MAX ONE
999 COMPANY LIMITED

MAX ONE 999 CO., LTD.

Company profile Brand

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ABOUT US

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MISSION STATEMENT

To enhance human health and awaken hidden energy of all mankind

VISION

A Part of your daily energetic movement

POWER ENERGY DRINK

DRINK

MAX ONE

ใหม่

맥스원 해마

VITAMIN

B6

B12

HIGH

ADDED SEAHORSE EXTRACT

เพิ่มสารสกัดจากม้าน้ำ

150 ML

MAX ONE 999 CO., LTD. | LAST UPDATED : 2023-08-25

POWER ENERGY DRINK

DRINK MAX ONE™

ใหม่

맥스원 해마

VITAMIN B6 B12 HIGH

ADDED SEAHORSE EXTRACT

เพิ่มสารสกัดจากม้าน้ำ

250 ML

MAX ONE 999 CO., LTD. | LAST UPDATED : 2023-08-25

VITAMIN B6

Vitamin B6

Vitamin B6, or pyridoxine, is a water-soluble vitamin found naturally in many foods, as well as added to foods and supplements. Pyridoxal 5' phosphate (PLP) is the active coenzyme form and most common measure of B6 blood levels in the body. PLP is a coenzyme that assists more than 100 enzymes to perform various functions, including the breakdown of proteins, carbohydrates, and fats; maintaining normal levels of homocysteine (since high levels can cause heart problems); and supporting immune function and brain health.

Vitamin B6 and Health

Vitamin B6 has been widely studied for its role in disease prevention. The vitamin in supplement form shows the most promise for the treatment of pregnancy-induced nausea,but such use should only occur under the supervision of a physician.
Adequate blood levels of B6 may be associated with lower risk of cancers, compared to low blood levels. However, the use of separate B6 supplements (apart from the RDA amounts in typical multivitamin preparations) is inconclusive and not recommended.

Signs of Deficiency and Toxicity

Cardiovascular disease

High homocysteine levels are associated with an increased risk of heart disease and stroke as it may promote the formation of blood clots and excess free radical cells, and may impair normal blood vessel function. A lack of adequate vitamin B6, along with vitamin B12 and folic acid, can increase homocysteine levels. Although epidemiological studies have found that vitamin B supplementation can decrease homocysteine levels, they have not consistently shown a decreased risk of cardiovascular events in taking the vitamins. Therefore the American Heart Association does not advocate for the use of B vitamin supplements for reducing cardiovascular disease risk.

- In a study from Norway, results from two randomized double-blinded controlled trials were combined, consisting of 6,261 participants followed for more than three years. The participants were given either a B-vitamin complex with folic acid, B12, and B6; folic acid and B12; 40 mg B6 alone; or a placebo. There were no differences in cardiac events or cardiac deaths in the vitamin B6 groups compared with the placebo group.
- A Cochrane review was conducted with randomized controlled trials looking at the effects of homocysteine-lowering interventions (B6, B12, folic acid supplements alone or in combination) for the prevention of cardiovascular events (stroke, heart attack). The follow-up period was one year or onger. The review did not find a difference in heart attacks or deaths between the B supplements or placebo; it did find a small association with reduced strokes with the supplements given alone or in combination, compared with placebo.

Cognitive function

Vitamin B6 may indirectly help with brain function by lowering levels of homocysteine, as high levels of this protein in the body have been linked with a higher incidence of dementia, Alzheimer's disease, and cognitive decline. However, there is a lack of controlled trials showing that supplementation can slow cognitive decline.

A Cochrane review looked at 14 randomized controlled trials that evaluated the cognitive effects on people who used B vitamin supplements for at least three months. It did not find an effect of the supplements (B6 alone or in combination with B12 and folic acid) on cognition in older adults (60+ years) without dementia at baseline, compared with a placebo.

https://www.hsph.harvard.edu/nutritionsource/vitamin-b6/

VITAMIN B6

Cancer

A systematic review of both epidemiological and clinical studies looked at the relationship of dietary intake or blood levels of vitamin B6 and all cancers. The epidemiological studies found that a high intake of vitamin B6 foods and higher B6 blood levels were significantly associated with a lower risk of all cancers, most notably gastrointestinal cancers. However when total B6 intake from food and supplements was assessed, the protective effect weakened. The clinical studies did not find a protective effect of B6 supplements but the quality of these studies was rated low because B6 was not given alone and because cancer was not the main outcome studied. The authors concluded an unclear role of vitamin B6 in cancer prevention because of the discrepancy between results from epidemiological and intervention studies. They suggested that higher levels of B6 in the body may reflect the status of other nutrients that are protective against cancer.

Vitamin B6 is believed to play a role in colorectal cancer through its enzyme activity that may reduce oxidative stress and the spread of tumor cells. Vitamin B6 deficiency is associated with chronic inflammation, a risk factor for colorectal cancer.

A meta-analysis of epidemiological studies did not find that B6 supplements reduced colorectal cancer risk. However, when measuring pyridoxal 5' phosphate (PLP) blood levels, participants who had higher PLP showed a 30-50% reduced risk of the cancer. The authors noted potential confounding factors in these studies such as healthy lifestyle behaviors (higher level of exercise, no smoking, higher intakes of other vitamins) that could have protected against colorectal cancer.

- Two prospective studies of men and women from the Physicians' Health Study and Nurses' Health Study cohorts found a protective effect of higher PLP blood levels and B6 intakes (from food and supplements) on colorectal cancer risk. This result remained after adjusting for intakes of folate, multivitamins, and methionine (nutrients that may be protective against colorectal cancer).

Morning sickness

Vitamin B6 has long been documented as a remedy to help relieve pregnancy-related nausea and its most severe form, hyperemesis gravidarum, which sometimes necessitates hospitalization due to severe dehydration.

- A blinded randomized controlled trial of 77 pregnant women found that 40 mg of vitamin B6 taken twice daily reduced the severity of mild to moderate nausea compared with a placebo.

- A review of randomized controlled trials showed that vitamin B6 supplements (up to 10 mg daily) was associated with improved symptoms of mild pregnancy-related nausea compared with a placebo. For moderate to severe nausea, a combination of vitamin B6 and doxylamine (an antihistamine) taken preventively before symptoms started was more effective than taking it after the nausea started.

- Over-the-counter vitamin B6 and B6 with doxylamine are recommended as safe and effective first-line treatments for pregnancy-related nausea by the American College of Obstetricians and Gynecologists.

https://www.hsph.harvard.edu/nutritionsource/vitamin-b6/

VITAMIN B6

Signs of Deficiency and Toxicity

Deficiency

A vitamin B6 deficiency most often occurs when other B vitamins in the body are low, particularly vitamin B12 and folic acid. A mild deficiency may have no symptoms, but a more severe or prolonged deficiency can exhibit the following:

- Microcytic anemia
- Skin conditions
- Depression
- Confusion
- Lowered immunity

Certain conditions can increase the risk of developing a deficiency by interfering with the absorption of vitamin B6:

- Kidney disease
- Autoimmune intestinal disorders like celiac disease, ulcerative colitis, and Crohn's disease
- Autoimmune inflammatory disorders such as rheumatoid arthritis
- Alcoholism

Toxicity

It is quite unlikely to reach a toxic level of vitamin B6 from food sources alone. Vitamin B6 is a water-soluble vitamin so that unused amounts will exit the body through the urine. However, a toxic level can occur from long-term very high dose supplementation of greater than 1,000 mg daily.Symptoms usually subside after stopping the high dosage. Symptoms include:

- Neuropathy in feet and hands
- Ataxia (loss of control of body movements)
- Nausea

https://www.hsph.harvard.edu/nutritionsource/vitamin-b6/

VITAMIN B12

Vitamin B12, or cobalamin, is naturally found in animal foods. It can also be added to foods or supplements. Vitamin B12 is needed to form red blood cells and DNA. It is also a key player in the function and development of brain and nerve cells.

Vitamin B12 binds to the protein in the foods we eat. In the stomach, hydrochloric acid and enzymes unbind vitamin B12 into its free form. From there, vitamin B12 combines with a protein called intrinsic factor so that it can be absorbed further down in the small intestine.

