

Chapter

Improvement of Cognitive Function by Wasabi Component “Hexaraphane”

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Abstract

Hexaraphane (6-methylsulfinylhexyl isothiocyanate; 6-MSITC) is an isothiocyanate present in the rhizomes and roots of wasabi (*Eutrema japonicum* (Miq.) Kiudz.). It is known to induce detoxifying and antioxidant enzymes by activating the Keap1-Nrf2 system, ameliorating oxidative damage in the body. Hexaraphane was shown to inhibit brain damage and improve dementia symptoms in Alzheimer's model mice. Moreover, in two randomized controlled trials conducted on middle-aged and elderly subjects, the extract powder “Wasabi sulfinyl™” containing 0.8% hexaraphane improved memory, attention, and judgment. In a clinical study of fifteen patients with chronic fatigue syndrome, treatment with Wasabi sulfinyl™ for 3 months improved brain fog and other symptoms.

Keywords: wasabi, hexaraphane, clinical study, brain function, dementia, brain fog

1. Introduction

Wasabi (*Eutrema japonicum* (Miq.) Kiudz.) is a plant species belonging to the Cruciferae family, native to Japan. It is used primarily as a condiment for its distinctive pungent and stimulating flavor. It is sometimes referred to as *Wasabia japonicum*, a synonym for *Eutrema*. *E. yunnanense*, also belonging to the genus *Eutrema*, grows in the wild in mainland China and is similar in form to *E. japonicum*, but lacks its characteristic pungent flavor. Genetic analyses indicated its separation from its Japanese counterpart approximately 5 million years ago, and the Japanese plant subsequently acquired the distinctive pungent flavor [1].

Since ancient times, wasabi has grown in mountain streams throughout Japan and has been used as a medicinal herb for centuries because of its characteristic pungent flavor (**Figures 1** and **2**). The earliest written record is “Wasabi” written on a wooden strip unearthed from a ruin in the village of Asuka, Nara Prefecture, dating from the Asuka period [2]. As the area was a medicinal herb garden, it is believed to have been used as a medicinal herb since ancient times.

“Wasabi” is also mentioned in “Honzo Wamyo,” Japan's oldest encyclopedia of medicinal plants 1100 years ago. According to the dictionary of medicinal food published more than 300 years ago, “Gleanings from the Materia Medica,” it disperses depression, generates sweating, dispels wind (shallow evil poison as the cause of the

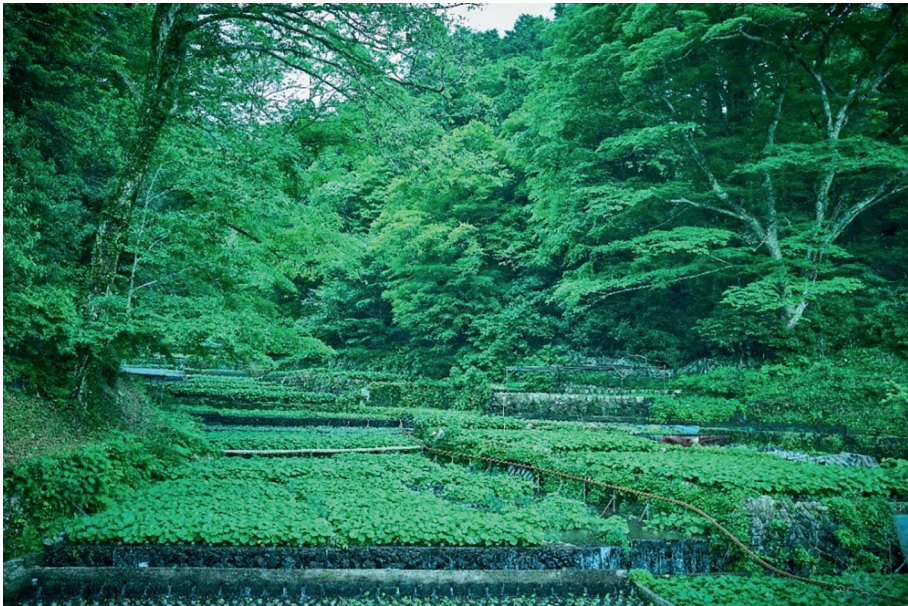


Figure 1.

