DESCRIPTION

TITLE

COMPOSITION COMPRISING ORGANIC SELENIUM COMPOUND

FIELD

[0001]

The present invention relates to a composition containing an organic selenium compound as an active ingredient. More specifically, the invention relates to a composition, food, food composition and medical composition for reduction of oxidative stress, prevention of ischemic disease, improvement of fatty liver, improvement of impaired glucose tolerance, improvement of metabolic syndrome including abnormal lipid metabolism, protection and improvement of disorders associated with metabolic syndrome, suppression of carcinogenesis, suppression of general immunity disorders, suppression of arteriosclerosis, prevention of cognitive disorder and improvement of brain function, protection of corneal epithelial cells, improvement of dry eye and/or suppression of inflammation.

BACKGROUND

[0002]

The organic selenium compound selenoneine has been discovered to have powerful antioxidant activity (free radical-eliminating activity) and to exhibit an antioxidant effect on human cells in the body (PTL 1). Substances with antioxidant effects remove active oxygen species in the body and exhibit various forms of physiological activity. Selenium is a trace element that is essential for the human body, and intake of selenoneine, which is a form of organic selenium derived from seafood, is one way for deficiency of selenium to be supplemented by diet. Applications of functional foods with selenoneine as the active ingredient are therefore anticipated for the future.

[0003]

A diet of fish is known to have a preventive effect against lifestyle diseases such as cancer and diabetes, as well as an anti-aging effect, and therefore selenoneine is thought to contribute to preventing lifestyle diseases and also to anti-aging, but the effect of selenoneine for preventing lifestyle diseases, as well as its molecular mechanisms and the interaction of selenoneine with other biomolecules, has not yet been verified. In particular, no studies have yet demonstrated the effect that ingesting selenoneine has on improving antioxidant activity including free radical-eliminating activity, and thereby promoting healthy function, by selenoneine taken up into the human body or animal cells. It was therefore found necessary to

verify the physiological activity of selenoneine on biomolecules, model cells and the body, determining its effectiveness across a wide scope for development and implementation of novel uses of selenoneine.

CITATION LIST

[PATENT LITERATURE]

[0004]

[PTL 1] Japanese Patent Publication No. 5669056

DISCLOSURE OF THE INVENTION

PROBLEMS TO BE SOLVED BY THE INVENTION

[0005]

It is an object of the present invention to study the health function of the organic selenium compound selenoneine and analyze its actual effectiveness in the body using mammalian cells and rodents, and to demonstrate its effect of improving the damage of oxidative stress in the body and alleviating the stress, which includes countering metabolic syndrome, preventing carcinogenesis, protecting from ischemic disease, preventing cognitive disorder, improving dry eye and suppressing inflammation. The invention thus provides a selenium compound-containing composition, containing selenoneine, that has a novel food function with an effect of countering metabolic syndrome, for example.

[0006]

Oxidative stress is defined as a breakdown in the balance between oxidation reaction in the body, caused by active oxygen and active nitrogen species or free radicals, and antioxidation reaction, thus creating an undesirable condition in the body, and generation of active oxygen and free radicals, as well as oxidative stress, are known to contribute to a host of diseases including inflammation, arteriosclerosis, cancer, aging, cranial nerve disease, respiratory disease, cataract, skin disease, gastrointestinal disease, heart disease and hypertension, and to play a central role in the onset and pathogenesis of numerous diseases. Verifying that selenoneine, which has powerful free radical-eliminating activity, reduces active oxygen, free radicals and oxidative stress generated in the body and has a preventive effect on diseases caused by oxidative stress, can help lead to the development of novel food products containing selenoneine as an active ingredient.

MEANS FOR SOLVING THE PROBLEMS

[0007]

The present inventors conducted biochemical analysis of the health functions of

selenoneine, and have completed this invention upon finding that ingestion of a selenium compound-containing composition has effects of alleviating stress to protect against brain damage resulting from cerebral ischemia, and of preventing stress-induced damage.

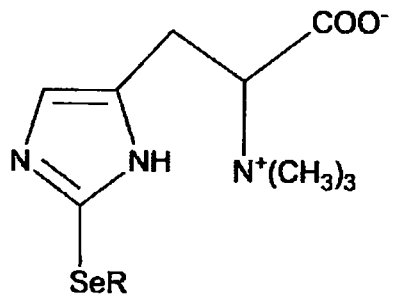
[0008]

Specifically, the present invention provides the following.

[1] A composition for reduction of oxidative stress, suppression of oxidized lipids, prevention of ischemic disease, improvement of metabolic syndrome, improvement of impaired glucose tolerance, protection from and improvement of damage associated with metabolic syndrome including abnormal lipid metabolism, suppression of carcinogenesis, suppression of general immunity disorders, suppression of vascular endothelial damage, suppression of arteriosclerosis, prevention of cognitive disorder, improvement of brain function, protection of corneal epithelial cells, improvement of dry eye or suppression of inflammation, the composition comprising an organic selenium compound represented by formula I:

[0009]

[Chemical Formula 1]



I

[0010]

wherein

R is hydrogen or an ergothionyl, glutathionyl or cysteinyl group,

as an active ingredient.

[0011]

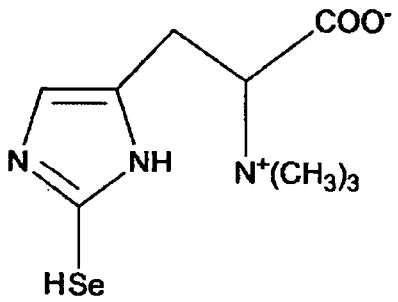
[2] The composition according to [1] above, wherein the organic selenium compound is 3-(2-hydroseleno-1H-imidazol-5-yl)-2-(trimethylammonio)propanoate,

[0012]

wherein the compound is represented by formula II:

[0013]

[Chemical Formula 2]



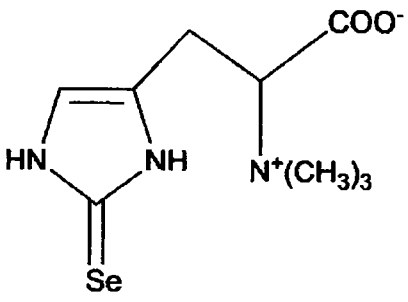
II

[0014]

[3] A composition for reduction of oxidative stress, suppression of oxidized lipids, prevention of ischemic disease, improvement of metabolic syndrome, improvement of impaired glucose tolerance, protection from and improvement of damage associated with metabolic syndrome including abnormal lipid metabolism, suppression of carcinogenesis, suppression of general immunity disorders, suppression of vascular endothelial damage, suppression of arteriosclerosis, prevention of cognitive disorder, improvement of brain function, protection of corneal epithelial cells, improvement of dry eye or suppression of inflammation, the composition comprising an organic selenium compound represented by formula III:

[0015]

[Chemical Formula 3]



III

[0016]

as an active ingredient.

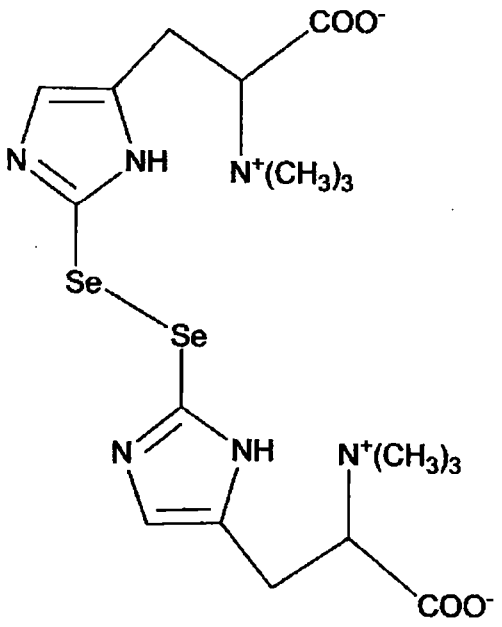
[0017]

[4] A composition for reduction of oxidative stress, suppression of oxidized lipids, prevention of ischemic disease, improvement of metabolic syndrome, improvement of impaired glucose tolerance, protection from and improvement of damage associated with metabolic

syndrome including abnormal lipid metabolism, suppression of carcinogenesis, suppression of general immunity disorders, suppression of vascular endothelial damage, suppression of arteriosclerosis, prevention of cognitive disorder, improvement of brain function, protection of corneal epithelial cells, improvement of dry eye or suppression of inflammation, the composition comprising an organic selenium compound represented by formula IV:

[0018]

[Chemical Formula 4]



IV

[0019]

as an active ingredient.

[0020]

[5] A food composition for reduction of oxidative stress, suppression of oxidized lipids, prevention of ischemic disease, improvement of metabolic syndrome, improvement of impaired glucose tolerance, protection from and improvement of damage associated with metabolic syndrome including abnormal lipid metabolism, suppression of carcinogenesis, suppression of general immunity disorders, suppression of vascular endothelial damage, suppression of arteriosclerosis, prevention of cognitive disorder, improvement of brain function, protection of corneal epithelial cells, improvement of dry eye or suppression of inflammation, containing a composition according to any one of [1] to [4] above.