Supplements and fortified foods contain B12 in its free form, so they may be more easily absorbed. There is a variety of vitamin B12 supplements available. Although there are claims that certain forms—like sublingual tablets or liquids placed under the tongue to be absorbed through the tissues of the mouth—have better absorption than traditional tablets, studies have not shown an important difference. Vitamin B12 tablets are available in high dosages far above the recommended dietary allowance, but these high amounts are not necessarily the amount that will be absorbed because an adequate amount of intrinsic factor is also needed. In cases of severe vitamin B12 deficiency due to inadequate intrinsic factor (pernicious anemia), doctors may prescribe B12 injections in the muscle.

Vitamin B12 and Health

Cardiovascular disease

Vitamin B12 is involved in the breakdown of a protein called homocysteine. High homocysteine levels are associated with an increased risk of heart disease and stroke as it may promote the formation of blood clots and excess free radical cells, and may impair normal blood vessel function.
A lack of adequate vitamin B12 can increase homocysteine levels.

Although epidemiological studies have found that vitamin B12 supplementation can decrease homocysteine levels, they have not consistently shown a decreased risk of cardiovascular events in taking the vitamin. Therefore the American Heart Association does not advocate for the routine use of B vitamin supplements in reducing cardiovascular disease risk. [1] However, vitamin B12 supplements can be important for some individuals with genetic variants that lead to high homocysteine levels.

Conitive function

High homocysteine levels are linked with a higher incidence of Alzheimer's disease, dementia, and cognitive decline. Similar to cardiovascular disease, although the research has shown that vitamin B12 supplementation reduces homocysteine blood levels, this has not translated into reduced rates of cognitive decline. A Cochrane review on folic acid supplements and cognition, with or without vitamin B12, did not find a significant effect of the supplements vs. placebo on cognitive function in healthy elderly people or people with dementia. [3] Another review of 14 randomized controlled trials also did not consistent evidence of benefit with the use of vitamin B12 supplements, alone or with other B vitamin supplements, on cognitive function in people with either normal or impaired cognition.
[4] These findings do not preclude a possible benefit among some individuals with low vitamin B12 levels, and more research is needed.

https://www.hsph.harvard.edu/nutritionsource/vitamin-b12/

VITAMIN B12

Signs of Deficiency and Toxicity

Deficiency

Measuring vitamin B12 in the blood is actually not the best way to determine whether someone is deficient, as some people with a deficiency can show normal B12 blood levels. Blood levels of methylmalonic acid, a protein breakdown product, and homocysteine are better markers that capture actual vitamin B12 activity. These values increase with a vitamin B12 deficiency. It is estimated that up to 15% of the general population has a vitamin B12 deficiency. [1]

Factors that may cause vitamin B12 deficiency:

Avoiding animal products. People who do not eat meat, fish, poultry, or dairy are at risk of becoming deficient in vitamin B12, since it is only found naturally in animal products. Studies have shown that vegetarians have low vitamin B blood levels. [5] For this reason, those who follow a vegetarian or vegan diet should include B12-fortified foods or a B12 supplement in their diets. This is particularly important for pregnant women, as the fetus requires adequate vitamin B12 for neurologic development and deficiency can lead to permanent neurological damage.

Lack of intrinsic factor. Pernicious anemia is an autoimmune disease that attacks and potentially destroys gut cells so that intrinsic factor is not present, which is crucial for vitamin B12 to be absorbed. If vitamin B12 deficiency ensues, other types of anemia and neurological damage may result. Even the use of a high-dose B12 supplement will not solve the problem, as intrinsic factor is not available to absorb it.

Inadequate stomach acid or medications that cause decreased stomach acid. A much more common cause of B12 deficiency, especially in older people, is a lack of stomach acid, because stomach acid is needed to liberate vitamin B12 from food. An estimated 10-30% of adults over the age of 50 have difficulty absorbing vitamin B12 from food. [1] People who regularly take medications that suppress stomach acid for conditions like gastroesophageal reflux disease (GERD) or peptic ulcer disease—such as proton-pump inhibitors, H2 blockers, or other antacids—may have difficulty absorbing vitamin B12 from food. These drugs can slow the release or decrease production of stomach acid. In theory this can prevent the vitamin from being released into its free usable form in the stomach; however, research has not shownan increased prevalence of a deficiency in people using these medications. Anyone using these medications for an extended time and who are at risk for a vitamin B12 deficiency for other reasons should be monitored closely by their physician. They may also choose to use fortified foods or supplements with vitamin B12, as these forms are typically absorbed well, and do not require stomach acid.

Intestinal surgeries or digestive disorders that cause malabsorption. Surgeries that affect the stomach where intrinsic factor is made, or the ileum (the last portion of the small intestine) where vitamin B12 is absorbed, can increase the risk of a deficiency. Certain diseases including Crohn's and celiac disease that negatively impact the digestive tract also increase the risk of deficiency.

Medications that interfere with absorption. Long-term use of metformin, a drug commonly prescribed for type 2 diabetes, is strongly associated with vitamin B12 deficiency and lower folic acid levels as it can block absorption, which may lead to increased homocysteine levels and risk for cardiovascular disease. [6] Proton pump inhibitors and histamine blockers prescribed to reduce stomach acid are also associated with lower vitamin B12 levels.

https://www.hsph.harvard.edu/nutritionsource/vitamin-b12/

VITAMIN B12

Signs of deficiency may include:

- Megaloblastic anemia—a condition of larger than normal sized red blood cells and a smaller than normal amount; this occurs because there is not enough vitamin B12 in the diet or poor absorption

- Pernicious anemia—a type of megaloblastic anemia caused by a lack of intrinsic factor so that vitamin B12 is not absorbed

- Fatigue, weakness
- Nerve damage with numbness, tingling in the hands and legs
- Memory loss, confusion
- Dementia
- Depression
- Seizures

https://www.hsph.harvard.edu/nutritionsource/vitamin-b12/

PROPERTIES OF SEAHORSES

Seahorses have many properties such as

- nourishing the kidneys because seahorses help warm the kidneys.
- Treatment of pus abscesses
- nourish energy.
- is an elixir
- treat asthma
- Enhance memory, enhance immunity
- flu treatment
- bruises abnormalities
- The seahorse helps to calm the nerves,
- reduce swelling
- regulate the Qi (energy) and blood
- dilate the blood vessels
- relieve coughing and ease labored breathing.
- Treatment of lumbar and knee pain and back pain.
- It increases the weight of the uterus and ovaries
- Treatment of uterine Helping infertile women to have children more easily
- The properties of seahorses enhance sexual performance
- helps build muscles in the prostate, seminal vesicle and anus,
- In the 'Marine Resources and Oriental Medicine' by Professor Se-kwon, Kim of Pukyong National University, the pharmacology of the seahorse is presented as follows.

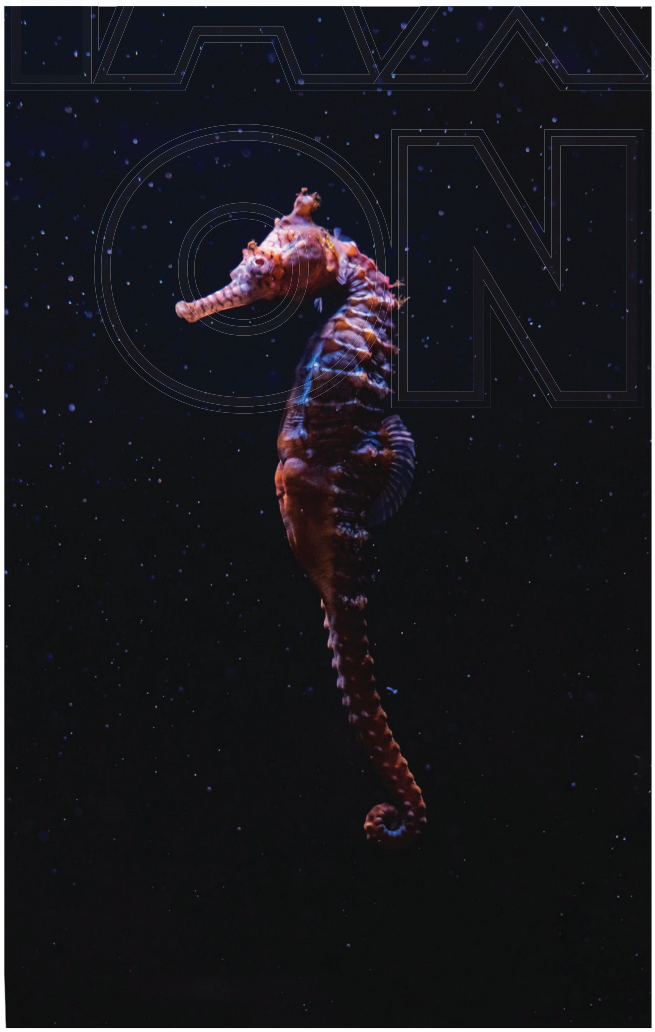
http://www.haechunma.co.kr/cont/0401.php?s=41&lang=2