Wasabi cultivation. The wasabi fields in Izu City, Shizuoka Prefecture, Japan. The wasabi fields stretch from the upper reaches to the lower reaches, and the cold but abundant clear water nurtures the wasabi. Source: Kinjirushi Co., Ltd.



Figure 2.

Wasabi. Wasabi grows slowly for more than a year until the rhizome reaches about 15 centimeters. It has a distinctive flavor; sweetness, and consistency when ground. Source: Kinjirushi Co., Ltd.

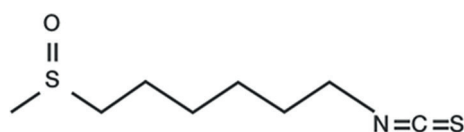


Figure 3.

Hexaraphane. It is found in trace amounts in the rhizomes of wasabi. It is a nonvolatile amphipathic substance with a slightly astringent flavor, but no pungent taste. 6-methylsulfinylhexyl isothiocyanate; molecular weight: 205.3, C₈H₁₅NOS₂, CAS number: 4430-35-7. Source: Kinjirushi Co., Ltd.

disease), purges dampness (the first of the five tinctures as the cause of the disease), eliminates stagnation (pain-causing), and removes plumpness (plumpness, one of the five stagnations, refers to the spleen stagnation). It is the best of the seven flavors of colic herbs. It is also used to treat biofilms and is recognized as a medicinal plant [3]. In addition to its appetite-boosting properties, modern catalogs of medicinal plants state that the rhizomes of wasabi, when ground and thinly applied to a cloth and applied to the areas affected by rheumatism, neuralgia, and tonsillitis, can be effective in relieving pain and that the sap of the rhizomes is effective in preventing fish and horsemeat poisoning [4]. Finely chopped leaves can also be used as a moisturizing bath additive by placing them into a bathtub in bags [5].

Although wasabi has been used in Japan since ancient times, its medicinal properties have little scientific basis and have been regarded only folklore. Recent studies have elucidated the active ingredients in wasabi and explored their functions. These functional ingredients have been used to promote health and beauty. In particular, hexaraphane (6-methylsulfinylhexyl isothiocyanate, 6-MSITC) (**Figure 3**), which is relatively abundant in wasabi rhizomes and roots, has been reported to exhibit antioxidant, anticarcinogenic, and detoxifying enzyme-inducing effects. Moreover, it was reported to inhibit platelet aggregation and ameliorate dementia. Here, I would like to focus on the clinical data on the effect of hexaraphane in improving brain function. I would also like to discuss some of the other functional ingredients in wasabi.

2. Functional components of wasabi

The extracts of leaves, stems, and roots of wasabi were analyzed by MS chromatography, and 42 major peaks were detected, including glucosinolates, phenylpropanoid glycosides, flavone glycosides, and hydroxycinnamic acids [6]. Some of these constituents had the strongest anti-inflammatory, antimicrobial, and cytotoxic effects compared to others.

Wasabi is known to contain many isothiocyanates, responsible for its characteristic pungent flavor. In addition, its leaves contain many polyphenols, such as isosaponarin (4'-O-glucosyl-6-C-glucosyl apigenin) and isovitexin. In addition, several new components with various functional properties are being elucidated [7].

Wasabi contains at least 21 kinds of isothiocyanates [8, 9]. Among them, the most abundant isothiocyanate is allyl isothiocyanate (AITC), which is the main component responsible for the pungent flavor. Hexaraphane is another isothiocyanate that has recently attracted attention as a functional ingredient. It has been shown to induce detoxification of metabolic enzymes [10] and exhibit anticancer [11], anti-inflammatory [12], antidiabetic [13], and antiallergenic/anti-atopic [14] effects. Recent studies have demonstrated its ability to activate hair papilla cells and improve dementia [15].