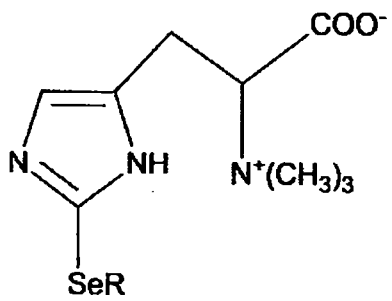
[6] A food containing a composition according to any one of [1] to [4] above.

[0021]

[7] A medical composition for reduction of oxidative stress, suppression of oxidized lipids, prevention of ischemic disease, improvement of metabolic syndrome, improvement of impaired glucose tolerance, protection from and improvement of damage associated with metabolic syndrome including abnormal lipid metabolism, suppression of carcinogenesis, suppression of general immunity disorders, suppression of vascular endothelial damage, suppression of arteriosclerosis, prevention of cognitive disorder, improvement of brain function, protection of corneal epithelial cells, improvement of dry eye or suppression of inflammation, the medical composition comprising an organic selenium compound represented by formula I:

[0022]

[Chemical Formula 5]



I

[0023]

[wherein R is hydrogen or an ergothionyl, glutathionyl or cysteinyl group], as an active ingredient.

[0024]

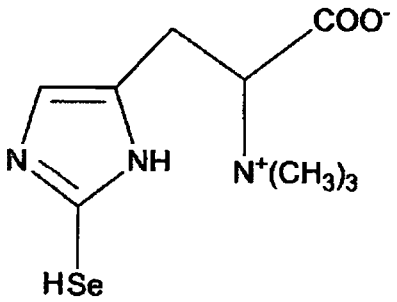
[8] The medical composition according to claim 7, wherein the organic selenium compound is 3-(2-hydroseleno-1H-imidazol-5-yl)-2-(trimethylammonio)propanoate,

[0025]

wherein the compound is represented by formula II:

[0026]

[Chemical Formula 6]



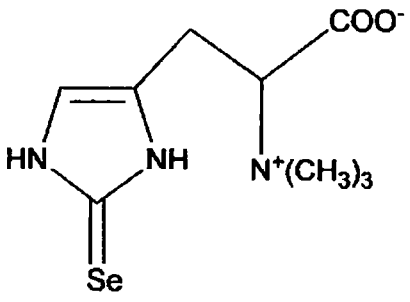
II

[0027]

[9] A medical composition for reduction of oxidative stress, suppression of oxidized lipids, prevention of ischemic disease, improvement of metabolic syndrome, improvement of impaired glucose tolerance, protection from and improvement of damage associated with metabolic syndrome including abnormal lipid metabolism, suppression of carcinogenesis, suppression of general immunity disorders, suppression of vascular endothelial damage, suppression of arteriosclerosis, prevention of cognitive disorder, improvement of brain function, protection of corneal epithelial cells, improvement of dry eye or suppression of inflammation, the medical composition comprising an organic selenium compound represented by formula III:

[0028]

[Chemical Formula 7]



III

[0029]

as an active ingredient.

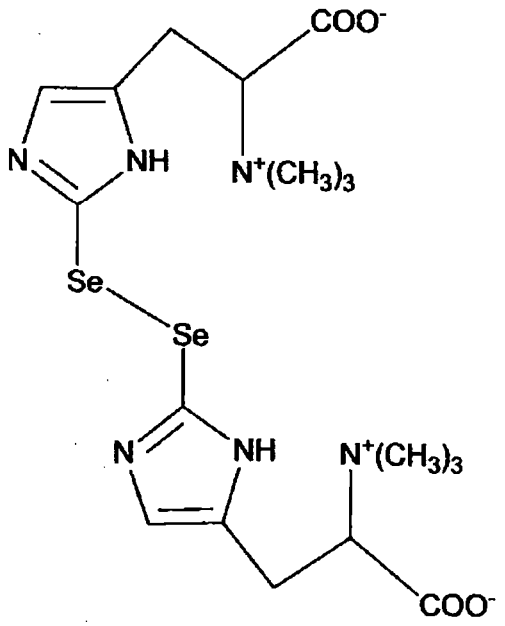
[0030]

[10] A medical composition for reduction of oxidative stress, suppression of oxidized lipids, prevention of ischemic disease, improvement of metabolic syndrome, improvement of impaired glucose tolerance, protection from and improvement of damage associated with

metabolic syndrome including abnormal lipid metabolism, suppression of carcinogenesis, suppression of general immunity disorders, suppression of vascular endothelial damage, suppression of arteriosclerosis, prevention of cognitive disorder, improvement of brain function, protection of corneal epithelial cells, improvement of dry eye or suppression of inflammation, the composition comprising an organic selenium compound represented by formula IV:

[0031]

[Chemical Formula 8]



IV

[0032]

as an active ingredient.

ADVANTAGEOUS EFFECTS OF INVENTION

[0033]

The organic selenium compound of the invention is already known to have antioxidant activity that inhibits generation of peroxides and radicals, functioning as an antioxidant based on free radical-eliminating activity, but verification of its effectiveness when used in mammalian bodies has not yet been published. The continuing verification of its anticancer and antiarteriosclerotic effects by effectiveness testing by administration to human cells and living organisms allows its utilization for novel purposes such as preventing and improving lifestyle diseases and aging-associated diseases.

BRIEF DESCRIPTION OF DRAWINGS

[0034]

FIG. 1 shows (A) a chromatogram for selenium 82 as a standard selenoneine product, and (B) a chromatogram for the selenoneine-containing fish extract obtained in Example 1.

FIG. 2 is a graph showing evaluation of the effect of a selenium-containing composition (selenoneine-containing fish extract) as a test substance in a gerbil model subjected to ischemic stress load followed by measurement of spontaneous movement, and examination of influence on cerebral ischemia reperfusion.

FIG. 3 is a graph showing evaluation of the effect of a selenium-containing composition (selenoneine-containing fish extract) as a test substance in a gerbil model subjected to cerebral ischemia reperfusion load followed by a Y maze test, and examination of influence on cerebral ischemia reperfusion (7th day).

FIG. 4 is a graph showing evaluation of the effect of a selenium-containing composition (selenoneine-containing fish extract) as a test substance in a gerbil model subjected to cerebral ischemia reperfusion load followed by a Y maze test, and examination of influence on cerebral ischemia reperfusion (14th day).

FIG. 5 is a graph showing results of examining the improving effect on metabolic syndrome by ingestion of a selenium-containing composition, in mice with abnormal glucose metabolism.

FIG. 6 is a graph showing results of examining the improving effect on damage associated with metabolic syndrome by ingestion of a selenium-containing composition, in mice with abnormal glucose metabolism.

FIG. 7 is a graph showing results of examining the anticancer effect and suppressing effect against general immunity disorders by ingestion of a selenium-containing composition using a colorectal cancer primary tumor model.

FIG. 8 is a graph showing results of examining the suppressing effect against general immunity disorders by ingestion of a selenium-containing composition using a colorectal cancer primary tumor model.

FIG. 9 is a graph showing the results of examining the effect of preventing cancer relapse by ingestion of a selenium-containing composition, based on inhibition of Treg cells.

FIG. 10 is a graph showing the results of examining the effect of inhibiting progression of atherosclerosis by ingestion of a selenium-containing composition, based on suppression of smooth muscle cell migration.

FIG. 11 is a graph showing the results of examining the effect of ingestion of a selenium-containing composition for protecting cells from amyloid-β toxicity.

FIG. 12 is a graph showing the results of examining the change in viable cell count after

dry stress by ingestion of a selenium-containing composition.

FIG. 13 is a graph showing the results of examining the change in mucin gene expression by ingestion of a selenium-containing composition.

FIG. 14 is a graph showing the results of examining the effect of suppressing NFκ-B activation by ingestion of a selenium-containing composition.

MODE FOR CARRYING OUT THE INVENTION

[0035]

The present invention relates to a selenium-containing composition for improvement of metabolic syndrome, containing an organic selenium compound as an active ingredient.

[0036]

(1) Organic selenium compound

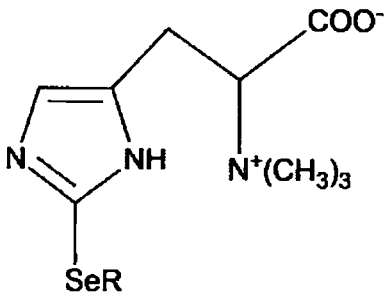
The selenium-containing composition of the invention can be obtained following the method described in Japanese Patent Publication No. 5669056, for example. Unless otherwise specified, the term “organic selenium compound” as used herein refers to the selenoneine monomer represented by chemical formula II and its tautomer represented by chemical formula III, the oxidized dimer of selenoneine represented by chemical formula IV and the chemically modified form of selenoneine represented by chemical formula I, and is also intended to include a “selenium-containing composition” that contains these compounds. An organic selenium compound can be obtained by extracting a sample from an organism such as a fish using an aqueous solvent or organic solvent to obtain a selenium concentrate, and then separating and purifying it by chromatography. The “selenium concentrate” is a selenium concentrate containing an organic selenium compound represented by chemical formulas I to IV according to the invention (described below), and it is obtained by extracting a sample with an organic solvent or water and then concentrating it with a rotary evaporator or the like. When the selenium concentrate is in solution form, it is preferably one containing the organic selenium compound of the invention at 5 μg/mL or greater, and more preferably it is a dry powder solidified by concentration under reduced pressure.