PROPERTIES OF SEAHORSES

Records of using the seahorse for human health have been found in the Compendium of Materia Medica: Ben Cao Gang Mu, China's most complete and comprehensive medical book consisting of 52 volumes completed by Li Shizen in 1578 during the Ming dynasty, as well as Donguibogam: Principles and Practice of Eastern Medicine compiled by Heo Jun in 1610. Despite being listed in the Compendium of Materia Medica about 400 years ago, it is believed till now that the seahorse has an excellent effect in the treatment of diseases and in health maintenance, resulting in seahorses being constantly traded as an expensive medicinal ingredient in China and its neighboring countries.

Uses of the seahorse differ by country and region and traditional uses vary greatly. In the southern regions of China, the value of the seahorse is ranked just below wild ginseng, reflecting the deep trust the Chinese have in the seahorse. With regards to the efficacy of the seahorse, modern oriental medicine practitioners explain that in the Donguibogam, the seahorse is considered to be good for strengthening the kidneys and enhancing male virility, as well as for cooling the semen and treating penis shrinkage.

The seahorse also helps to calm the nerves, reduce swelling, regulate the Qi (energy) and blood, dilate the blood vessels, relieve coughing and ease labored breathing. It increases the weight of the uterus and ovaries and helps build muscles in the prostate, seminal vesicle and anus, and is recommended for the treatment of knee and back pain. In the 'Marine Resources and Oriental Medicine' by Professor Se-kwon, Kim of Pukyong National University, the pharmacology of the seahorse is presented as follows.



http://www.haechunma.co.kr/cont/0401.php?s=41&lang=2

CERTIFICATE OF REGISTRATION

■ 야생생물 보호 및 관리에 관한 법률 시행규칙 [별지 제26호의3서식] <신설 2014.7.17>

※ [ ]에는 해당되는 곳에 √표를 합니다. (앞쪽)

증명번호 BC-EK2020-00088

국제적 멸종위기종 인공증식증명서

신청인	상 호(명 칭)	어업회사법인 (주)해천마		
	성명(대표자)	노 섬	생년월일	1942-04-16
	주 소 (사업장 소재지)	제주특별자치도 제주시 구좌읍 종달동길 52-22 어업회사법인 (주)해천마 (전화번호064-782-0015)		
국제적 멸종위기종 내역	보통명	Big-belly seahorse	학 명	Hippocampus Abdominalis
	국제적 멸종위기종 등급	[ ] I급 [√] II급 [ ] III급		
	수량(단위: 개)	1,000,000		
	증식 · 재배 장소	상동		
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	목적 및 용도	상업용(T)		
	보관시설 명세	육상수조식 양식 시설 수면적 954.42㎡		

「야생생물 보호 및 관리에 관한 법률」 제16조제7항 및 같은 법 시행규칙 제23조의2제2항에 따라 인공증식한 국제적 멸종위기종임을 증명합니다.

2020년 12월 23 일

영산강 유역환경청장 (지방환경청장)

210mm×297mm(백상지 120g/㎡)

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CERTIFICATE OF REGISTRATION

(Translation)

CERTIFICATE OF PATENT

Patent No.	10-2011747
Application No.	10-2017-0058908
Filing Date	11 May 2017
Registration date	12 August 2019

Title of the Invention

The compounds for developing muscle growth or athletic ability by using Alkalase Hydrolysate from the Hippocampus Abdominalises

Patentee

Recorded in the registration data list.

Inventor

Recorded in the registration data list.

This is to certify that, in accordance with the Patent Act, a patent for the invention has been registered at the Korean Intellectual Property Office.

August 12, 2019

(QR code)

Please check the data list of the current registration with QR code

(Emblem)

COMMISSIONER,

KOREAN INTELLECTUAL PROPERTY OFFICE

- Signature -

RESEARCH

(Translation)

KFAS Journal of Fisheries and Aquatic Sciences of the Republic of Korea Original Content

Korean Journal of Fisheries and Aquatic Sciences Korean J Fish Aquat Sci 52(2),127-133,2019

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Antioxidant and antihypertensive effects of Edible Enzyme Hydrolysate from Hippocampus Abdominalis.

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Antioxidant and Antihypertension Effects of Enzyme Hydrolysate from Hippocampus Abdominalis

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Seahorses (Hippocampuses) have long been used as ornaments and medicines. The (Large-bellied Seahorses) Hippocampus Abdominalises have their beautiful colors and unique shapes. They are also used in decoration and traditional medicine in China as well. This study was to test the utility of the Hippocampus Abdominalises as a functional food or feed additive. Hippocampus Abdominalises were hydrolyzed using seven proteases: Flavorzyme, Neutrase, Alkalase, Trypsin, Koji Enzyme, Pepsin and Protamax as the total hydrolysate yield was higher than that of the aqueous extract among those enzymes, Seahorse Protamex Hydrolysate (SHP) produced the highest yield with antioxidant activity as well as having an inhibitory effect on the conversion of Angiotensin-1 Enzymes (Angiotensin-I). It also protects Vero Cells from oxidation by AAPH [2 ,2 -azobis-(2 -amidinopropane) dihydrochloride] and used as anyihypertension in rats that have high blood pressure by nature as well. This study has shown that the Hippocampus Abdominalises could be used as a functional food or feed additive in the future.

Keywords: Hippocampus Abdominalis, Enzyme Hydrolysate, Protamex, Antioxidant, Antihypertension

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RESEARCH

(Translation)

Introduction

Nowadays, young people are concerned related to the rapidly increasing number of aging population. At the same time, the interest in health and the demand for healthy food is constantly increasing (Cha et al., 2012). Therefore, there have been studies on antioxidant activity and antihypertensive effect to improve human quality of life for a long time. Reactive Oxygen Species are formed in the body by various cellular metabolic processes or by the activation of reactions (Such as cytokines). For this reason, oxidative stress occurs in the body and causes various diseases. (Such as aging, obesity). It is well known that these Active Oxygen-depleting antioxidants can work in the human body such as preventing aging, old prevention of aging, etc. (Farag et al., 1989; Lee et al., 2003). Well-known synthetic antioxidants, including : Butylated Hydroxy Anisole : BHA, Butylated Hydroxy Toluene : BHT, and Propyl Gallate (PG), are widely used. However, the toxicity and side effects of these synthetic antioxidants have limited their use. For these reasons, people are increasingly avoiding and interested in natural antioxidants (Seog et al., 2002). In addition, hypertension, another health threat of young people, is a chronic condition to make blood pressure rise consistently. It is also a risk factor for various diseases such as atherosclerosis and myocardial infarction, which require continuous care and treatment. The best known treatments for hypertension are Angiotensin Converting Enzyme Inhibitors : ACEi and ARBs (Angiotensin II Type1 Receptor Blockers), which can inhibit hypertension artery function (Kang et al., 2018; Kim et al., 2018). However, although there are many types of ACEIs and have different ejection positions but it has common side effects such as dry cough, itchy rash and high blood potassium levels. But due to ARBs still require different antihypertensive tablets for various risk groups, As a result, interest in natural ACE inhibitors is increasing (Kim et al., 2000). Seahorses (Hippocampuses) have been used as traditional medicines in China since ancient times. At present, Hippocampuses are used as sexual enhancement drugs and cosmetics in China including other countries that are Chinese cultural zones, thereby increasing demand for them (Kang et al., 2017). Even though Hippocampus Histrix, Hippocampus Kuda, Hippocampus Mohnkei and Hippocampus Trimacularta are sold in Korea but it is difficult to acquire hippocampuses as a natural resource because it is an illegal trade and overfishing as well as destroying the ecosystem (Huh et al., 2014). Currently, large-scale culturing of Hippocampus Abdominalises on Jeju Island is very successful. They also have a large size and beautiful shape. This keeps hippocampuses with great value as pets and of high commercial value (Ko et al., 2015). Although many studies have been conducted on common hippocampuses, such as their antioxidant properties, anti-cancer, anti-aging and antihypertensive effects. However, in the case of Hippocampus Abdominalises