In addition, 6-methylthiohexyl isothiocyanate (6-MTITC) is a volatile component with the characteristic green note of wasabi. It is mainly used as a flavoring agent in processed food, but it has also been reported to have functional properties including antimicrobial and anticarcinogenic effects [16], detoxification and antioxidant effects [17], anticarcinogenic effects [18], and antiallergic effects [12].

To date, wasabi leaves have been partially used as food, but mostly discarded. However, studies have gradually identified the components of wasabi leaves and their functional properties, such as anti-influenza virus [19], anti-obesity [20, 21], and anticancer [22] effects.

Experimental and clinical studies have demonstrated the effects of wasabi leaves on the skin. For example, wasabi leaf extract was shown to decrease tyrosinase activity, melanin content, and formation of advanced glycation end products in vitro and to improve skin condition in a clinical trial [23].

Further research on the functional ingredients may enable more efficient use of wasabi leaves.

2.1 Allyl isothiocyanate (AITC)

Studies have reported the antimicrobial properties of AITC. Its pungent flavor is believed to have antimicrobial properties. It is a relatively volatile ingredient with a low molecular weight of 99.15 and a boiling point of 152°C. Its antimicrobial activity is also very effective in gas-phase exposures. Its minimum growth inhibitory concentrations against bacteria, yeasts, and molds ranged between 16 and 110 ng/mL [24]. Industrially, AITC-based antimicrobial products for lunch boxes and rice storage container and antimicrobial deodorizers for car air conditioners are available.

Due to its highly irritant nature, an acute toxicity LD₅₀ of 310 mg/kg has been reported in mice [25]. Moreover, it causes irritation of skin and mucous membranes on contact [26]. In addition, there are reports of suspected mutagenicity and genotoxicity [27]. Therefore, due caution should be exercised when using it as a functional material.

2.2 Hexaraphane

Hexaraphane is a nonvolatile isothiocyanate, mainly found in the rhizome and root, but at low levels of 200–500 µg/g [28]. The dietary intake ranges from 2.5 to 5.0 g per meal, with an estimated hexaraphane content of 0.5 to 2.5 mg per meal.

The LD₅₀ values for hexaraphane in rats ranged from 338 to 451 mg/kg, but the LD₅₀ values for the natural and synthetic extracts differed. Hexaraphane is detected in the blood 2 hours after ingestion, and approximately 50% of the ingested amount is excreted in the urine within 24 hours [29]. Animal studies have shown that sulforaphane (4-methylsulfinylbutyl isothiocyanate; 4-MSITC), an analog of hexaraphane, crosses the blood-brain barrier 15 minutes after ingestion [30].

Therefore, hexaraphane is considered to be an effective functional ingredient because it is also rapidly absorbed by the body. It is important to note that the most commonly used products are room temperature tubular ones. However, these processed products often contain no rhizomes or only a small amount of rhizomes and therefore very little hexaraphane [28]. Therefore, the consumption of ground rhizomes or the use of products or supplements primarily containing rhizomes is essential to utilize the benefits of hexaraphane.

Hexaraphane acts on the transcription factor Nrf2 (NF-E2-related factor 2)/Keap1 (Kelch-like ECH-associated protein 1) system and promotes the transcriptional expression of antioxidant response elements. It is known to increase the expression of phase 2 enzymes such as GST and NAD(P)H quinone reductase (NQO1), and its GST-inducing activity was reported to be the highest among 20 vegetables [31].

Hexaraphane has been reported to inhibit the production of reactive oxygen species (ROS) by neutrophils stimulated with phorbol myristate acetate (PMA) [32]. A study suggested that hexaraphane does not directly eliminate the ROS produced by stimulation, but inhibits the ROS production by neutrophils by acting on the site of ROS production. Studies have shown that benzyl isothiocyanate (BITC), a typical papaya isothiocyanate, modifies cytochrome b558 [33] of the NADPH oxidase complex on leukocyte membranes. Hexaraphane is also believed to have the same mechanism of action.