[0037]

Organic selenium compounds to be used for the invention are limited to compounds of formulas I to IV. They “organic selenium compounds” are compounds represented by formula I:

[0038]

[Chemical Formula 9]



I

[0039]

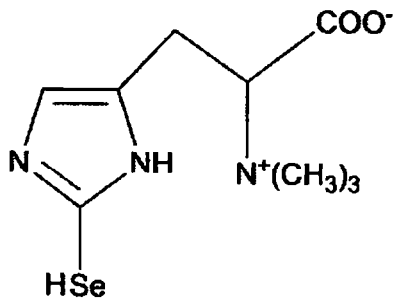
where the bonded substituent R is hydrogen, ergothioneine, glutathione, cysteine, acetylcysteine, homocysteine, methylmercury or a thiol compound assumed to be formed in the body, with "organic selenium compounds" also including metals or polymer materials bonded via selenol groups.

[0040]

According to one embodiment, the organic selenium compound of the invention may be 3-(2-hydroseleno-1H-imidazol-5-yl)-2-(trimethylammonio)propanoate represented by formula II:

[0041]

[Chemical Formula 10]



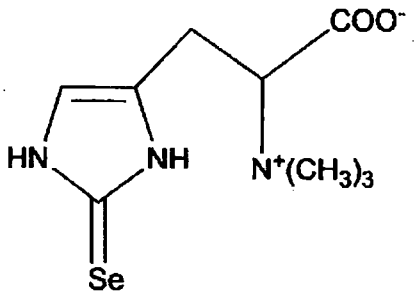
II

[0042]

or 3-(2-selenoxo-2,3-dihydro-1H-imidazol-4-yl)-2-(trimethylammonio)propanoate represented by formula III:

[0043]

[Chemical Formula 11]



III

[0044]

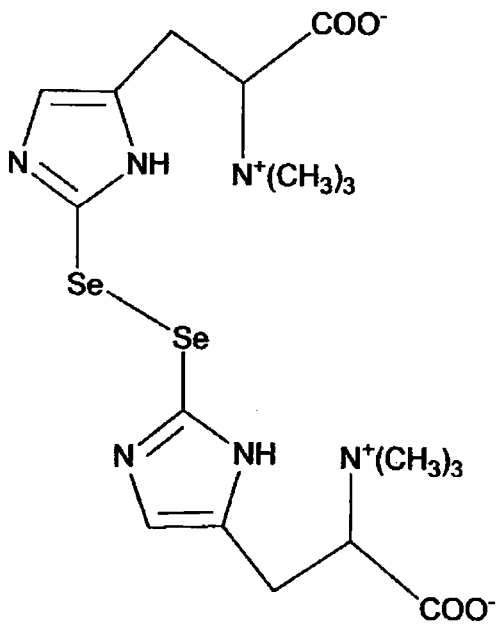
As mentioned above, the organic selenium compounds represented by formula II and formula III are all organic selenium compound monomers. The organic selenium compounds of formula II have a molecular structure with a selenol group bonded to the 2-position carbon atom of an imidazole ring, the selenol group forming a tautomer in equilibrium with the selenoketone group, and therefore in a solution state it has two chemical forms, the selenol form (formula II) and selenoketone form (formula III), depending on the conditions of the solvent. In the presence of a nonpolar solvent where the equilibrium is shifted toward the selenol-type isomer, an oxidized dimer readily forms, but in a polar solvent, the chemical equilibrium becomes biased toward the selenoketone isomer form, so that it exists mainly as a monomer.

[0045]

The organic selenium compounds of the invention also include dimers wherein the base unit is a compound having a molecular structure wherein a selenol group and a trimethylammonium group are bonded to an imidazole ring, in the oxidized dimer (3,3'-(2,2'-diselenediylbis(1H-imidazole-5,2-diyl))bis(2-(trimethylammonio)propanoate)) represented by formula IV:

[0046]

[Chemical Formula 12]



IV

[0047]

the compound forming a diselenide via the selenol groups.

[0048]

According to the invention, the selenium content of the selenium-containing composition can be measured by a fluorescence method using 2,3-diaminonaphthalene (DAN) (see J. H. Watkinson, Anal. Chem., 38, 92-97 (1966), for example), or HPLC-ICP-MS analysis using a GPC column (see H. Ge, et al., Anal. Commun., 33, 279-281 (1996), for example).

[0049]

According to another embodiment, the selenium-containing composition of the invention may be provided in the form of a composition containing a compound represented by formula II ("selenoneine"). The composition containing selenoneine according to the invention is not restricted, and may be in the form of a tablet, paste or beverage containing a selenoneine concentrate, its dried form or purified selenoneine, with a selenium content of 0.1 to 100 μg. For use as a food such as supplement or beverage, it is preferably molded into a form suitable for ingestion of the required amount of selenium per day. It may also include other food materials or food additives such as sweeteners, flavors, diluents, thickeners or preservatives.

[0050]

Examples of selenoneine-derived materials, as typical organic selenium compounds for the invention, include fish, such as tuna (bluefin tuna, southern bluefin tuna, yellowfin tuna,

bigeye tuna and albacore), swordfish (swordfish, marlin, Indo-Pacific sailfish, blue marlin), bonito (bonito, auxis, frigate tuna, striped bonito, mackerel tuna), mackerel (chub mackerel, blue mackerel, Norwegian mackerel, double-lined mackerel, Indian mackerel), yellowtail, horse mackerel (Japanese horse mackerel), sardine (Japanese sardine, anchovy) and seabream (red seabream), marine mammals such as toothed whales, and microbes such as yeast (*Schizosaccharomyces pombe*) (see "Methylmercury and selenium in marine products, Kagaku to Seibutsu 50(11), 807-817, 2012-11-01; Genetic and Metabolomic Dissection of the Ergothioneine and Selenoneine Biosynthetic Pathway in the Fission Yeast, *S. pombe* and Construction of an Overproduction System, PLoS One., 2014 July 31; 9(7): e105177).

[0051]

(2) Selenium-containing composition

The invention provides a selenium-containing composition containing the organic selenium compound described above as an active ingredient. The content of the organic selenium compound in the composition of the invention may be 0.000001 to 99 wt% based on the total weight of the composition, and a person skilled in the art can appropriately adjust the content of the organic selenium compound to obtain a desired effect. When the selenium-containing composition is used as an active ingredient, it may be in a form containing 0.1 to 100 μg selenium, as mentioned above. The starting material for the composition is not particularly restricted so long as it contains selenoneine, and for example, fish, marine mammals and microbes such as yeast, as well as hyphae, plants, seaweed, algae and chemically synthesized products, may be used as starting materials.

[0052]

Depending on the purpose, the organic selenium compound of the invention may also contain other components in addition to the organic selenium compound as the active ingredient. Other components that may be included in the selenium-containing composition of the invention are not limited and may be water; alcohol; processed meat products; common food materials and their powders such as rice, wheat, corn, potato, sweet potato, soybean, tangle weed, wakame and Gelidiaceae; saccharides such as starch, rice jelly, lactose, glucose, fructose, sucrose and mannitol; proteins such as fish meat powder, egg white or dried whole egg, fish eggs, fish meat protein, plant protein, collagen, myosin and gluten; common food additives such as spices, sweeteners, edible oils and vitamins; surfactants; diluents; coloring agents; preservatives; coating auxiliary agents; lactose; dextrin; corn starch; sorbitol; crystalline cellulose; polyvinylpyrrolidone; oils; humectants; thickeners; preservatives; flavorings and combinations of the foregoing. The composition of the invention may also contain other chemical agents as necessary. The contents of such other components and/or other chemical agents are not particularly restricted and may be selected as appropriate by a person skilled in the art.

[0053]

(3) Food, food composition and medical composition containing selenium-containing composition

The present invention provides a food, food composition and medical composition containing the composition of the invention. When the composition of the invention is to be used as a food or food composition, it may include a carrier that is acceptable as a food and suited for the desired product form, as well as other additives. Examples of such carriers and additives include diluents, binders, flavorings, buffering agents, thickeners, coloring agents, stabilizers, emulsifiers, dispersing agents, suspending agents, disintegrants, lubricants and preservatives. The medical composition of the invention may be in the form of a solid formulation such as a food, food additive, tablet, powder, fine granules, granules, capsules, pill or sustained-release agent or a liquid formulation such as a solution, suspension or emulsion, for example, as an orally ingestible form. Such forms can be formulated or manufactured by methods commonly used in the technical field, together with the necessary carriers and additives.

