rate of oxidation of GSH to GSSG and H_2O_2 was used as the substrate.[30] All the reactions of supernatants were performed at 37°C for 3 min in the presence of 0.4 mM NADPH, 0.2 mM H_2O_2, 1 mM of L-glutathione reduced (GSH) and 1 unit/mL of GSH disulfide reductase (GSSG-R). Following then, the changed NADPH concentration was determined by microplate reader at 340 nm. All chemicals are purchased from Sigma-Aldrich (USA).

Statistical analysis

All data of the results are expressed as the means ± standard deviation. Each experiment was repeated three times or more than that. The determination of significance treatment effects was based on the one-way analysis of variance. Values of $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ were considered statistically significant compared to only glutamate-treated group. The whole data were expressed as percentage compared to nontreated group setting on 100%.

RESULTS

Identification of phycocyanin from water extracted *Spirulina maxima* by High-performance liquid chromatography-DAD

HPLC-DAD analysis of water extracted *S. maxima* sample was performed to detect the phycocyanin. The HPLC results reveal that *S. maxima* sample analysis contains all of four same peaks as phycocyanin [Figure 1]. At the result of phycocyanin, the four peaks were appeared at the retention time of 3.3, 3.9, 14.7, and 16.0 min [Figure 1a]. *S. maxima* sample also contains four peaks at the almost same retention time and their UV chromatograms were not presented in this paper but also showed similar forms as phycocyanin [Figure 1b]. This suggested that the water extraction of *S. maxima* can effectively contain the phycocyanin.

Neuroprotective effects of water extracted *Spirulina maxima* in glutamate-injured HT22 cells

To investigate the protection effect of water extracted *S. maxima* on the cell viability verses glutamate toxicity, HT-22 cells were treated with three different concentrations of sample overnight, and then, cell viability was monitored by the MTT assay [Figure 2]. This assay is based on the reduction of MTT to a purple formazan product by survived mitochondrial dehydrogenase enzyme. Because the experiment pointed at the prevention effect of *S. maxima* over toxicity, the samples were pretreated before 1 h than glutamate treat. As resulted, about 53.01% of the glutamate-stimulated cells remained viable compared to those not stimulated. However, pretreatment with dose-dependent manner (1–100 μg/mL) of *S. maxima* gradually raised the viability against the cells only treated with glutamate. At the 10 and 100 μg/mL concentration of *S. maxima*, the sample showed significant protection effect. Each

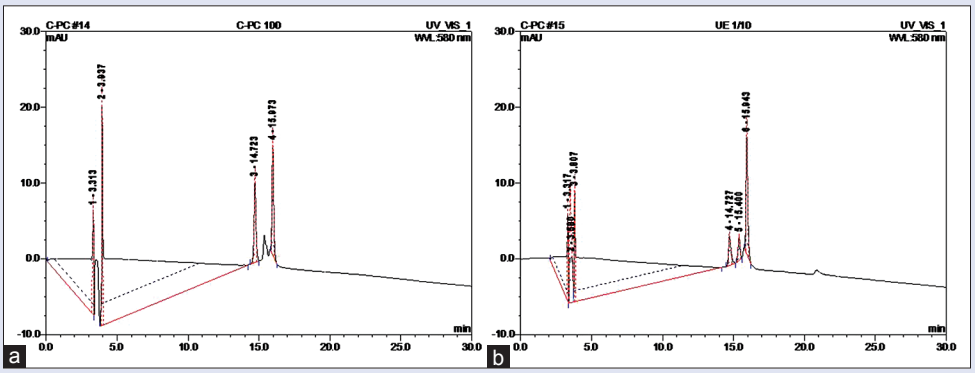

Figure 1: High-performance liquid chromatography-DAD analysis (λ = 580 nm) of phycocyanin (a) and *Spirulina maxima* (b) using a C_5-column with 20% (v/v) acetonitrile solution containing 0.1% (v/v) trifluoroacetic acid as eluent

protective percentage was 67.18% and 106.97%. The cells were survived about two times more than glutamate-treated group at 100 μg/mL. This result implied a first indication that *S. maxima* could prevent glutamate-induced neuronal cell death.