(Translation)

that they have recently been successful in breeding, more studies are still needed (Alagumuthu et al., 2016; Kim et al., 2016). Thus, the aim of this study is to prove the excellent efficacy of the Hippocampus Abdominalises by confirming their antioxidants and the antihypertensive effect of protamex hydrolysate that are the best among the hydrolysate enzymes of each species of Hippocampus Abdominalises by experimenting under suitable extraction conditions.

Materials and Methodology

Test Reagents and Experimental Equipment

The hippocampuses used in this test are Hippocampus Abdominalises), provided by the Haecheonma Co., Ltd., located in Gujwa-dong, Jeju City, Jeju Province, and used after the hippocampuseses are proceeded to keep them dry as Lyophilization then having ground. Digestive enzymes used for the digestion of 7 types of proteins. (Protamax, Nutrase, Alkalase, Koji Enzyme, Flavorzyme, Trypsin and Pepsin), purchased from the Novozyme Co., Ltd. (Denmark) 1,1-Diphenyl-2- Pickrilhydrazil (DPP) Alpha-(4-Pyridine-1). -Oxide)-N-Tert-Butylnitrone (4-POBN) 2, 2-Azobis-(2 -Amidinopropane) Dihydrochloride (AAPH) 3 -(4 , 5 -Dimethylthiazol-2 -yl)-2 , 5 -Diphenyltetrazolium Bromide (MTT) and Dimethyl Sulfoxide (DMSO), where they are bought from the Sigma-Aldrich Co., Ltd. (St. Louis City, Missouri State, USA) and used them for testing. Besides, We used the ACE Inhibition Potential Evaluation Kit (The Dojindo Co., Japan) to confirm their antihypertensive effect. For other test chemicals, we use the test reagents to confirm the results.

Method for producing Protein Hydrolysate Enzyme

After the lyophilization and having ground the Hippocampus Abdominalises, then the ratio of substrate to enzyme will be determined as a 100:1 in the form of dry powder. The hydrolysate of Hippocampus Abdominalises are then produced and compared with the Distilled Water : DW by using seven protein degrading enzymes, which are performed for 24 hours according to the temperature and pH value suitable for each enzyme. Distilled water will be extracted at room temperature for 24 hours with hydrolysate enzyme. The hydrolysis is then stopped by closing the Water Bath at 100 ℃ for 10 min after centrifugation (12,000 rpm, 15 min, 4 ℃). Then filtering only the part above the sediment by using filter paper and keeping them dry in the form of the lyophilization to obtain all 7 enzymatic protein hydrolysates and distilled water extract in dry powder form.

RESEARCH

(Translation)

Measurement of Antioxidant Activity of DPPH and Alkyl Free Radicals Activity

DPPH and alkyl free radicals are measured by following the method of Heo et al. (2005) where corresponding samples are measured by Electron Spin Resonance: ESR method. In this regard, we have measured the antioxidant activity of DPPH by mixing 60 μl of DPPH solution and 60 μl of sample substance in a 1:1 ratio, having allowed to react for 2 min and transferred to a capillary tube then the alkyl antioxidant activity will be measured by mixing 20 μl of distilled water, 20 μl of sample substance, 20 μl of 40 mM AAPH, and 20 μl of 40 mM 4-POBN and allowing them to react in a water bath with the temperature of 37°C for 30 min, then transferred to the capillary tube and taking measurement of the concerned example substance. Besides, we use distilled water instead of the sample substance as it is the control group for each type of free radical.

Measurement of Angiotensin-1 Enzyme (ACE) Inhibitory Activity

ACE inhibitory activity is measured by following the protocol of Kim et al. (2018), using the ACE Inhibition Potential Evaluation Kit. We have made the Enzyme Working Solution by injecting 2 ml of distilled water into the Enzyme B of the kit and dissolving it. Then 1.5 ml of Enzyme B is injected into enzyme A. The indicator working solution is prepared by injecting 3 ml of distilled water into the enzyme C and coenzyme bottles, respectively, and dissolving. Then, 2.8 ml of each sample substance is added to the indicator solution and mixed them together. 20 μl of distilled water is poured into Blank1 and Blank2 of the 96-well microplates according to the protocol. The sample substance is then diluted with a volume of 20 μl for each concentration. After 20 μl of substrate buffer are diluted in all the microplate wells, 20 μl of Enzyme Working Solution will be diluted in the microplate wells except Blank2 and 20 μl of distilled water are diluted in Blank2 after reaction for 60 min in a Shaking Incubator with the temperature of 37 °C and having diluted 200 μl of the indicator working solution in all the microplate wells. The reaction is then left at room temperature for 10 min and the absorbance is measured at 450 Nanometre.

Vero Cells Culture

Viro cells are cultured in cell culture dishes using RPMI 1640 medium, as sub-culture in an incubator, containing 5% carbon dioxide at 37 °C for 2–3 days according to cell conditions and then used.

RESEARCH

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Active Oxygen Inhibition Test - DCFH-DA Assay

The measurement of the inhibitory effect of active oxygen generation by using the DCFH-DA assay testing. The measurement method is performed in 24-well microplates with 5.0 × 104 cells per well and cultured for 24 hours according to the method of Wang et al. (2018), then having diluted the sample reagent into the cells. After the reagent is incubated for 30 min, it will be diluted with AAPH (Stock, 200 mM) and having left to react in the incubator for 1–3 h. After the reaction, then diluting DCFH-DA (Stock, 500 μg/mL) and fluorescence is measured at 485-530 nm.

Assessment of Toxicity and Measurement of Protective Effects against Oxidation

Vero cells are used to assess the intracellular toxicity for each type of hydrolysate. Measurements are then performed by using MTT assay according to Wang et al. (2018) method by dividing cells 1x105 cells/ml to 96 wells per microplate and 190 microliters are instilled each time. Subcultures are then carried out in an incubator containing 5% carbon dioxide at 37 °C for 24 hours, then the sample reagent will be diluted with concentration. After 24 hours, 50 μl of MTT is diluted. After 3 hours of culture, using DMSO (Dimethyl Sulfoxide) to dissolve completely and stored for 12 hours out of light. The absorbance must be then measured at 540 nm.

Animal Environment and Culture

Test animals are rats with spontaneous hypertension (Spontaneously hypertensive rat : SHR, whey they are purchased from Center Lab Animal Co., Ltd. (Trade License No. 2017-0017). The rats with natural hypertension (SHR) are developed with the method of Ko et al. (2012) in which they are cultured for 2 weeks at 24 ± 1 °C with exposure to light for 12 hours. They are used in the test after acclimatization by Solid Food and drinking water can be given while measuring blood pressure with a blood pressure monitor 1 day before providing the test reagent. In the event that the animals with systolic blood pressure values greater than 160 mmHg. They will be excluded. Animals of similar body weight are selected and used for testing.

RESEARCH

(Translation)

Table 1. Ideal conditions for hydrolysate preparation from Hippocampus Abdominalises with 8 proteases.