In a study, rat basophil-like cells RBL-2H3 were stimulated with specific IgE antibodies, and the released histamine and leukotrienes were measured by ELISA. The addition of hexaraphane [12] significantly inhibited the release of histamine and leukotrienes.

In addition, we investigated the effects of hexaraphane on dermal papilla cells (DPCs) and showed that hexaraphane activated hair papilla cells and upregulated vascular endothelial growth factor and adenosine receptors. This suggests that hexaraphane may promote the growth of hair [34].

It is important to note here that wasabi is primarily sold in tube form as processed, but horseradish is used as a substitute for it. In other words, many products are made by coloring dried horseradish powder, kneading it with water, and then putting it into tube form, which contains almost none of wasabi's functional ingredients, including hexaraphane. Some wasabi products are made using the stems and leaves, but as the wasabi rhizomes and roots contain large amounts of hexaraphane, only processed products containing large amounts of these parts have these functional ingredients in adequate amounts.

2.3 Isosaponarin

Wasabi plants accumulate C-glucosyl flavones, including isosaponarin, in their leaves [7, 35]. Isosaponarin is one of the major flavonoids in wasabi and has been rarely reported from other species in the Plant Kingdom (Figure 4) [36].

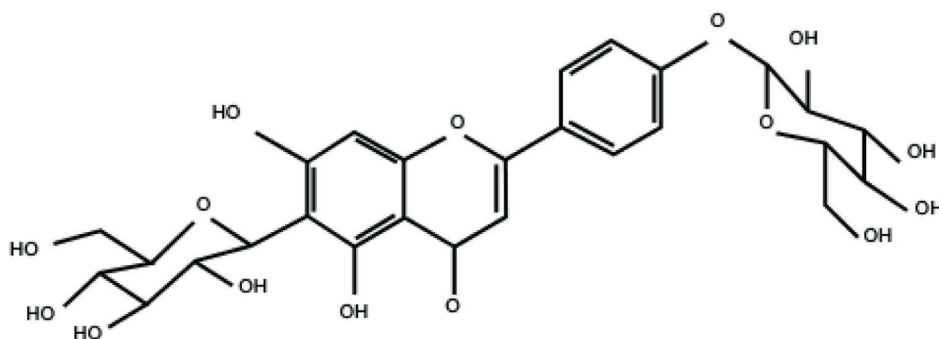


Figure 4. Isosaponarin. It is a unique flavonoid with C-bond found in the leaves of wasabi and has the structure of apigenin with two sugars attached. An enzymatic reaction removes the sugar and converts it to isovitexin. Molecular weight: 594.5, $C_{27}H_{30}O_{15}$, CAS number: 19416-87-6. Source: Kinjirushi Co., Ltd.

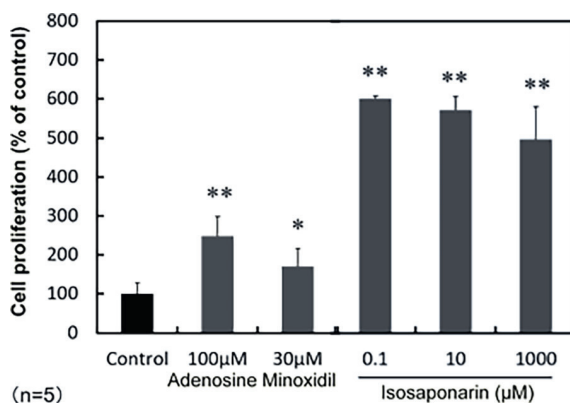


Figure 5.

*Effect of isosaponarin on the proliferation of DPCs. The effect of isosaponarin on amounts and activity of mitochondria in DPCs. DPCs were cultured for 72 hours with the indicated concentrations of isosaponarin, solvent control (DMSO), and positive controls (adenosine and minoxidil). The amounts of mitochondria were measured by Mito Tracker staining and further divided by the number of nuclei obtained by Hoechst staining to evaluate the number per cell. The data represent mean $\pm$ SD of five replicates. * and ** indicates significant differences versus control ($p < 0.05$ and $p < 0.01$ respectively; t -test). Source: Author.*

Isosaponarin and other flavonoids in wasabi leaves were shown to promote collagen production [37] and activate hair papilla cells.