Inhibition of glutamate-induced cellular peroxide production and Ca^{2+} influx by *Spirulina maxima*

The initial stimulation of ROS and Ca^{2+} influx is seemed to be important indicators for neuronal cell death. It is known that glutamate-induced ROS generation involves a Ca^{2+} influx mechanism.[31] As shown in Figure 3a, *S. maxima* significantly decreased DCF-DA fluorescence intensity – an indicator of ROS production at the concentration of 100 μg/mL especially. The amount of ROS accumulation recovered nearly 106.13% compared to 129.13% of glutamate group. In addition, it also dramatically attenuated the intracellular Ca^{2+} level which is presented by Fura-2 AM intensity at all concentration [Figure 3b]. The fluorescence intensity of Ca^{2+} level decreased from 127.30% to 102.77%, 99.92%, and 92.42% at 1 μg/mL, 10 μg/mL, and 100 μg/mL perspective. All of the results have high significance toward the negative control. The effect of water extracted *S. maxima* treatment dose-dependently expressed at both ROS and Ca^{2+} results. These results suggest that *S. maxima* could effectively contribute to diminish the neurotoxicity by inhibiting ROS generation and Ca^{2+} influx.

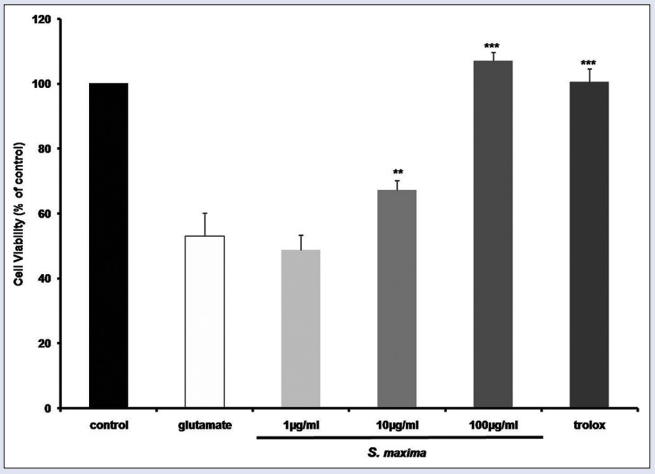

Figure 2: Cytoprotective effects of *Spirulina maxima* on glutamate-injured HT22 cells. Cells were pretreated with three different concentrations of *Spirulina maxima* (1, 10, or 100 μg/mL) and trolox as positive control for an hour followed by stimulation with 2.5 mM glutamate. Cell viability was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Data are presented as means ± standard deviation ($n = 5$, $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ verses only glutamate-treated group)

Recovery of mitochondrial membrane potential loss by *Spirulina maxima*

The mitochondrial damage during apoptosis drops the level of MMP. The active mitochondria disrupted in the early apoptosis, and due to mitochondrial oxidation, the MMP level is diminished. Rho 123 was used to detect this change. It aggregates in normally polarized mitochondria but in the case of apoptotic depolarized cell, where MMP level is lowered, Rho 123 is diffused throughout the cell and emits a green fluorescence. Therefore, if cells were protected from the oxidative stress, the fluorescence intensity increases. As shown in Figure 4, *S. maxima* treatment fairly augmented the fluorescence intensity. It showed the strongest fluorescence at the highest concentration with the value of 91.85% while 74.97% at glutamate group. There is no significance in 1 μg/mL and 10 μg/mL but it also gradually increased proportionally to concentration. Thus, this result demonstrates that *S. maxima* can block the mitochondrial pathway in glutamate-induced apoptosis.

The changes of glutathione contents and antioxidant enzyme activities in glutamate-injured HT22 cells by *Spirulina maxima*

To further elucidate the antioxidant effects of *S. maxima*, the changes on the cellular GSH contents and activities of antioxidant enzymes were determined. The sulfide group of GSH reacts with DTNB and produces a yellow-colored TNB. The mixed disulfide – GSTNB recycles the GSH and produces more TNB. The total rate of TNB production is in turn directly the proportional to the concentration of GSH.[32] GR activity was measured by detecting the reduction of GSSG in the presence of NADPH,[29] and GPx was evaluated by quantifying the content of oxidation of GSH to GSSG.[30]

The total GSH amount was plenty enough at all concentrations with the value of 98.59%, 96.48%, and 97.03% per each concentration compared to those treated with only glutamate – 66.92% [Figure 5a]. The decrease of GR activity by glutamate was significantly affected at the concentration of 100 μg/mL *S. maxima* [Figure 5b] while GPx activity was affected at 10 μg/mL, slightly higher than 100 μg/mL [Figure 5c]. The activity of GR increased