(Enzymatic Digests)	Ideal Conditions	
	(PH)	Temperature (Celsius)
Distilled water	7.0	28
Protamex	6.0	40
Nutrase	6.0	50
Alkalase	8.0	50
Koji Enzyme	6.0	40
Flovorzyme	7.0	50
Trypsin	8.0	37
Pepsin	2.0	30

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(Translation)

Dosing of Test Reagents and Measuring Blood Pressure

Blood pressure is measured four times, 12 hours in total, starting at hour 0 when the blood pressure is first measured. Thereafter, measurements are made every 3 hours. In this test, food groups are divided into a general food group (Controlled), a sardine peptide food group. (Positive Controlled) and a food group of Hippocampusess' protamex hydrolysates are then given the test reagent. The Hippocampusess' protamex hydrolysate food group is divided into 50 mg/kg and 100 mg/kg for the test run. Blood pressure measurements are performed after accustoming the animals to the temperature of 30-32 °C as well as the plastic fittings in order for the experimental animals to fully dilate the blood vessels, therefore the position of the caudal artery is then confirmed and measuring by inserting a Volume Pressure Recording (VPR) sensor and together with collecting systolic blood pressure data, then the systolic blood pressure is measured by using a blood pressure monitor (CODA® High Throughput System, U.S.A).

Statistical Analysis

The statistical analysis of the results of this assay is expressed as the mean ± standard deviation for each sample reagent. One-way analysis of variance (ANOVA-test) is performed by using SPSS version 14, and having tested significance between items tested at the significance level of $P<0.01$, 0.05 and 0.1 by using Turkey's test.

Study Results and Discussion

Production of protein hydrolysate enzyme, antioxidant activity of DPPH, alkyl free radicals and measurement of ACE inhibitory activity.

We have hydrolyzed the Hippocampus Abdominalisess, seven protein enzymes and distilled water by setting the appropriate temperature and pH for each enzyme. The yield (Table 1) of the hydrolysate extracted with distilled water is 36.00±0.87%, which is slightly lower than that of other enzyme hydrolysates. However, when hydrolysis is done by using proteolytic enzyme, it is found that the yield is significantly much higher than the distilled water extract.

Table 2. Yield, antioxidant activity and ACE inhibitory effect of protease hydrolysate from Hippocampus Abdominalisess.

Sample Reagents	Yield (%)	ACE inhibition effect (IC_{50}, mg/mL)	Antioxidant Effect (IC_{50}, mg/mL)	
			DPPH	Alkyl
Flovorzyme	57.67±0.76	0.13±0.00	3.45±0.23	0.32±0.03

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Sample Reagents	Yield (%)	ACE inhibition effect (IC_{50}, mg/mL)	Antioxidant Effect (IC_{50}, mg/mL)	
			DPPH	Alkyl
Trypsin	57.17±0.76	0.11±0.00	2.35±0.22	0.33±0.03
Nutrase	65.50±3.04	0.06±0.01	3.04±0.11	0.48±0.02
Protamex	70.67±1.89	0.06±0.00	3.02±0.07	0.47±0.03
Koji Enzyme	39.33±0.58	0.14±0.01	2.20±0.18	0.40±0.0
Pepsin	55.67±1.04	0.16±0.00	4<	1.29±0.03
Alkalase	67.17±1.15	0.06±0.01	2.63±0.13	0.43±0.03
Distilled Water (DW)	36.00±0.87	0.95±0.10	1.41±0.01	0.58±0.02

Note: ACE (Angiotensin Convert Enzyme : Angiotensin Enzyme Converting), DPPH (1,1-Diphenyl-2-Picrylhydrazyl : 1,1-Diphenyl-2-Picrylhydrazyl), DW (Distilled Water : Distilled water)

However, when the seahorses are extracted as lyophilization and having ground into form of a dry powder of 2 g. and 100:1 ratio of substrate to enzyme are obtained. The Seahorse Protamex Hydrolysate : SHP (SHP), giving the highest yield among the seven protein hydrolysate enzymes at 70.67±1.89% (Table 2), followed by alkalase, nutrase, flavorzyme, pepsin, trypsin and koji enzyme decreased respectively. As the alkalase hydrolysate yield is 78.00±1.00% and the nutrase hydrolysate yield is 78.33±2.00%, which having presented a significant difference from the main test while the yield of protamex hydrolysate is 67.00±2.00% and that of distilled water hydrolysate (Distilled water) is 37.45. There are the samples reagents, showing similar trends at ±0.35. % which are consistent with the results of the study of Kim et al. (2016) as well. In this regard, due to the Electron Spin Resonance (ESR) is a method for detecting the inhibition effect against oxidative stress precisely and accurately. The antioxidant activity of the protein hydrolysate is thus confirmed. The IC50 value for the DPPH inhibitory activity is 1.41±0.01 mg/ml in the distilled water extract where it has shown higher activity than other hydrolysate enzymes (Table 2). Seahorse Protamex Hydrolysate (SHP) which has a high yield of 3.02±0.07 mg/ml. It is approved to have a relatively high IC50 value compared to other hydrolysates, as alkalase and nutrase are 2.63±0.13 mg/ml, 3.04±0.11 mg/ml, respectively and followed by trypsin: 2.35±0.22 mg/ml, flavorzyme: 3.45±0.23 mg/ml, and coenzyme: 2.20±0.18 mg/ml. The pepsin hydrolysate measurements of IC50 is more than 4 mg/ml. Besides, IC50 of alkyl antioxidant activity as shown in Table 2 presented that flavozyme hydrolysate has the best IC50 value at 0.32±0.03 mg/ml, followed by trypsin: 0.33±0.03 mg/ml, koji enzyme: 0.40±0.05 mg/ml., alcalase:

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(Translation)

0.43±0.03 mg/ml, protamax: 0.47±0.03 mg/ml, nutrase: 0.48±0.02 mg/ml, pepsin: 1.29±0.03 mg/ml and distilled water (DW): 0.58± 0.02 mg/mL as a result of the confirmation for the antioxidant activity of seahorses (Hippocampuses) extract with high-pressure hot water shown that IC50 of the antioxidant activity of DPPH is 87.46 µg/ml. while our protamex hydrolysate has presented the excellent antioxidant activity which is corresponding with the study of Jang et al. (2010). Looking at the ACE inhibition effect, it can be confirmed that the enzymatic hydrolysate is excellent, when compared with IC50 value of 0.95±0.10 mg/ml of seahorses' distilled water extract. In addition, the high yield of Seahorse Protamex Hydrolysate : SHP has presented an IC50 of 0.06±0.00 mg/ml. Both nutrase and alkalase are equal at 0.06 ± 0.01 mg/ml. Besides, the hydrolysates produced by other enzymes are not only relatively low yields, but also presented an ACE inhibition effect of lower than 0.1 mg/ml. The results of assessing the alkyl antioxidant activity and ACE inhibitory activity of alkalase hydrolysates from seahorses presented that the IC50 of each reaction is at 0.58±0.09 mg/ml and 0.32±0.06. mg/ml, which is in line with the results of a study by Kim et al. (2018). These results have demonstrated that the Hippocampus Abdominalisess have excellent antioxidant and antihypertensive effects from previous studies. When we have measured the alkyl antioxidant activity and ACE inhibitory activity by using protamax hydrolysate, the IC50 values are 0.47±0.03 mg/ml and 0.06±0.00 mg/ml, respectively, which having differed from previous studies and when comparing the alkalase hydrolysate results from the previous studies with that of our protamex hydrolysate. The protamex has been found to have excellent effects. Based on these results, we therefore, having assessed the antihypertensive activity in vivo, using the protamex hydrolysate from Seahorse Protamex Hydrolysate : SHP model. It that has not been evaluated in past studies.