[0054]

(4) Use

The selenium-containing composition comprising a selenium compound of formula I to IV according to the invention as an active ingredient, and a food or food composition containing the composition, are expected to exhibit effects of reducing oxidative stress, suppressing oxidized lipids, preventing ischemic disease, improving metabolic syndrome, improving impaired glucose tolerance, protecting from and improving damage associated with metabolic syndrome including abnormal lipid metabolism, suppressing carcinogenesis, suppressing general immunity disorders, suppressing vascular endothelial damage, suppressing arteriosclerosis, preventing cognitive disorder, improving brain function, protecting corneal epithelial cells and improving dry eye, and also suppressing inflammation. The form in which it is used as a food is not restricted, and it may be in the form of a health food, functional food, special health food, nutritional function food, nutritional supplement, or functional designated food or beverage. The word “improvement”, as used throughout the present specification, means improvement or alleviation of the state of a disease, symptoms or condition, prevention of aggravation of a disease, symptoms or condition, or reversal, prevention or delay of progression of a disease, symptoms or condition. The term “reduction” means delay of the appearance, decrease in frequency or reduction in severity of a disease, symptom or condition, and it may be used synonymously with “improvement”. The term “inhibition” means complete or partial suppression of a disease, symptom or condition. The terms “improvement”, “reduction” and “inhibition” may be used herein in a mutually synonymous manner. However, “prevention” means prevention or delay of onset of a disease or its symptoms in an individual, or reduction in

the risk of onset of a disease or its symptoms in an individual. The term “protection” means that onset of various clinical symptoms does not occur, and it is understood to include “prevention”.

[0055]

In order to obtain the aforementioned desired effect with the selenium-containing composition of the invention, the organic selenium compound as the active ingredient may be included at 0.1 ng to 10 mg, more preferably 1 ng to 5 mg, even more preferably 2 ng to 3 mg, yet more preferably 3 ng to 1 mg, even yet more preferably 5 ng to 500 μg, still yet more preferably 5 ng to 100 μg and most preferably 10 ng to 50 μg, per 1 kg of body weight of an adult. More specifically, administration or ingestion per day is preferably 2 ng to 2550 μg, more preferably 5 ng to 47 μg and even more preferably 10 ng to 14.5 μg. For the purpose of the invention, this amount of active ingredient may be administered or ingested over one or several times in a day, with the compound in its original form or in a desired form such as a food, composition, drug or beverage.

[0056]

a. Preventive effect on ischemic disease and effect of reducing oxidative stress

Blood reperfusion for ischemia is known to be associated with high production of active oxygen, and damage. Ingestion of a selenium-containing composition of the invention reinforces antioxidant effects in the body and can reduce oxidative stress in the brain and prevent ischemic brain disease. The preventive effect on ischemic disease and effect of reducing oxidative stress can be confirmed by the effect of reduced ischemia reperfusion injury following administration of the selenium-containing composition, in a cerebral ischemia reperfusion model test using gerbils. More specifically, a selenium-containing composition (for example, a selenoneine-containing fish extract) is administered to gerbils for 2 weeks, and reperfusion is carried out after stopping blood flow of the bilateral common carotid artery for 5 minutes. Administration of the organic selenium compound is then continued for 2 weeks, and spontaneous movement and working memory ability are examined for evaluation (see Example 2 below). After slaughtering, the brains and hearts may be extracted and the sites of ischemia biochemically confirmed. The selenium-containing composition of the invention may be used as an active ingredient in a food starting material and composition for reduction in oxidative stress caused by oxygen deficiency and prevention of damage by ischemic heart disease and cerebral infarction.

[0057]

b. Effect of improving protection against metabolic syndrome and its associated damage

The number of patients with hepatitis due to alcohol overconsumption and viruses, as well as non-alcoholic hepatitis, has been increasing in recent years. Ingestion of a selenium-containing composition of the invention can suppress lipid peroxides (oxidized LDL) and prevent onset of non-alcoholic hepatitis, fatty liver, impaired glucose tolerance, metabolic

syndrome and diabetes associated with these conditions. The animal model used may be a non-alcoholic hepatitis mouse model with diabetes induced by administration of streptozotocin and a high fat diet, allowing confirmation that administration of a selenium-containing composition of the invention by admixture with feed can provide a preventive effect against oxidative stress and liver damage associated with non-alcoholic hepatitis. For example, upon ingestion of a selenoneine-containing fish extract, as a selenium-containing composition of the invention, and a high fat diet, and extraction of the livers after 4 weeks and measurement of the hepatitis score, it is possible to evaluate the effect of the selenium-containing composition of the invention on alleviating oxidative stress, reducing fat accumulation in the liver and improving protection against onset of hepatitis and metabolic syndrome and their associated damage (see Example 3 below).

[0058]

c. Carcinogenesis prevention effect and effect of suppressing systemic immunity reduction

Ingestion of a selenium-containing composition of the invention can augment suppressing effects against cancer growth, metastasis and carcinogenesis, and suppressing effects against reduction in systemic immunity. The effect of preventing carcinogenesis and suppressing effect against systemic immunity reduction can be confirmed using animal models. The cancer cell growth inhibition effect can be confirmed by transplanting cancer cells into mice, and measuring the reduction at the tumor site after administration of a feed containing the selenium-containing composition of the invention (with a selenium concentration of 3 mg/kg, for example).

[0059]

The effect of mutagenicity suppression by the selenium-containing composition of the invention can be confirmed by a reverse mutation test using bacteria. The transformation-suppressing effect of the selenium-containing composition of the invention can also be confirmed under more *in vivo*-like conditions using mammalian cells (for example, Bhas42 cells). This test method is used for detection of carcinogenic substances, as established by the Japan Food and Drug Safety Center, and it allows detection while distinguishing carcinogenic initiation activity and promotion activity. The transformation-inducing agent 3-methylcolanthrene is added to culture medium 1 day after seeding cells, for 3 days of treatment. After exchange with selenoneine-containing fish extract-added medium and culturing to the 21st day after cell seeding, the number of transformed foci is counted. Since this allows effects on the different stages of cancer initiation, promotion and progression to be confirmed, it becomes possible to evaluate whether or not the composition can be used as a food material with a preventive effect against cancer (see Example 4 below).

[0060]

The selenium-containing composition of the invention may also be administered to mice with bile cancer to confirm the improving effect on systemic immunity. In regard to suppression of general immunity disorders, the carcinogenic suppression effect of a selenium-containing composition can be verified by analyzing regulatory T (Treg) cells that have immunosuppressive function and promote tumor growth (see Example 4 below). Ingestion of a selenium-containing composition of the invention can provide improved immunity against disease, suppressed growth of cancer, and prevention of cancer relapse.

[0061]

d. Suppressing effect on arteriosclerosis

Oxidized LDL is thought to be part of the mechanism of progression of arteriosclerosis, and migration of smooth muscle cells by oxidized LDL has been reported. It has been found that the selenium-containing composition of the invention has a preventive effect against arteriosclerosis, by inhibiting dedifferentiation of smooth muscle cells and their migration into the vascular lumen, which is a cause of stenosis during progression of arteriosclerosis. By administration of a selenium-containing composition of the invention to smooth muscle cells it is possible to confirm its effect of inhibiting migration of smooth muscle cells and suppression of arteriosclerosis. For example, human aorta smooth muscle cells may be cultured in medium to which the selenium-containing composition has been added, and the number of migrating cells counted, to evaluate whether or not the composition can be used as a food material with a preventive effect against arteriosclerosis (see Example 5 below). The invention can inhibit smooth muscle cell migration and inhibit progression of atherosclerosis, for example. Vascular endothelial injury related to arteriosclerosis can also be ameliorated.

[0062]

e. Effect of improving brain function

Known risks for Alzheimer's include aging, genetic factors and lifestyle diseases (such as diabetes). The selenium-containing composition of the invention has been found to have an effect of improving brain function, whereby generation of active oxygen species due to aging or diabetes is alleviated, and onset of cognitive disorder is prevented. Throughout the present specification, “cognitive disorder” means Alzheimer-type cognitive disorder, Lewy body dementia, cerebrovascular cognitive disorder and frontotemporal dementia, and the like. Also, “brain function” means memory function (for example, working memory and short-term memory), affect, speech function, cognitive function, motor function, learning function and attention function. Brain functions are varied, and reduction in brain function can be inhibited by protecting from ischemia reperfusion injury or protecting cranial nerves. Diabetes is also known to increase the risk of depression, and protecting against reduction in brain function due to

diabetic injury can help to prevent not only cognitive disorder but also depression. These effects of improving brain function, such as an effect of reducing progression of Alzheimer's, can be confirmed by preparing a mouse model of Alzheimer's pathology by streptozotocin administration and administering a selenium-containing composition of the invention (for example, a selenoneine-containing fish extract). Direct administration of streptozotocin into the brain is known to increase intracerebral oxidative stress and cause accumulation of amyloid-β and tau protein, resulting in a condition similar to Alzheimer's disease. To prepare the model, streptozotocin is administered 1 week after ingestion of the selenium-containing composition of the invention. After 2 weeks, the selenium-containing composition is ingested, and evaluation is conducted by examining working memory and brain tissue. The inhibiting effect against amyloid-β accumulation can be biochemically confirmed by a test using cells. Ingestion of a selenium-containing composition having an effect of preventing and improving reduction in brain function, and a food containing it as a starting material, may be expected to have a brain function-protecting effect not only for patients but also for healthy elderly persons (see Example 6 below).