Isosaponarin was found to increase collagen production in fibroblasts and enhance expression of transforming growth factor β II-type receptor (T β R-II) and prolyl 4-hydroxylase (P4H). These results suggested that isosaponarin promotes collagen synthesis by increasing T β R-II and P4H [37].

We also reported the effect of isosaponarin on DPCs. This was tested by adding wasabi leaf extract and isosaponarin to the culture of DPCs and assessing the cellular activity by the water-soluble tetrazolium method. Minoxidil and adenosine, which are used as ingredients in hair growth products, were used as positive controls. The results showed activation of DPCs at a low concentration of 0.1 μ M, as well as an increase in vascular endothelial growth factor and other substances (**Figure 5**) [38]. Although further validation is needed, it is conceivable that isosaponarin could be used as a material to promote hair regrowth and inhibit hair loss. Indeed, formulations containing isosaponarin are now available as hair care materials.

3. Cognitive function improvement by hexaraphane

3.1 Animal studies

Hexaraphane is known to express detoxification and antioxidant genes by activating the Keap1/Nrf2 system. Activation of Keap1/Nrf2 increases antioxidant enzymes such as HO-1, thereby inhibiting oxidative damage to neurons [39]. In the study by Morroni et al., the administration of hexaraphane to Parkinson's disease model mice was found to improve symptoms [15].

Uruno et al. conducted animal studies on Alzheimer's Disease model mice. The Keap1-Nrf2 system was shown to protect neurons in the hypothalamus against oxidative damage [40]. They also demonstrated that Nrf2 induction improved the impairment in AD model mice in the passive avoidance test (PAT) and suppressed

the elevation of the oxidative stress marker 8-OHdG. Administration of hexaraphane improved the impaired cognition of AD model mice in the PAT [41].

These studies suggest that hexaraphane may inhibit oxidative damage in the brain, preventing the onset of dementia or alleviating symptoms.

3.2 Safety

The characteristic pungent component of wasabi is AITC, which is approximately five times more abundant than hexaraphane [28, 42]. Therefore, a wasabi extract prepared to obtain hexaraphane would contain large amounts of AITC and its original glycoside, sinigrin. Ingestion of this extract may cause abdominal pain due to their high stimulant properties. We developed a special manufacturing process to remove AITC and sinigrin and developed “Wasabi Sulfinyl™ (WS),” an extract containing 0.8% hexaraphane and almost no AITC or sinigrin. Four-week overdose studies of WS confirmed the safety, with intake ranging from 500–2000 mg (containing 4–16 mg of hexaraphane) [43, 44]. No adverse effects were observed in these overdose trials demonstrating the safety of WS.

3.3 Randomized controlled trial

Cognitive function is known to be influenced by diet [45–47] and lifestyle. Intake of vegetables, fruits, and spices was shown to improve cognitive function [48–51]. Hexaraphane has antioxidant and anti-inflammatory properties and has been shown to improve memory and other functions in a mouse model of dementia. Thus, hexaraphane is expected to improve cognitive function. Below, we discuss several clinical trials on this subject.

3.3.1 Neurocognitive functions in cognitively intact middle-aged and older adults

Based on the antioxidant effects of hexaraphane and the results of animal studies, we conducted a randomized controlled trial for 8 weeks (UMIN000027407) [52]. Fifty cognitively intact elderly adults with memory complaints between the ages of 45 and 69 years were enrolled in this randomized double-blind, placebo-controlled trial. Subjects were randomized to receive 100 mg WS or placebo daily for 8 weeks. The effect on neurocognitive functions was assessed using a battery of four neuropsychological tests at baseline and after 4 and 8 weeks of treatment.