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RESEARCH

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Cell Viability Rate (%)

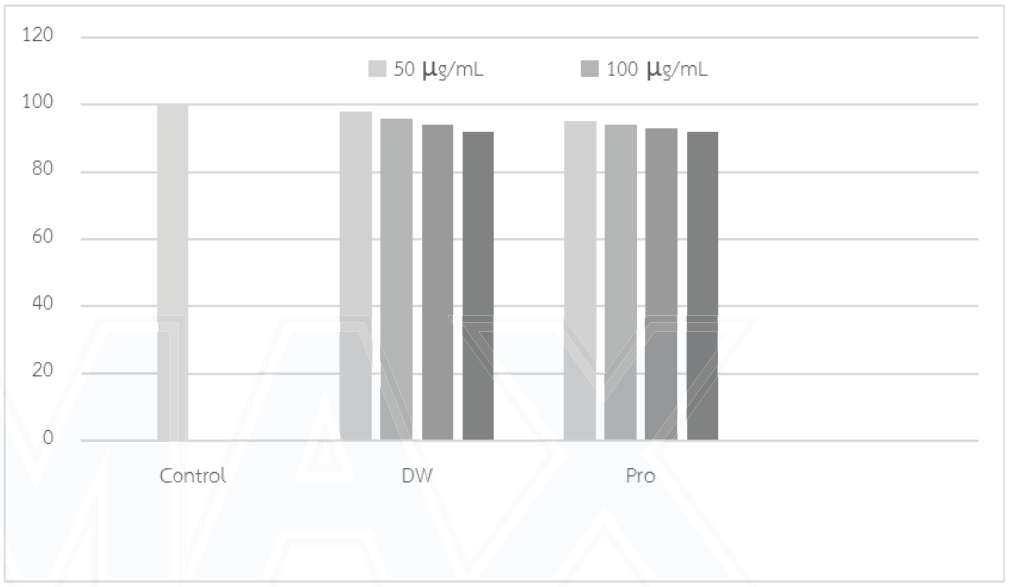

Figure 1. Cytotoxicity of distilled water extract and seahorses' protamex extract on viro cells and cell viability are measured by MTT assay. This assay have been repeated 3 times and data are displayed as the mean ± standard deviation (S.D), where *P<0.05 compared to the control group; MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide : 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyl Tetrazoleum Bromide DW (Distilled water) Pro (Protamex hydrolysate)

RESEARCH

(Translation)

Oxidative Stress Inhibition by AAPH from Seahorse Protamex hydrolysate: SHP

Currently, the antioxidant activity of hydrolysate has been extensively studied (Chung et al., 2006; Ko et al., 2010; Kwon et al., 2010; Kim et al., 2016). In those studies, Kim et al. (2016) had studied the production rate of reactive oxygen species (ROS) in viro cells by using pepsin hydrolysate from Hippocampus Abdominalisess and the rate of cell viability. The results of the study thus confirm that hydrolysate has excellent intracellular antioxidant activity. On the basis of this study, the antioxidant activity of protamex hydrolysate from the Hippocampus Abdominalisess could be predicted and the inhibitory effect of oxidative stress by AAPH could be confirmed using viro cells as well we have measured cell viability by using the MTT assay, which is a model method for measuring cell number along with accessing the toxicity of distilled water hydrolysate (Distilled water) from seahorses and Seahorse Protamex Hydrolysate (SHP) as shown in Fig.1. The control group is divided into the experimental group in which does not receive the sample reagent. The toxicity is confirmed 24 hours after dilution of the sample reagents at concentrations of 50 μg/ml, 100 μg/ml, 200 μg/ml and 400 μg/ml of each of the sample reagent as shown in Fig. 1. It is found that there is significant toxicity ($P<0.05$) at the maximum concentration of 400 mcg/mL. but no toxicity is observed at other concentrations. For distilled water hydrolysate (Distilled water) and Seahorse Protamex Hydrolysate are diluted at 50 μg/ml, 100 μg/ml, and 200 mcg/mL at each concentration to confirm the inhibitory activity of reactive oxygen species (ROS) with 10 mM AAPH in viro cells as shown in Fig. 2. When 10 mM AAPH is diluted at each concentration level. It is found that distilled water hydrolysate (Distilled water) from those seahorses having inhibited the formation of reactive oxygen species (ROS), depending on the concentration as well as presenting a significant difference at 200 μg/ml ($P<0.01$).

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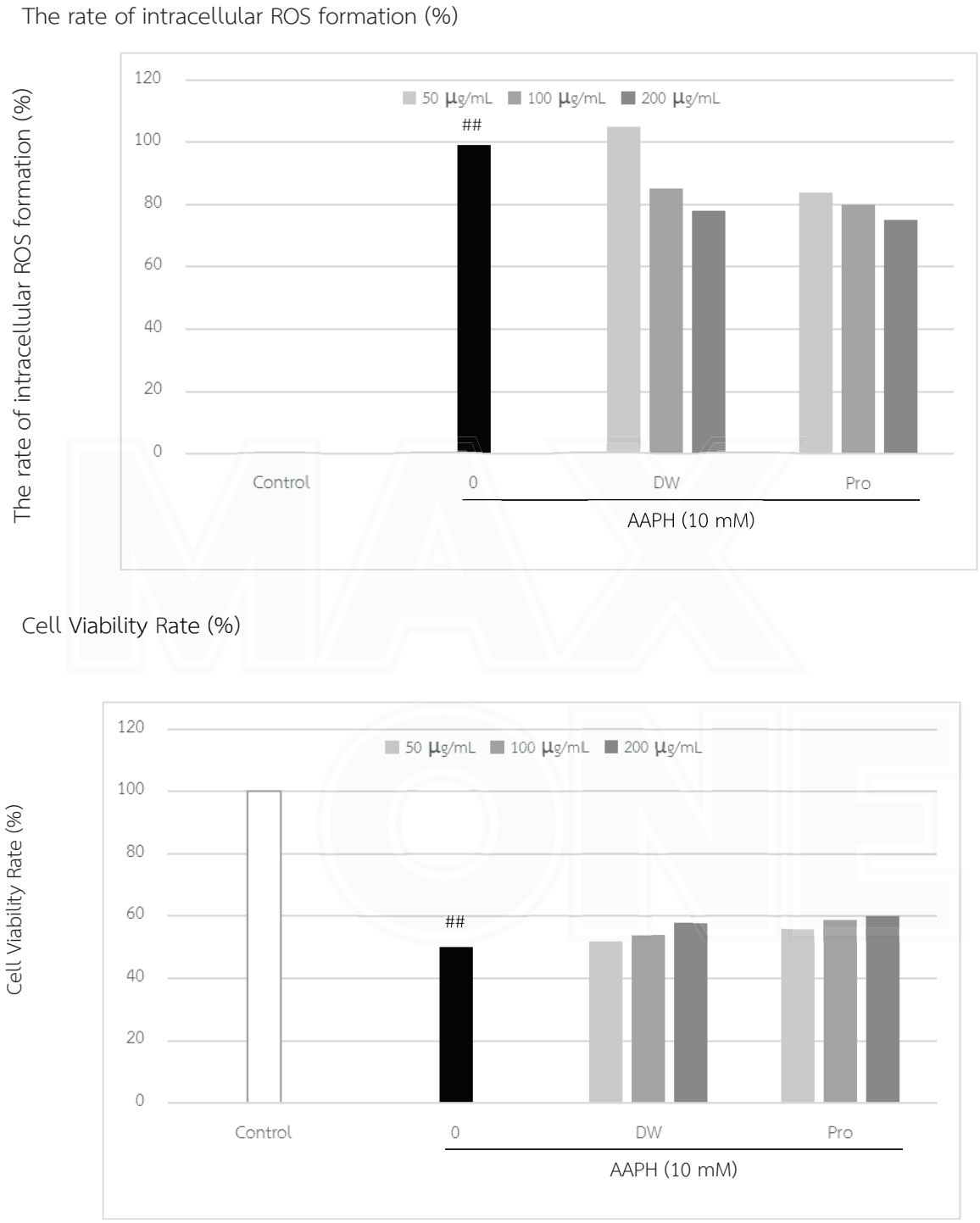

Fig. 2 Protective effect of Distilled Water (DW) extract and Seahorse Protamex Extract against AAPH-Induced Oxidative Stress in viro cells; (A) Rate of intracellular ROS formation; (B) Cell viability rate; The ROS incidence rate, which is measured by the DCF-DA assay, and the cell viability rate, is measured by the MTT assay, with this assay have performed 3 replicates and data were displayed as the mean ± standard deviation (S.D)

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(Translation)