[0063]

f. Effect of improving dry eye

Ingestion of a selenium-containing composition alleviates and protects from drying of corneal epithelial cells and oxidative stress, thus allowing a preventive effect against dry eye to be obtained. The number of dry eye patients in Japan is estimated to be approximately 8 to 22 million. Inflammation and persistent oxidative stress have been reported to exacerbate dry eye and reduce mucin, and to lead to mitochondrial dysplasia in mouse dry eye models (Kawai, M., et al., Sci. Rep., vol.3, 2455 (2013)). Human immortalized corneal epithelial cells may be cultured in medium containing an added organic selenium compound (for example, a selenoneine-containing fish extract), and evaluation may be conducted by measuring mucin production, for example. Since dry eye is not a serious condition it is often overlooked, but improvement of dry eye is important for improved QOL of patients, and it is possible to evaluate whether or not the composition is utilizable as a food material for improvement of dry eye (see Example 7 below). Using a selenium-containing composition of the invention can protect against dry stress of cornea cells and can prevent and improve dry eye.

[0064]

g. Effect of suppressing inflammation

Ingestion of a selenium-containing composition of the invention can suppress redness, swelling, heat release, pain and dysfunction associated with inflammation, and can prevent rheumatoid arthritis, hemolytic anemia and myasthenia gravis associated with inflammation. Immune regulatory mechanisms function in a balanced manner in a normal adult, but when an

abnormality occurs in the host immune regulatory mechanism due to some cause, resulting in autoantibodies being produced that recognize the individual's own biological components as antigen, various immunoreactions are elicited, leading to fibrotic disorders and dysfunction. Such autoimmune diseases include rheumatoid arthritis, hemolytic anemia and myasthenia gravis. Therapeutic agents for such autoimmune diseases that are used include immunosuppressive agents such as azathioprine, cyclophosphamide and vincristine, but administration of these drugs is also known to be associated with numerous side-effects. Almost all topical inflammation therapeutic agents that are directly applied to affected areas are steroidal, and therefore their usage has been restricted due to side-effects. An organic selenium compound of the invention can be used as a food and is expected to suppress a variety of inflammatory responses without side-effects. The target of suppressing inflammation by ingestion of a selenium-containing composition of the invention is primarily NFκ-B activation, and the composition is effective against allergic disease, cancer, arthritis, autoimmune disease, inflammatory intestinal disease, systemic lupus erythematosus, ankylosing spondilitis and celiac disease (see Example 8 below).

[0065]

The following examples serve as illustration of different modes of the present disclosure. It will be apparent to a person skilled in the art that various modifications may be made to both the materials and methods while still being within the scope of the present disclosure. All of the reagents and solvents purchased from commercial product suppliers are preferably further purified for use by a method such as a distillation, recrystallization or filtration in order to remove any contaminating peroxides and radicals.

EXAMPLES

[0066]

Example 1: Preparation of organic selenium compounds

For the organic selenium compounds, selenoneine-containing extracts were obtained from tuna braised meat-exposed water following the method previously reported in Japanese Patent Publication No. 5669056 (PTL 1), and were concentrated to 55 Brix to obtain selenoneine-containing fish extracts.

[0067]

Analysis of selenoneine content

The selenoneine content of the selenoneine-containing fish extract (55 Brix) obtained in Example 1 was calculated in terms of selenium (μgSe/g) based on the measured value obtained in LC-ICPMS as described below.

[0068]

(1) Apparatus

Separation column: Ultrahydrogel 120 (Waters)

LC pump: Pu712 (GL Sciences, Inc.)

Sample injector: 9725i (Rheodyne)

ICPMS: ELAN DRC II (Perkin-Elmer)

[0069]

(2) LC measuring conditions

Moving bed: 0.1% IGEPAL-CA63, 0.1 M ammonium acetate (pH 5.3)

Flow rate: 1.0 mL/min

Sample injection rate: 0.01 mL

[0070]

(3) ICP-MS measuring conditions

Measured element: Se

Mass number: 82

RF output (W): 1200

Nebulizer gas (Ar, L/min): 1.0

Auxiliary gas (Ar, L/min): 1.3

Plasma gas (Ar, L/min): 17

Makeup gas (Ar, L/min): 0

Reactive gas (Ar, L/min): 0

Pulse stage voltage (eV): 1050

RPq: 2.5

Residence time (sec/amu): 0.4

[0071]

(4) Sample preparation

After adding a 19-fold volume (380 μL) of ultrapure water (Milli-Q Element, Millipore) to 20 μL of the selenoneine-containing fish extract obtained in Example 1 and mixing, the supernatant obtained from centrifugation at 12,000 rpm for 10 minutes was used for HPLC-ICPMS analysis.

[0072]

(5) Preparation of standard selenoneine product

The standard selenoneine product used was obtained by purification from swordfish red muscle by the following method:

(5-1) Frozen swordfish fillet was thawed overnight at 5°C and the red muscle was cut out and collected for use.

[0073]

(5-2) After adding 0.1 g of cysteine hydrochloride (Wako Pure Chemical Industries, Ltd.) to 100 g of the red muscle, it was pulverized for 15 seconds with a Polytron homogenizer (PT MR 6000, Kinematica) at 6800 rpm in a 10-fold volume of acetonitrile (Wako Pure Chemical Industries, Ltd.), while cooling with ice water.

[0074]

(5-3) The pulverized product was centrifuged at 6000 × g for 10 minutes and the supernatant was concentrated with a rotary evaporator under reduced pressure to obtain concentrate 1.

[0075]

(5-4) An acetonitrile:tetrahydrofuran (Wako Pure Chemical Industries, Ltd.) (1:1, v/v) mixed solution was added to concentrate 1 to separate the concentrate into two layers.

[0076]

(5-5) Of the two separated layers, the acetonitrile layer was separated out and concentrated with a rotary evaporator under reduced pressure to obtain concentrate 2.

[0077]

(5-6) After adding 5 ml of cooling water to concentrate 2, it was supplied to an Atlantis^{R} dC18300 Angstrom column (19 × 150 mm, Japan Waters, K.K.) that had been equilibrated with 0.1% acetic acid, and fraction 1 was collected from an elution amount of 40 ml to 50 ml under a 0% to 50% linear acetonitrile gradient.

[0078]

(5-7) Fraction 1 was concentrated with a rotary evaporator under reduced pressure to obtain concentrate 3.

[0079]

(5-8) Concentrate 3 was supplied to an Ultrahydrogel^{R} 120 column (7.8 × 300 mm, Japan Waters, K.K.) that had been equilibrated with 0.1% acetic acid and 30% acetonitrile, and the selenoneine-containing fraction from an output of 7.5 ml to 9.0 ml was collected to obtain fraction 2.

[0080]

(5-9) Fraction 2 was passed through an Ultrahydrogel^{R} 120 column (7.8 × 300 mm, Japan Waters, K.K.) that had been equilibrated with 0.1% acetic acid and the selenoneine-containing fraction was collected and concentrated with a rotary evaporator under reduced pressure to obtain a standard selenoneine product.

[0081]

(5-10) The total selenium content of the standard selenoneine product was measured by the fluorescence method described below to be 0.476 μg/mL.

[0082]

The standard selenoneine products obtained in (5-1) to (5-9) include the compounds represented by formulas (1) to (4), with the compound represented by formula (2) known to be a major component (Thesis by University of Tokyo professor (of agriculture), pp17-19 (Yamashita, Y. 2012)).

[0083]

(6) Identification and quantitation of selenoneine

The selenium 82 in the sample and standard selenoneine product of (4) was measured with the LC-ICPMS apparatus described for (1) to (3) to obtain chromatograms, which were used to identify and determine the contents of selenoneine.

[0084]

(6-1) A peak was detected in the standard selenoneine product near an elution time of 10.3 minutes. For standard selenoneine product in which a signal for unidentified selenium compound was detected, the integrated value of all of the selenium signals from 6 to 11 minutes was measured and considered to be the peak area corresponding to the total amount of selenium injection as quantified by the fluorescence method described below.

[0085]

(6-2) The peak area near an elution time of 10.3 minutes in the obtained chromatogram was identified as selenoneine, and was read using software (TotalChrom, Perkin-Elmer).

[0086]

(6-3) Formula (5):

$$X = (A/B) \times (1000/C) \times D/1000 \qquad \text{formula (5)}$$

(where:

X: selenoneine content (μgSe/g) of sample

A: peak area at 10.3 min for sample

B: detection area of standard selenoneine product per mass of injected selenium (/ngSe)

C: amount in microL of sample injected in LC-ICPMS (C = 5 for this test)

D: dilution factor with ultrapure water during preparation of sample (D = 20 for this test).

[0087]

(6-4) 10 microL of standard selenoneine product was injected into the LC-ICPMS apparatus, the integrated value of all of the selenium signals from 6 to 11 minutes was calculated, and the value obtained by dividing the integral by the amount of selenium injected (4.76 ng for this test) was recorded as the area detected per weight of selenium (coefficient C in formula (5)).

[0088]

(6-5) Measurement results

Fig. 1 shows (A) a chromatogram for selenium 82 as a standard selenoneine product, and

(B) a chromatogram for the selenoneine-containing fish extract obtained in Example 1.