All subjects completed the study although four were excluded from the efficacy analysis due to adverse events or protocol violations. Analysis of the fully evaluable population indicated no significant differences between WS and placebo groups on any outcome measure. Because of the relatively small sample size, the unexpectedly high scores in the placebo group, the fact that all subjects were cognitively intact, and the fact that the eight-week treatment period was too short, it may not have been sensitive enough to discern the relatively subtle differences between the two groups. However, there were trends favoring WS in almost all parameters of the Group version of the Stroop Color Word Test (G-SCWT), of which the magnitude of improvement in correct responses in the Stroop interference task and the interference rate reached near-significant levels ($P = 0.083$ and 0.091 , respectively) at the end of 8-week treatment.

Exercise has been suggested to contribute to improved cognitive function [53, 54]. Therefore, we performed a subgroup analysis stratified according to their exercise

habits or lack. A subset of nonexercisers exhibited significantly better performance in both the reverse-Stroop control task and the Stroop interference task of the G-SWCT, which assesses attention and judgment, at week 8. The Stroop test is divided into four levels of difficulty, and in this study, there was a significant difference between difficulty level 1 and difficulty level 4 (**Figure 6**). In difficulty level 1, an improvement in performance was observed after 8 weeks even with placebo, suggesting a learning effect; however, in difficulty level 4, there was no improvement in performance after a period of time with placebo, and only the WS intake group showed an improvement in performance. This suggests that the effect of WS is not just to maintain function by preventing impairment of brain function, but also to improve function. However, due to the small number of subjects and the stratified analysis of the results, more robust studies are required to obtain definitive evidence. Of note, no WS-induced side effects were observed in the study.

3.3.2 Memory functioning in healthy adults aged 60 years and older

Nouchi et al. assessed cognitive function in 72 healthy subjects (19 males, 53 females; mean age: 65.4 years [range, 60–69 years]; mean MMSE score: 28.5) who received a capsule containing either 100 mg WS (0.8 mg hexaraphane/day) or a placebo for 12 weeks (UMIN000032694) [55].

A wide range of cognitive functions (e.g., executive function, episodic memory (immediate and delayed), processing speed, working memory, attention, reasoning, short-term memory, and visuospatial cognitive function) were assessed before and after the intervention.

There were no significant differences between the two groups baseline characteristics with respect to age (WS; 65.9 ± 3.9 years, placebo; 64.9 ± 3.6 years), education (WS; 12.5 ± 0.8 , placebo; 12.9 ± 1.1), Mini-Mental State examination (WS; 28.5 ± 1.5 , placebo; 28.8 ± 1.6), Frontal Assessment Battery at bedside (WS; 15.4 ± 1.9 , placebo; 15.4 ± 1.9), Japanese Reading Ability Test (WS; 20.3 ± 3.9 , placebo; 20.8 ± 3.6), and Geriatric Depression Scale (WS; 2.4 ± 1.4 , placebo; 2.1 ± 1.1). And, the duration of supplement intake also did not differ between the two groups.

The WS group showed a significant improvement in working memory, measured by digit span backward ($p = 0.000$), and in episodic memory performance, measured by logical memory (LM) immediately ($p = 0.00$), LM delayed ($p = 0.03$), and the face and second name test ($p = 0.00$) (**Figure 7**). However, they did not find significant

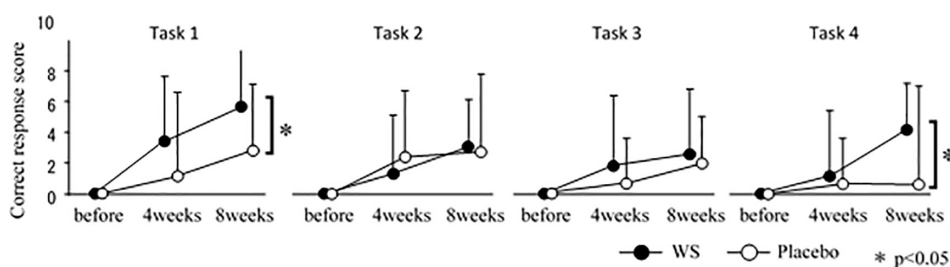


Figure 6.