##P<0.01, compared to the control group; *P<0.05 and **P<0.01, compared with AAPH; DW : Distilled water Pro : Protamex hydrolysate SHP (Seahorse Protamex hydrolysate : Seahorse Protamex Hydrolysate) ROS (Reactive Oxygen Species : Generation of free oxygen radicals DCF-DA : 2',7'-Dichlorofluorescin Diacetate MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide AAPH (2,2-Azobis-(2-Amidinopropane) Dihydrochloride

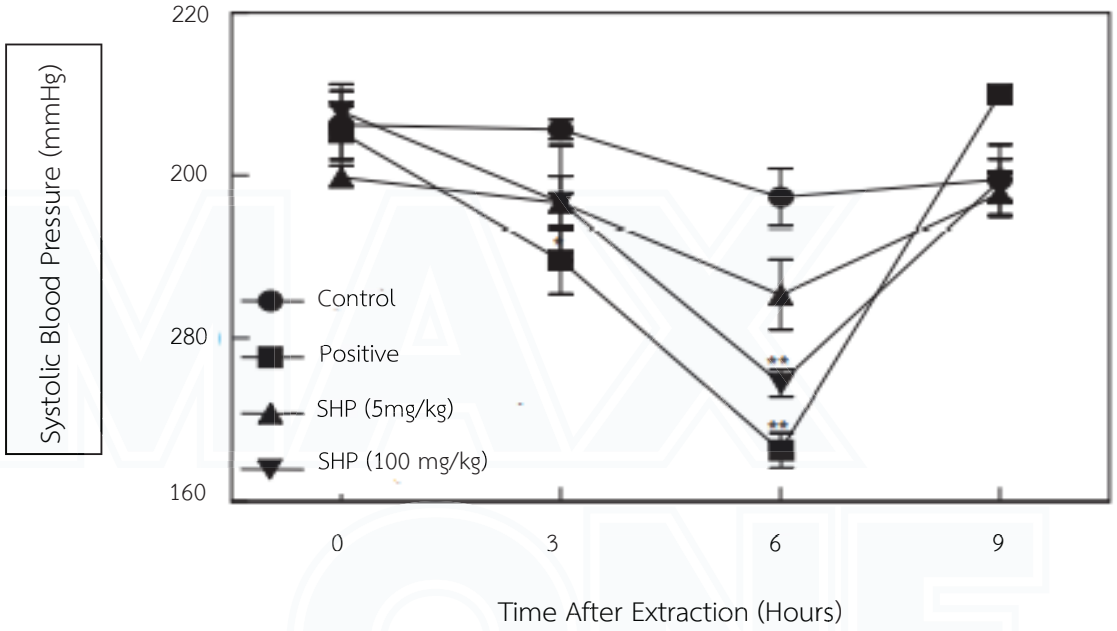

Fig. 3 Changes in systolic blood pressure of SHRs after Oral Administration of SHP (Protamex extract from seahorses); The positive control is sardine peptide (50 mg/kg); Indicator symbols : ● Controller; ■ Positive control (Sardine peptide) ▲ SHP (50 mg/kg) ▼ SHP (100 mg/kg); *P<0.1 and **P<0.0, compared to the control group; SHR (Spontaneously Hypertensive Rat SHP (Seahorse Protamex Hydrolysate).

Seahorse Protamex Hydrolysate (SHP) presented a relatively better effect in inhibiting Reactive Oxygen Species (ROS), when compared to Distilled Water Hydrolysate (Distilled water) where having obtained from sea horses as well as being significantly different (P<0.01) at 100 µg/ml and 200 µg/ml. In order that, 10 mM of AAPH are diluted with Seahorse Protamex Hydrolysate : SHP, , Distilled Water Hydrolysate (Distilled water) and Protamex Hydrolysate at a concentration of 50 µg/ml.,100 µg/ml., and 200 µg/

ml., respectively to confirm the anti-oxidation effect of AAPH. So that, the control group is divided into experimental group in which is activated the stress from the oxidation reaction without taking the sample reagents and the experimental group that is not taken the stress caused by the oxidation reaction. In the experimental group treated with only AAPH, the cell viability rate is significantly lower than that in the untreated group treated with AAPH ($P<0.01$) and it is found that the Seahorse Protamax Hydrolysate : SHP at a concentration of 200 µg/ml. has significantly excellent anti-oxidation effect of AAPH. For this reason, we can confirm that Protamex Hydrolysate from the Hippocampus Abdominalisess has an excellent antioxidant activity in viro cells which is different from previous studies.

Measurement of Antihypertensive Effect in Spontaneously Hypertensive Rat : SHR

To measure the antihypertensive effect of Seahorse Protamex Hydrolysate (SHP), we have followed the protocol of Ko et al. During the time of testing, it is found that rats in the control group with hypertension, having consistently high systolic blood pressure. As the results of measuring systolic blood pressure is measured at h 0, h 3, h 6 and h 9, after administration of 50 mg/kg of sardine peptide as a positive control to rats in which having spontaneous high blood pressure (Spontaneously Hypertensive Rat : SHR), a decrease in systolic blood pressure is observed at h 3 by confirming a significant difference ($P<0.1$) and the greatest decrease in systolic blood pressure is at h 6, confirming a significant difference ($P<0.05$). When treating spontaneously hypertensive rats (SHR) at concentrations as low as 50 mg/kg. It is found that blood pressure will be the lowest at h 6, but the difference is quite large when **having** comparison with the positive control. However, when the extract is given to rats with spontaneous hypertension (Spontaneously hypertensive rat: SHR) at a concentration of 1,000 mg/kg., the blood pressure is the lowest at h 6 as the same in the other treatment groups and being significantly different ($P<0.05$), compared to the positive control. In addition, the spontaneously hypertensive rats (SHR) have displayed their blood pressure values in 9 hours which are lower than the sardine peptide used as a positive control. However, there are many studies on the effects of the protein in the spontaneously hypertensive rats, where corresponding with this study. Some of which have been studied in such areas as Chlorella Ellipsoidea, Paralichthys Olivaceus and royal jelly etc. (Matsui et al., 2002; Ko et al., 2012; Ko et al. , 2016). From this study, the yield efficiency of each sample reagent is approved. Antioxidant activity of DPPH and alkyl free radicals including the inhibitory effect on ACE (Angiotensin Convert Enzyme) by hydrolyzing the Hippocampus Abdominalisess by using protein enzymes. For this reason, we have chosen Protamex Hydrolysate from Hippocampus Abdominalisess and can confirm that Protamex

(Translation)

Hydrolysate from edible Hippocampus Abdominalisess, has antioxidant and antihypertensive effects. It also has excellent performance as a health food or food additive in the future.

Acknowledgment

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06749

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The Rule of Patent Issuing

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Application No : 10-2019-0169228

A name that represents the invention : Compounds for lowering blood pressure based on Protamax Hydrolysate or an effective peptide of Hippocampus abdominalises.

Listed Number of Application
: 6

The ruling to issue a patent for this application is in accordance with Section 66 of the Patent Act. (The power of the patent will arise only after the payment of the patent fee and the establishment of the right under Section 87 of the Patent Law).

[Applying for Priority]

Prior Applying Country: KR, Priority Application No : 102190155167 (Prior Application Date : 28 November 2019)

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[Remark]

The review regarding the previous submission of this invention is the one filed on 27 May 2020 and has not yet ruled out whether the application passed a treaty prior application after the aforementioned date, it violates the Patent Act, Article 29 paragraph three and four, or Article 36 paragraph one to paragraph three in any way. – End -

[Reference]

1. KR102008620 B1

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-Translation-

28 May 2020

Bureau of Chemical and Biotechnology Inspection, the Office of Intellectual Property, the Republic of Korea

Pharmaceutical Chemicals Inspection Department Inspector: Sora Yun (-Signature-) (Seal)

<<Instructions>>

[Payment of Registration Fees, Enabling Service for Issuing Electronic Registration Certificates, Guidelines of Applying for Patent Technology Awards and Verification of Registration Notices]

1. Instructions for paying the registration fee

- Estimated amount for patent fees (1st-3rd year period) : 279,000 Won (In case of the 1st -3rd year fee is paid within the normal payment period) : 139,500 Won (In case of being eligible for a 50% reduction) : 83,700 won (In the case of being eligible for a 70% reduction).
- The above payment amount is subject to change when an additional payment is made after the normal patent fee payment period is overdue or when the patent fee is changed in the patent fee payment period.
- For detailed instructions, please see the attachment below. (Guidelines for paying patent fees).