[0089]

(6-6) A peak corresponding to a selenoneine dimer (formula IV) was observed in the chromatogram (B) at a retention time of 10.6 minutes (arrow), as the main selenium compound. This indicated that most of the selenium compound in the selenoneine-containing fish extract obtained in Example 1 is composed mainly of the compound represented by formula IV. The selenoneine-containing fish extract obtained in Example 1 had a calculated selenoneine content of 32.5 mgSe/L.

[0090]

(7) Total selenium content

The selenoneine-containing fish extract obtained in Example 1 and the standard selenoneine products obtained in (5-1) to (5-9) were wet-decomposed by the method described in published literature (Fluorometric determination of selenium in nanogram amounts in biological materials using 2,3-diaminonaphthalene. R. Hasunuma, T. Ogawa, Y. Kawanishi, Analytical biochemistry (1982), 126:242-245), and after converting all of the selenium compound to an inorganic state, it was reacted with 2,3-diaminonaphthalene (DAN) and quantified by measuring the fluorescence of 4,5-benzopiaselenol (Se-DAN) produced by Se(IV) complex-forming reaction. The obtained measured value was used to calculate the selenium weight (mg/kg) per weight of sample. Specifically, the measurement was performed under the following conditions.

[0091]

(7-1) A 10 mg portion of the selenoneine-containing fish extract obtained in Example 1 was weighed out and wet ashed in a test tube for 2 hours at 210°C together with 1.5 mL of a mixed acid (nitric acid:perchloric acid = 2:1), and then 0.25 mL of saturated aqueous ammonium oxalate was added to the solution and the mixture was heated for 5 minutes in a 100°C water bath.

[0092]

(7-2) After water-cooling the sample obtained in (7-1), 0.25 mL of 6 M hydrochloric acid was added and the mixture was heated for 30 minutes in a water bath at 100°C and water-cooled, after which 0.25 mL of 0.1 M ethylenediaminedisodium tetraacetate salt was added, and a 6 M aqueous sodium hydroxide solution was used to adjust the pH to between 1.0 and 1.5.

[0093]

(7-3) 1 mL of 1 mg/mL DAN dissolved in 0.1 M hydrochloric acid was added to the sample obtained in (7-2), and the mixture was heated at 50°C for 20 minutes. After water-cooling, it was shaken with 1 mL of cyclohexane and the fluorescence of the cyclohexane layer was measured with 379 nm excitation light and a fluorescent wavelength of 521 nm.

[0094]

(7-4) In parallel with the sample, tubes containing 5, 10, 50 and 100 μL of a procedural blank test containing no sample and a 1 mg/L selenium standard solution were also used to prepare calibration curves for calculation of the total selenium content of the sample.

[0095]

The total selenium content of the selenoneine-containing fish extract obtained in Example 1 was 95.3 mg/kg.

[0096]

Example 2: Examining effect of reducing ischemia reperfusion injury

After subjecting gerbils to a cerebral ischemia reperfusion load, spontaneous movement was measured and a Y maze test was conducted, to examine the effect of cerebral ischemia reperfusion and evaluate the effect of the test substance (selenoneine-containing fish extract) in the model.

[0097]

1. Test system

Seven-week old gerbils (MON/Jms/Gbs Slc, male) were purchased from Japan SLC, Inc. and conditioned for 5 days, after which the body weights were measured and they were divided into groups. During the test period there were given free access to ordinary feed (CRF-1, Oriental Yeast Co., Ltd.) and water. Division was into the following 6 groups, as test groups.

[0098]

[Table 1]

Table 1. Test groups

Group No.	Test group	Course	Number of animals
1	22-day normal group	1st	8
2	22-day cerebral ischemia reperfusion (control) group	1st	8
3	22-day cerebral ischemia reperfusion + selenoneine-containing fish extract-administered group	1st	8
4	29-day normal group	2nd	8
5	29-day cerebral ischemia reperfusion (control) group	2nd	9
6	29-day cerebral ischemia reperfusion + selenoneine-containing fish extract-administered group	2nd	9

[0099]

The initial day of administration of the test substance was designated as Day 0, and on Day 14, the common carotid artery was threaded under isoflurane anesthesia and the incision site was sutured, on the day before ischemia. On the following day, the animal was laid down on a hot plate set to 40°C±3°C to maintain a body temperature of 37°C, the thread at the incision site was cut and the bilateral common carotid artery was pulled, and hemorrhage of the artery was

stopped for 5 minutes with a klemme. The klemme was then removed, reperfusion was carried out, and the incision site was sutured. In the normal group, both common carotid arteries were exposed under isoflurane anesthesia and the skin incision site was sutured without treatment. In the test substance-administered groups, 6.2 mL/kg of the test substance was forcibly administered with a polypropylene disposable glass syringe and a feeding tube each day during the test period. Water was similarly administered in an amount of 6.2 mL/Kg to the normal group and control group. The forced administration was carried out for 22 days or 29 days, at a frequency of once per day. Ischemia reperfusion causes behavior disorder (spontaneous hyperactivity) and learning memory impairment due to hippocampal cell death. Measurement of spontaneous movement and a Y maze test were conducted to evaluate the effect of the test substance for preventing brain damage caused by ischemia reperfusion.

[0100]

(1) Measurement of spontaneous movement

Measurement of spontaneous movement was carried out every hour on days 6, 13, 15, 17, 19, 21 and 27 (with the second course alone on day 27), designating the initial day of administration of the test substance as day 0. A SUPERMEX multichannel spontaneous movement system measuring system (product of Muromachi Kikai Co., Ltd.) was used for the measurement.

(2) Y maze test

A Y-shaped maze test was conducted on Day 21 (first course) and Day 28 (second course), placing the animals in the maze and allowing free behavior for 6 minutes, and then measuring the percentage of selection of new arms, to evaluate the memory learning behavior.

(3) Measurement of selenoneine

Upon completion of the first course, the surviving test individuals in group 1, group 2 and group 3 were euthanized by bleeding, by collecting whole blood from the ventral aorta of the animal with a heparin-containing syringe under 2.5% isoflurane inhalation anesthesia. The collected blood was centrifuged (3000 rpm, 10 minutes, 4°C) to separate the blood plasma and blood clots. The selenoneine content in the blood clots was analyzed by the method of Example 1.

[0101]

[Table 2]

Table 2. LC-ICPMS analysis results for gerbil blood clots in cerebral ischemia reperfusion test (μg/g per selenium)

Group No.	Number of specimens	Selenium protein	Selenoneine
Group 1	8	0.38 ±0.04	0.0
Group 2	5	0.35 ±0.02	0.0
Group 3	6	0.50 ±0.23	0.95 ±0.24

[0102]

2. Results

One day after ischemia reperfusion treatment, the degree of spontaneous movement had increased in group 2 ("22-day control group" in Fig. 2) and group 5 ("29-day control group" in Fig. 2), which were control groups, but it had been significantly suppressed in group 3 ("22-day selenoneine-containing fish extract-administered group" in Fig. 2) and group 6 ("29-day selenoneine-containing fish extract-administered group" in Fig. 2), which were test substance-administered groups (Fig. 2). On the other hand, when the Y maze test was conducted 7 days and 14 days after treatment, the rate of correct answers fell in each control group, whereas the reduction was significantly suppressed in the selenoneine-containing fish extract-administered groups to about the same level as the "normal group" (non-treated), thus confirming that the test substance has an effect of protecting from brain damage caused by ischemia reperfusion (Figs. 3 and 4).

[0103]

In regard to the selenium protein content in the blood clots, there was no significant difference between group 1, group 2 and group 3, but the selenoneine content was less than the detection limit (0.01 μg/g) in group 1 and group 2, while it was detected at 0.95±0.24 μg/g with respect to selenium in group 3, indicating a significant increase in selenoneine content ($p < 0.05$). These results indicated that 22 days of administration of the test substance leads to accumulation of selenoneine in the blood cells of gerbils.

[0104]

<u>Example 3: Examining effect of improving protection against metabolic syndrome and its associated damage</u>

(1) Effect of improving metabolic syndrome

A high fat diet was provided to mice with abnormal glucose metabolism simulating type I diabetes, induced by streptozotocin administration. After a prescribed period, the effect of administering selenoneine-containing fish extract for suppression of non-alcoholic hepatitis was verified using the NAFLD activity score, as a method allowing comprehensive evaluation of fat accumulation in the liver.

[0105]

Specifically, 10 female SPF mice (C57BL/6J, Japan SLC, Inc.) were purchased on the 14th day of pregnancy and conditioned. All of the animals were allowed to experience natural birth, and the male mice that were born were used to prepare animal models. Weaning was at 4 weeks of age after birth.

[0106]

Observation of newborns: Mice observed as having been born by a delivery observation time of 8:00 were recorded as being born on the day before observation, and mice observed as having been born by a delivery observation time of 20:00 were recorded as being born on the same day as observation, with counting being from day 0 after birth. Streptozotocin (Sigma Aldrich Japan, KK.), at a concentration of 10 mg/mL in physiological saline (Japanese Pharmacopoeia, Otsuka Pharmaceutical Factory, Inc.), was administered to the 2-day-old male mice once subcutaneously in the dorsal region, at 20 μL (200 μg) per mouse. They were then nursed by the mother until weaning. Weaning was at 28±2 days after birth, with rearing thereafter on a high fat diet [High Fat Diet 32 (radiation-sterilized, Clea Japan, Inc.)].