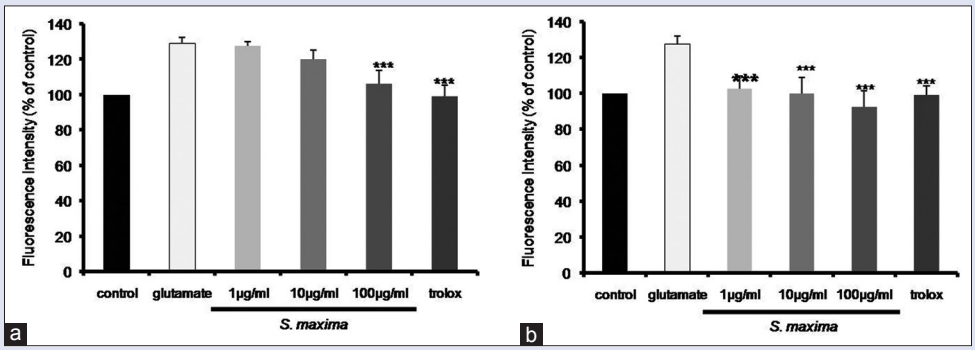

Figure 3: Effects of *Spirulina. maxima* on glutamate-induced intracellular reactive oxygen species accumulation (a) and Ca^{2+} influx (b). Cells were pretreated with three different concentrations of *Spirulina maxima* (1, 10, or 100 μg/mL) and trolox for an hour followed by stimulation with glutamate. Reactive oxygen species and Ca^{2+} were detected by fluorometer and expressed as relative fluorescence intensity. Data are presented as means ± standard deviation ($n = 6$, $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ verses only glutamate-treated group)

from 62.22% to 75.90% at 100 μg/mL statistically $P < 0.01$ significant. GPx significantly showed higher activity at 10 μg/mL of 83.27% and at 100 μg/mL of 81.26% when compared with 74.89% of glutamate group.

DISCUSSION

There are a plenty of researches addressing the neuroprotective effects of natural products against oxidative stress and their defense mechanisms. High content of glutamate may contribute to neuronal cell death and eventually cause the neurological disorder diseases such as dementia. Neurodegenerative diseases have gotten much attention because of their irreversible memorial deficit problem.[33] The hippocampus regarded as important organ for their contribution to short and long-term memory. Based on this functional importance, immortalized mouse hippocampal HT22 cell was selected to investigate whether water extracted *S. maxima* postpone the progress of glutamate-induced neurotoxicity due to its phycocyanin component.

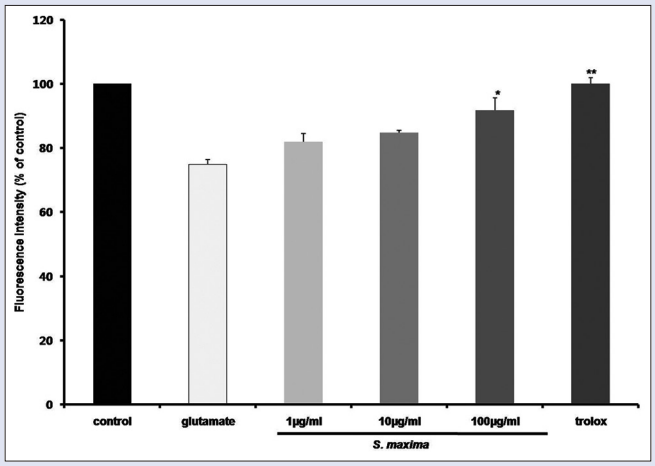

Figure 4: Effects of *Spirulina maxima* on glutamate-induced mitochondrial membrane potential levels. Cells were pretreated with three different concentrations of *Spirulina maxima* (1, 10 or 100 μg/mL) and trolox for an hour followed by stimulation with 2.5 mM glutamate. Changes of mitochondrial membrane potential levels were detected by fluorometer and expressed as relative fluorescence intensity. Data are presented as means ± standard deviation ($n = 3$, $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ verses only glutamate-treated group)

S. maxima has been previously experimented targeting for neuroprotection activity.[17,24,25] However, the extraction method in existing paper is different, and there is no previous study about protective effect of phycocyanin at glutamate damaged HT22 cell line.[26] In this study, we confirmed the mechanism related to protective effect of water extracted *S. maxima* at glutamate-induced neuronal damage using HT22 cell focusing on containing phycocyanin. The earlier study showed neuroprotective effect of ethanol extracted *S. maxima* in HT22 cell; however, we hypothesized that water-soluble protein pigment phycocyanin would be more extracted at distilled water. Therefore, *S. maxima* was extracted at distilled water by using ultrasonic extractor, and after extraction, the high contain of phycocyanin was exerted by HPLC-DAD.