The result of cognitive performance for the G-SCWT tasks 1 to 4 in the nonexercizer subgroup. Subjects who had a habit to perform exercise for 30 minutes or longer for more than twice a week were included in the exerciser subgroup, and the remaining subjects were included in the nonexercizer subgroup. Data presented as mean $\pm$ SD. Comparison of the mean change from baseline for independent samples. Source: Author.

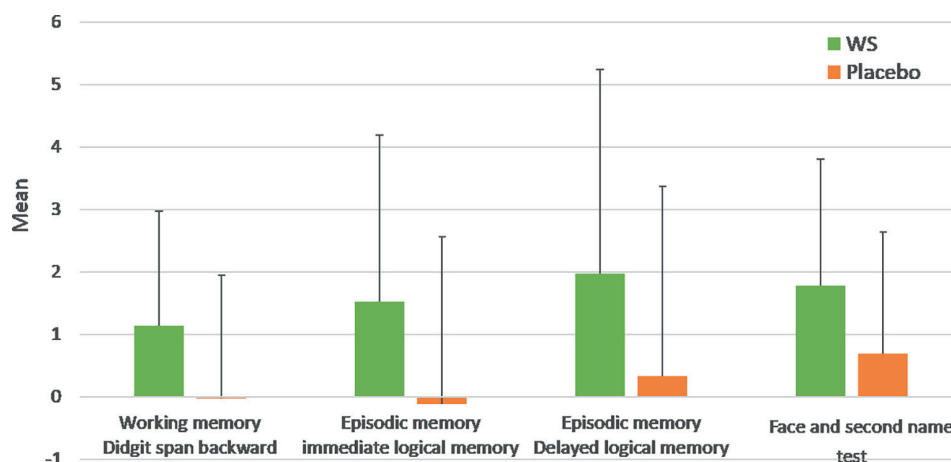


Figure 7.
 Change in scores of cognitive functions in both groups. * indicates significant differences versus placebo for multiple comparisons using the Bonferroni method ($p < 0.05$). This figure was constructed from Ref. ([55], Table 4). Source: Author.

improvements in other cognitive functions, such as processing speed, Cd, short-term memory, attention, inhibition, rST, reasoning, and visuospatial performances.

There were no significant changes in the inhibition and processing speed performances after hexaraphane intake in older adults. This is inconsistent with a previous study (3.3.1) which showed a beneficial effect of hexaraphane on inhibition performance. However, they noted some differences in methods between this study and previous studies, such as the intervention period (12 weeks or 8 weeks), subjective cognitive complaints (healthy, subjective memory complaints, patients with chronic fatigue), psychological tests (trail making test, SS, or Cd), and participants' age (older adults or middle-aged adults). Therefore, further studies are required to assess the effect of hexaraphane on inhibition and processing performances.

They further noted that antioxidant and anti-inflammatory effects are involved in the improvement of cognitive function [56, 57]. The hippocampus is involved in working and episodic memory [58], and the antioxidant and anti-inflammatory effects of hexaraphane on the hippocampus may improve cognitive functions. Antioxidant and anti-inflammatory effects are believed to be important in maintaining brain function, but as hexaraphane is thought to have a variety of effects, further studies are required to elucidate the underlying mechanisms.

3.4 ME/CFS and brain fog improvement

The antioxidant and anti-inflammatory effects of hexaraphane may be beneficial in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS).

ME/CFS is a disease with an unknown etiology and no known cure. However, chronic inflammation of the brain has been implicated in its causation [59, 60]. An estimated 17 million people worldwide suffer from the syndrome [61]. In addition to general malaise, low-grade fever, and muscle aches, patients also experience a loss of thinking ability, referred to as brain fog. Symptoms of brain fog include an inability to understand what others are saying, an inability to hold a conversation, an inability to read books and understand their meaning, and an inability to comprehend music.

Performance status (PS)	Pre (n)	Post (n)	P-value
0	0	0	
1	0	0	
2	0	2	
3	2	0	
4	0	1	
5	0	0	
6	1	1	
7	7	8	
8	4	2	
9	1	1	
average	6.8 ± 1.7	6.3 ± 2.1	*0.001

*indicates significant differences between pre- and post-treatment PS using Fisher's exact test. A parametric paired Student's t-test and nonparametric paired Wilcoxon signed rank tests yielded similar significant differences ($p < 0.05$). This table was adapted from Ref. ([62], Table 2). Source: Author.