[0107]

From the day of arrival of the animals until their weaning, both the mothers and children were freely provided with solid feed CE-2 (radiation-sterilized, Clea Japan, Inc.). After weaning, the streptozotocin-administered animals were freely provided with a high fat diet (High Fat Diet 32, radiation-sterilized) (Clea Japan, Inc.).

[0108]

A single dose was selenoneine-containing fish extract at 15 mg/kg body weight. The administration volume was 10 mL/kg body weight, with forced oral administration using an oral sonde. The control group was administered 10 mL/kg body weight in the same manner. The amount of dosing solution per individual was calculated based on body weight on the morning of the day of administration (units: 0.01 mL). The period was 28 days, once per day from the age of 5 weeks after birth. Upon completion of administration, the mice were bled to death by severing the ventral aorta, the livers were excised, the outer left lobe was divided into 6 pieces, two pieces of which were stained with hematoxylin-eosin, and the NAFLD activity score was calculated.

[0109]

The NAFLD activity score is a measure of the degree of pimelosis of the liver, inflammation of the liver parenchyma and hepatocyte damage (ballooning degeneration), and it is used for pathological diagnosis of non-alcoholic hepatitis. The control group had ballooning degeneration of hepatocytes, deposition of large and small fat droplets, and inflammatory cellular infiltration containing lymphocytes and neutrophils. The NAFLD Activity score was significantly lower in the selenoneine-containing fish extract-administered group compared to the control group. It has been suggested that selenoneine prevents and improves fat accumulation

due to metabolic disorders in metabolic syndrome, and it is thought to have effects of preventing and improving metabolic syndrome (Fig. 5, Table 3).

[0110]

[Table 3]

Table 3. NAFLD Activity Score (NAS) for each test group

Group	NAS
Control	5.1 ±0.8
Selenoneine-containing fish extract	3.0 ±1.0

[0111]

(2) Effect of improving damage associated with metabolic syndrome

An icv-STZ inducible Alzheimer-type cognitive disorder model is an Alzheimer-type cognitive disorder animal model that has a diabetes-like background produced by disturbance in intracerebral insulin signals, and in this particular model the primary factor is not genetic. The model is therefore useful for studying the therapeutic effect on sporadic Alzheimer-type cognitive disorder. For this Example, in order to study the effect of a selenoneine-containing fish extract as the test substance on Alzheimer-type cognitive disorder, it was forcibly administered orally to a mouse model of icv-STZ inducible Alzheimer-type cognitive disorder for 21 days, from 7 days before the model induction starting day until 14 days after the model induction starting day. Behavior was analyzed on the 13th day after the model induction starting day, and the mice were slaughtered and analyzed on the 14th day after the model induction starting day. The effect of the selenoneine-containing fish extract against Alzheimer-type cognitive disorder in the icv-STZ inducible Alzheimer-type cognitive disorder model was examined by comparing the solvent-administered group (RO water, control-administered group) and the selenoneine-containing fish extract-administered group.

[0112]

Streptozotocin (Sigma Aldrich Japan, KK.), at a concentration of 20 mg/mL in physiological saline (Japanese Pharmacopoeia, Otsuka Pharmaceutical Factory, Inc.), was administered to mice (C57BL/6J, male, 6 weeks old at time of purchase, Japan SLC, Inc.) through the lateral cerebral ventricle. On the model induction starting day, 3 μL was administered once into the left cerebral ventricle, and then 3 μL was administered once into the right cerebral ventricle on the 2nd day after the model induction starting day, to prepare an icv-STZ inducible Alzheimer-type cognitive disorder animal model. During the test period, solid feed CE-2 (radiation-sterilized, Clea Japan, Inc.) was made freely available, and on the day before the start of administration, 20 male mice were divided into an icv-STZ+control group and an icv-STZ+selenoneine-containing fish extract-administered group (10 mice/group), by a body

weight stratified random sampling method for equivalent average weights. The volume of test substance administered was 6.2 mL/kg body weight, and an oral sonde was used for forced oral administration once per day for 21 days. The control group was administered RO water at 6.2 mL/kg body weight in the same manner. The amount of dosing solution per individual was calculated based on body weight on the morning of the day of administration (units: 0.01 mL).

[0113]

Upon completion of the administration period, spontaneous alternation in a Y maze was analyzed. The mice were placed at the center of the maze one day before the final administration, and were allowed to freely explore for 5 minutes. After an elapse of 5 minutes, each mouse was returned to its home cage, the Y maze was disinfected with 50% ethanol, and then the behavioral test for the next mouse was conducted. On the day of final administration, the entrances to two of the three arms were partitioned off with walls, and the mice were again placed at the center of the maze. After each mouse entered the open arm, the walls set at the entrances to the remaining two arms were removed and free exploration was allowed for 8 minutes. A record was made during that time of the total number of times that each mouse entered the arms and the order in which the arms were entered. After an elapse of 8 minutes, each mouse was returned to its home cage, the Y maze was disinfected with 50% ethanol, and then the test for the next mouse was conducted. The alternation rate (%) was used as an index of SAB. The alternation rate was calculated by the following formula (Fig. 5, Table 4).

Alternation rate = 100 × (number of times different arms were consecutively entered 3 times)/(total number of times arms were entered - 2)

As a result, the selenoneine-containing fish extract group had a significantly higher alternation rate compared to the control ($P < 0.05$). This suggested that ingestion of selenoneine protects against reduced brain function due to diabetes.

[0114]

[Table 4]

Table 4. NAFLD Activity Score (NAS) for each test group

Group	Alternation rate (%)
Control	55.5 ±8.2
Selenoneine-containing fish extract	68.0 ±12.0

[0115]

Example 4: Examining anticancer effect or effect of suppressing general immunity disorders

(1) Verification of anticancer effect and effect of suppressing general immunity disorders using colorectal cancer primary tumor model

The effect of a selenoneine-containing fish extract on tumor growth or systemic

immunity in cancer patients was examined using an animal model. Each group was transitioned by feeding between 1.73 to 2.65 g each day. The consumption amount of the selenoneine-containing fish extract obtained from the selenoneine-containing fish extract feed was transitioned to 81.48 to 125.82 mg per day. As a result of using the colorectal cancer primary tumor model, there was a tendency toward reduced primary focus tumor growth, and the marked attenuation of IL-10 production seen in bile cancer animals was not observed in the selenoneine-administered group, indicating possible augmentation of regulatory immunity. Also, regulatory T (Treg) cells decreased by selenoneine administration, suggesting the possibility of improvement in antitumor immunity regulation by Treg cells. That is, it was suggested that selenoneine has an effect of improving systemic immunity and suppressing tumor growth in cancer patients (Figs. 7 and 8).

[0116]

Colorectal cancer cells from BALB/c female mice (Colon Tumor 26, CT-26) (ATCC No.CRL-2638) were cultured in RPMI-1640 medium (R8758, Sigma-Aldrich Corp.) containing 10% fetal bovine serum (171012, Nichirei Bioscience). Also, mouse splenocytes were cultured in RPMI-1640 medium containing 10% fetal bovine serum and 50 μM 2-mercaptoethanol (21417-52, Nacalai Tesque, Inc.). A water jacket incubator (APC-30D, Astec Co.) was used to culture the cells under conditions of 37°C, 5% CO_2, 100% humidity). The mice (BALB/c, female, Charles River Co.) were given selenoneine-containing feed or a control feed from 5 weeks to 8 weeks of age (see Table 5 below). After conditioning for 1 week (6 weeks of age), 500,000 CT26 cells were subcutaneously transplanted per mouse. At 14 days after transplantation (8 weeks of age), the mice were euthanized, the tumors were excised, and the tumor volumes were measured. The tumor volumes (mm^3) were calculated by the formula: width (mm) squared × length (mm) × 0.5. The spleens were also excised, and the CD4-positive, Foxp3-positive regulatory T cell (Treg) subset in the splenocytes was measured by flow cytometry using a fluorescent stained cell analyzer (AccuriC6, BD Bioscience) and FlowJo software (TreeStar). The IL-10 concentration of the culture supernatant from the splenocytes was measured by ELISA.

[0117]

[Table 5]

Table 5. Feed composition

Feed composition (%)	Control (AIN-93M)	Selenoneine-containing feed
Casein	14	14
L-Cystine	0.18	0.18
Corn starch	46.5692	41.8549143
Pregelatinized corn starch	15.5	15.5
Sucrose	10	10
Soybean oil	4	4
Cellulose powder	5	5
Mineral mixture	3.5	3.5
Vitamins	1	1
Choline	0.25	0.25
t-Butylhydroquinone	0.0008	0.0008
Selenoneine-containing fish extract	0	4.71

The materials other than AIN-93M and the selenoneine-containing feed fish extract were purchased from Oriental Yeast Co., Ltd.