Here, we showed definite neuroprotective effect of water extracted *S. maxima* on HT22 cell by MTT viability test. The protective effect was increased gradually correspond to the concentration of *S. maxima*. To compare the glutamate-injury on cell, high concentrations of glutamate-treated and evaluated at various series of assay. Excessive glutamate triggered oxidative neuronal cytotoxicity and mitochondrial dysfunction due to mechanism that independent to the glutamate receptor.[10] Mitochondrial damage caused by glutamate leads to both unusual generation of intracellular ROS and imbalance of Ca^{2+} influx. In addition to ROS, Ca^{2+} becomes another important mediator of apoptosis. One of the indicators of mitochondrial dysfunction is the loss of MMP. In this study, glutamate increased the level of ROS generation and Ca^{2+} concentration but decreased the MMP compared to nontreated group. In addition, followed by these procedures, the contents of GSH and activity of other antioxidant enzymes include GR and GPx are also decreased.

However, when previously treated with *S. maxima* samples on dose-dependent manner, the excessive generation of neuronal damaging factors is lowered, and on the contrary, shrinkages are recovered. For detailed description, *S. maxima* clearly attenuated the increased

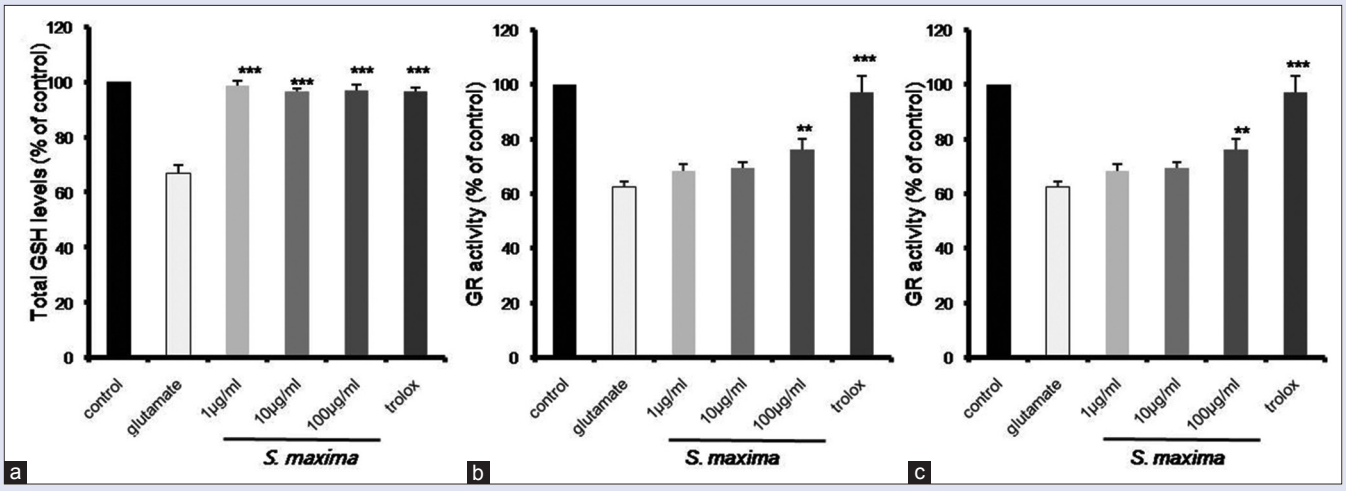

Figure 5: Effects of *Spirulina maxima* on total glutathione + glutathione disulfide amount (a) and activity of glutathione reductase (b) and glutathione peroxidase (c). Cells were pretreated with three different concentrations of *Spirulina maxima* (1, 10, or 100 μg/mL) and trolox for an hour followed by stimulation with 2.5 mM glutamate. The supernatant is collected and (a) glutathione + glutathione disulfide, (b) Glutathione reductase, and (c) Glutathione peroxidase were detected by microplate reader and each data are expressed by percentage absorbance. Data are presented as means ± standard deviation ($n = 3$, $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ verses only glutamate-treated group)

accumulation of ROS and Ca^{2+} influx. This may induce the recovery of MMP level. The pretreatment of *S. maxima* also recovered the total GSH content and GR/GPx activities. GSH levels were remarkably increased at all experiment under three different concentrations. GR and GPx activities are also significantly elevated.

In summary, we could demonstrate that water extracted *S. maxima* effectively scavenges ROS and influences on protective effect toward oxidative stress induced by glutamate through conserving the activities of antioxidant enzymes. Further study pointed at the activity of phycocyanin should be progressed.

CONCLUSION

The present study provides the neuroprotective activity of water extracted *S. maxima* based on mechanism of glutamate-induced injury using HT22 cell line. To prove this, cell viability test, intracellular ROS and Ca^{2+} content detection, total GSH content assay, GR assay, and GPx assay were performed. The whole results showed significant protective effect toward glutamate-injured group. These results suggested that water extracted *S. maxima* can be a powerful resource for the development of novel medicinal foods for neurodegenerative disorders through a mechanism related to antioxidant activity. The further study focused on activity of phycocyanin should be progressed again.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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