Table 1.
Change in PS after hexaraphane treatment.

In a 12-week study by Oka et al., 15 patients with ME/CFS (3 males and 12 females, mean age 37.5 years [range 20–58], mean duration of illness 5.1 years) were administered 1.2 grams of WS (9.6 milligrams of hexaraphane per day). The results showed a significant improvement in the patients' performance scores (PS), with six patients improving their scores (two patients improved their scores by 2 or more grades) (Table 1) [62].

These findings demonstrate the potential of hexaraphane, considering that none of the patients in this study had responded to standard treatment for at least 3 months. Specifically, they showed improvement in headache frequency (4.1 to 3.0 times/week, $p = 0.001$), myalgia frequency (4.1 to 2.4 times/week, $p = 0.019$),

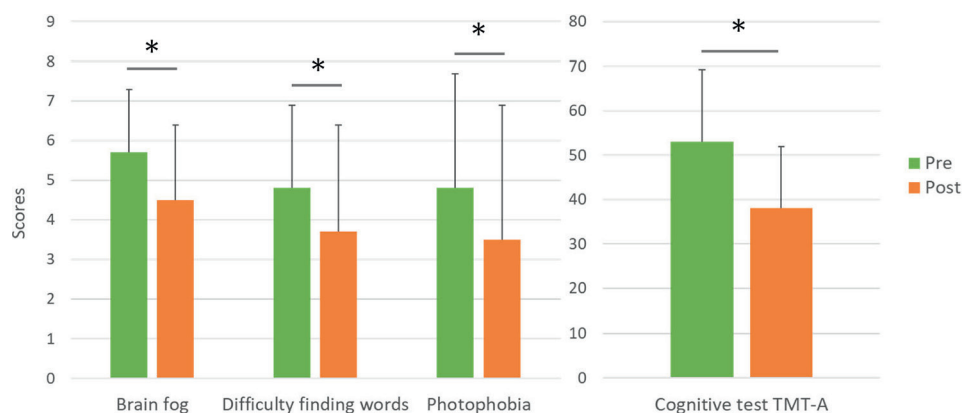


Figure 8.
Changes in neurocognitive symptoms and TMT-A test scores after Hexaraphane treatment. * indicates significant differences between pre- and post-treatment scores using a parametric paired Student's t-test. ($p < 0.05$). This figure was constructed from Ref. ([62], Table 5). Source: Author.

numerical rating scale brain fog scores (5.7 to 4.5, $p = 0.011$), difficulty in discovering the right words (4.8 to 3.7, $p = 0.015$), photophobia (from 4.8 to 3.5, $p = 0.008$), and mood state vigor scores (from 46.9 to 50.0, $p = 0.045$), right occipital pressure pain threshold (from 17.3 to 21.3, $p = 0.01$), and trail making test-A (TMT-A) (from 53.0 to 38.1, $p = 0.007$) (**Figure 8**). In particular, 14 out of 15 participants perceived improvement in brain fog and cognitive function, as well as significant improvement in general health perception, vitality, and vigor. While the mechanisms of improvement remain to be elucidated and need to be validated in a larger sample, 10 of the 15 patients continued to take WS at their own request after the completion of the study.

4. Conclusion

Wasabi is a pungent vegetable that contains several active ingredients, including hexaraphane. As wasabi is mainly grown and consumed in Japan, there is a paucity of research on its therapeutic effects in other parts of the world. Our basic research and clinical trials have demonstrated its efficacy in improving brain function, even in healthy individuals. The results of studies conducted in mice model of dementia also suggest its potential protective effect on the brain. It may not only help maintain brain function in healthy individuals but also improve symptoms in patients with MCI and dementia.

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Conflict of interest

Isao Okunishi is an employee of Kinjirushi Co., Ltd., and received a research grant from the company.

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