2. The applicant may file a separate application within 3 months as from the date of receiving the ruling on this patent. (Unless in the case where the date of application for registration of the establishment of a right under Section 79 of the Patent Law is less than 3 months, counting up to the aforesaid date).

3. Since 1 July 2018 onwards, electronic registration certificates have been issued.

In the event that you have applied for an electronic registration certificate in advance at (http://www.patent.go.kr) [Patent] or choose to receive electronic documents as a method of obtaining a registration certificate in a payment document (Form 25 of the Patent Registration Order Notice). Electronic registration certificate's files can be downloaded via If you do not submit an electronic registration certificate in advance and pay the registration fee with the attached payment slip. The registration certificate will be issued as the original document.

4. Koreans can apply for a patent technology award for an invention (Design) that meets the conditions below during the application period announced on the website. (http://www.patent.go.kr/jsp/kiponet/mp/patentprize/ index.htm) around February and August every year.

- Being as an invention (Design) registered within 5 years as from the date of application (1 February of each year for the first half of the year and 1 August of every year for the second half of the year).
- Implementation of a patented invention must be authorized or registered in accordance with "Pharmaceutical Law" or "Agricultural Chemicals Control Act" as well as being an invention that generates sales by launching the product within 5 years as from the date of application in the form of inventions that need to be tested for efficiency and safety in order to apply for permission or registration, etc.

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5. The applicant can register for an extension of the patent authority due to late registration within 3 months as from the date of registration of the right. (Except in the case of filing an application after 15 March 2012 and registering the establishment of patent rights within a period of 4 years as from the date of applying for a patent and 3 years as from the date of submission of the application for verification of the application only).

※ You may check the registration notice on the website of the Intellectual Property Office of the Republic of Korea. (Patent Topic → Making notice on the internet) within 7 days after paying the registration fee.

※ In the event that applying for the notification service at the Customer Center [Related to the Patent] You can receive notification of various civil complaints such as notification of appointing a responsible investigator and due date notification by email and mobile phone (SMS).

※ Since the International Intellectual Property's Training Institute of the Office of Intellectual Property, the Republic of Korea, offers free online education services, (www.ipacademy.net) to raise awareness of intellectual property among the general public. So please use the service to the fullest.

※ Please contact the Patent Applicant Consultation Center at Tel: 1544-8080 for follow-up procedures or the application form described above. And please contact the Intellectual Property Office of the Republic of Korea at Tel: 042-481-3367 (Responsible Investigator: Sora Yoon) for details of the decision of this notice.

※ The Office of Intellectual Property of the Republic of Korea and Officers of The Office of Intellectual Property of the Republic of Korea will not solicit or accept money, valuables, entertainment or any facilitation from the applicant or agent whatsoever that is in addition to the statutory fees set forth in the regulations. If you acknowledge or witness any such claim or circumstance, please report to the Government Corruption Reporting Center. (The Office of Intellectual Property of the Republic of Korea → Complaint/Participation → Public Report → Government Corruption Reporting Center) on the Republic of Korea's Intellectual Property Office website. (https://www.kipo.go.kr)

※ (The Office of Intellectual Property of the Republic of Korea (Daejeon Government Center, Dunsan-dong) 4-dong, 189 Cheongsa-ro Seo-gu Daejeon Postal Code 35208

The Special Investigation Division Police of the Intellectual Property Office of the Republic of Korea will be in charge and protect valuable intellectual property of the people.

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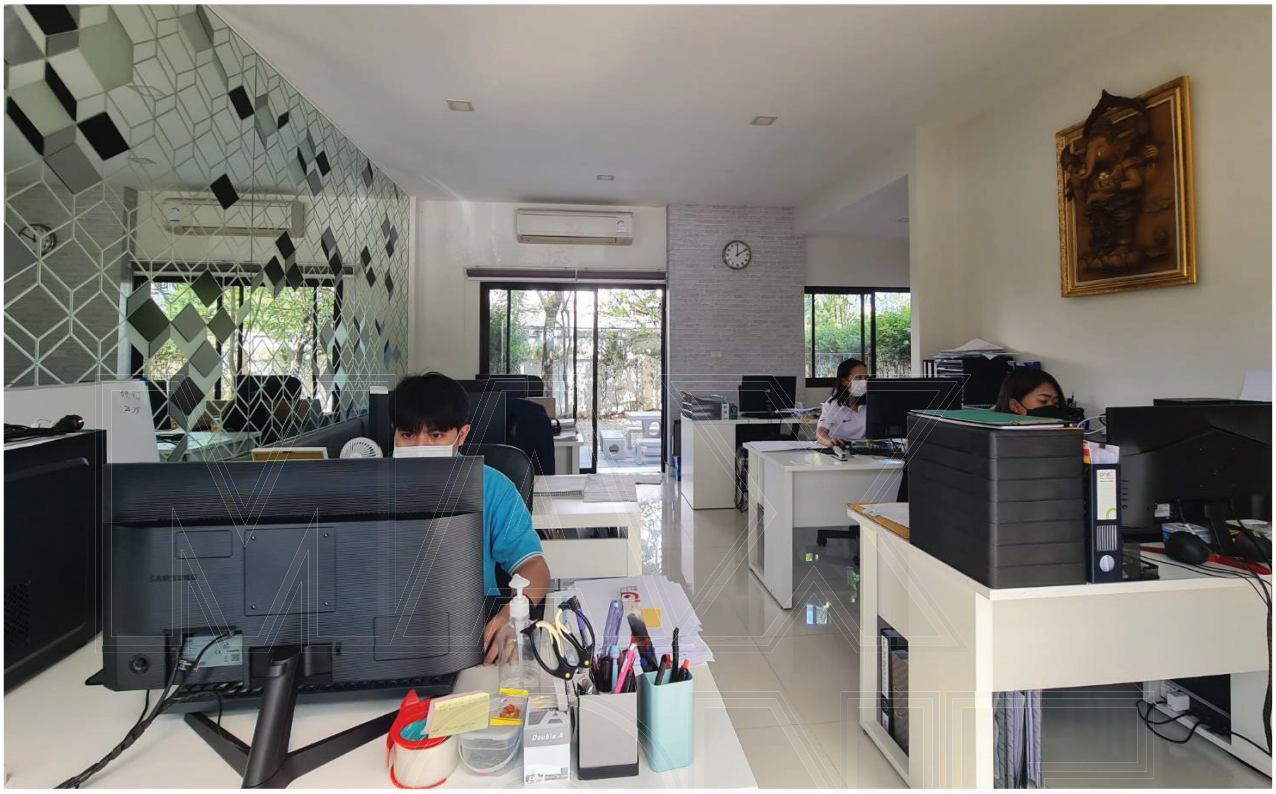

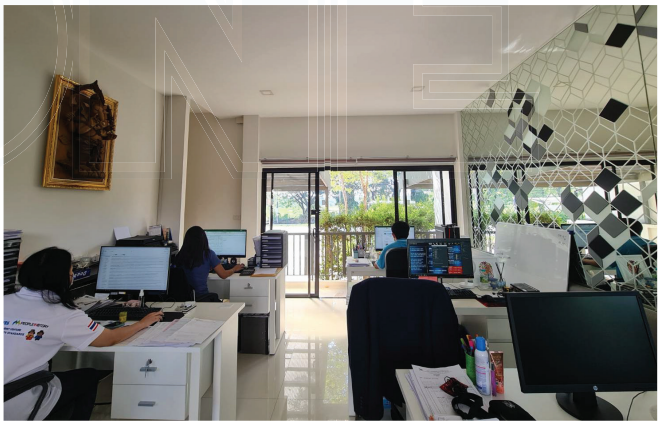


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