[0118]

Regulatory T (Treg) cells have an immunosuppressive function and promote tumor growth, and this strongly suggested the possibility that ingestion of selenoneine-containing feed can inhibit Treg cells in bile cancer mice. Moreover, while production of IL-10 that carries out regulation of antitumor activity in regulatory immunity was significantly attenuated in the bile cancer mice, it was not attenuated in the selenoneine-containing feed group. This also suggested that tumor growth is inhibited. It also suggests that selenoneine has an effect of improving systemic immunity and inhibiting tumor growth. The suppression of Treg cells was thought to also have an effect of preventing cancer relapse (Fig. 9).

[0119]

(2) Verifying effect of suppressing carcinogenesis using Bhas 42 cells

A transformation test was carried out using Bhas 42 cells, to examine whether selenoneine, as the main component of a selenoneine purified product, has a carcinogenesis-suppressing effect *in vitro*. In a dose-finding trial, with treatment of Bhas 42 cells with a selenoneine purified product (0.31, 0.63, 1.3, 2.5, 5.0, 10 μM), no cytotoxicity was found (data not shown). Based on these results and data from other experiments, the concentration for the transformation test was set at 1 μM. In the transformation test, it was examined whether the number of transformed foci induced by 1 μg/mL of 3-methylcolanthrene (MCA) (Sigma-Aldrich) is reduced by a 1 μM selenoneine purified product. Specifically, the transformation rates (transformed foci/well) in an MCA+ultrapure water group and an MCA+selenoneine purified product group were compared. As a result, the transformation rates were 15.8 and 10.2,

respectively, showing a significant reduction in the number of transformed foci. These results suggested that selenoneine has an effect of suppressing carcinogenesis *in vitro*.

[0120]

Bhas 42 cells (BALB/c 3T3 A31-1-1 cells from mouse whole fetuses with the v-Ha-ras gene introduced, Japan Food and Drug Safety Center) were released using 0.25% trypsin, and then suspended at a cell density of 2000/mL. A 2 mL portion of the cell suspension (4000 cells) was dispensed onto a plate (6 wells/group) and cultured for 1 day. One day after seeding, an MCA preparation was added and treatment was carried out for 3 days. Treatment with a selenoneine purified product and RA (retinoic acid, Sigma-Aldrich) was carried out by exchange of culture solution 4 days, 7 days and 11 days after seeding (2 mL per well). Exchange with fresh medium was carried out 14 days after seeding (10 days of treatment), and culturing was continued for 7 days. The plate for the transformation test was fixed with methanol 21 days after seeding, and then stained with a 5 vol% Giemsa solution, after which the number of transformed foci per well was counted.

[0121]

The transformation rate in the MCA+selenoneine purified product group (15.8/well) was reduced by approximately 2/3 compared to the transformation rate in the MCA+ultrapure water group (10.2/well), demonstrating a significant reduction. This result suggested that selenoneine, the main component of the selenoneine purified product, has an effect of suppressing carcinogenesis.

[0122]

Example 5: Examining effect of suppressing arteriosclerosis

Human aorta smooth muscle cells were used to examine the influence of the test substance on cell migration. As a result, 2 mg/ml of selenoneine-containing fish extract and 100 nM of purified selenoneine were found to inhibit migration in the presence of collagen.

[0123]

AoSMC cells (aorta smooth muscle cells) (LONZA, CC-2571) were prepared to the necessary cell count with dedicated medium (SmGM-2 Bullet Kit (LONZA, CC-3182)). Using a cell migration assay kit (CytoSelect^{TM} 96-well Cell Migration Assay (8 μm), Fluorometric, Cosmo Bio, CBA-106), the AoSMC cells were seeded in a cell migration assay kit-equipped Transwell upper chamber at 1×10^5 cells/0.1 mL/well, and after adding test medium containing the test substance (2 mg/ml selenoneine-containing fish extract or 100 nM purified selenoneine) and 300 μg/mL collagen peptide to the lower chamber, the cells were cultured for 48 hours. The cells were then detached from the Transwell membrane with the CyQuant GR Dye included in the cell migration assay kit, and dissolved, and the number of migrated cells was counted.

[0124]

Addition of purified selenoneine and selenoneine-containing fish extract significantly inhibited cell migration. This suggested that inhibition of smooth muscle cell migration can inhibit progression of atherosclerosis (Fig. 10).

[0125]

Example 6: Examining effect of improving brain function

Amyloid-β protein, known as a cause of Alzheimer's, is neurocytotoxic. Protecting cells from the toxicity of amyloid-β is expected to have an effect of preventing cognitive disorder. In this Example, PC-12 cells were used to verify whether the test substance can protect against amyloid-β toxicity.

[0126]

The PC-12 cells (Riken BioResource Center, RCB0009, Lot Nos. 52, 53, 54) were cultured in a CO_2 incubator (5% CO_2, 37°C) using 10% FBS, Horse Serum-added DMEM medium (prepared by adding 5 ml of penicillin-streptomycin solution, 50 ml of FBS (Biowest, Cat. No. S1820, Lot No. 516536) and 50 mL of Horse Serum (Gibco, 16050-122) to 395 ml of DMEM medium (Nacalai Tesque, Cat. No.08456-65)). Medium exchange was carried out every other day. A 0.25% Trypsin-EDTA solution was used for detachment of the cells.

[0127]

The neurons (PC-12) were seeded in the aforementioned DMEM medium in a 96-well plate (collagen-coated) at 2×10^3 cells/100 μL/well and cultured in a CO_2 incubator (5% CO_2, 37°C), and on the following day the culture was exchanged with medium containing the test substance (also including 1% FBS, 10 ng/mL NGF (Alomone Labs, PRODUCT #N-100)). After 24 hours of culturing, amyloid-β (1-40) (Sigma, Cat. No. A1075-.1 MG) (a solution prepared by dissolution in PBS (Nissui Pharmaceutical Co., Ltd., Code 05913) to a final concentration of 1 mM, and heat treatment at 37°C for 3 days) was additionally added to a final concentration of 2 μM (50 μL/well), and culturing was continued for 24 hours. After removing the culture supernatant for each well upon completion of culturing, it was exchanged with 100 μL of test medium (per well) containing 10% viable cell count-measuring reagent (WST-8) (Nacalai Tesque, Cat. No.07553-44), and culturing was continued for 30 minutes and 90 minutes. The absorbance in each well was then measured with a microplate reader (Precision microplate reader (Molecular Devices)) (absorption wavelength: 450 nm, reference wavelength: 630 nm), and both values were used to calculate the change in absorbance per hour, determining the viable cell count for measurement of the cell viability. For this Example, the test substances (final concentration) used were selenoneine-containing fish extract: 2 mg/mL, selenoneine: 100 nM.

[0128]

Addition of amyloid-β produced a viable cell count of about 70% of the unadded group. Upon treatment with each test substance under these conditions, the cell viability was found to

be significantly improved with the selenoneine-containing fish extract compared to the amyloid-β-added control (Fig. 11).

[0129]

Example 7: Examining effect of improving dry eye

HCE-T cells (human immortalized corneal epithelial cells) (Riken BioResource Center, RCB2280) were precultured in test medium (DMEM/F12 (ThermoFisher, Cat. No. 11330-032) (1:1), 10% FBS (Biowest, Cat. No. S1820, Lot No. 516536), 1% NEAA (Gibco, Cat. No. 11140-050), 1% antibiotic (Nacalai Tesque, Cat. No. 26253-84)) with a T75 flask in a CO_2 incubator (5% CO_2, 37°C), until reaching the necessary cell count. For subculturing of the cells, trypsin/EDTA solution (Nacalai Tesque, Cat. No. 32777-44) was used for detachment from the flask, and after neutralizing the trypsin with test medium, centrifugal separation was performed and the cells were collected. The cells were then resuspended in test medium and used as a cell suspension.

[0130]

The selenoneine-containing fish extract is water-soluble, and it was directly dissolved in the medium to prepare a stock solution for use.

[0131]

(1) Test of avoiding dry stress of corneal epithelial cells (dry eye)

HCE-T cells were seeded in a 96-well plate at 2.5×10^4 cell/0.1 mL/well and cultured for 5 hours in a CO_2 incubator (5% CO_2, 37°C), after which adhesion of the cells to the plate bottom was confirmed, and the medium was exchanged with test medium containing the test substance. Culturing was then carried out for 1 day. The culture supernatant was subsequently removed and the cells were exposed to dry conditions (dry stress treatment time: 10 minutes). Dedicated medium was then added and culturing was continued for 3 hours in a CO_2 incubator (5% CO_2, 37°C). The proliferative ability of the cells was measured using a viable cell count measuring reagent, SF (Nacalai Tesque, Cat. No. 07553-44) (WST-8).

[0132]

(2) Mucin gene expression analysis test

HCE-T cells were seeded in a 48-well plate at 6×10^4 cells/0.5 mL/well and cultured for 1 day in a CO_2 incubator (5% CO_2, 37°C), after which the medium was exchanged with test medium containing the test substance. After 5 hours from addition of the test substance, the total RNA was recovered from the cells and converted to cDNA, and the mucin gene expression was analyzed by real-time PCR (Light Cycler 96 System (Roche)). Analyzed gene: membrane-bound mucin gene MUC1, internal standard gene: GAPDH.

[0133]

Since addition of selenoneine-containing fish extract significantly increased the viable

cell count and mucin gene expression after dry stress, compared to the control, the selenium-containing composition of the invention exhibited an effect of protecting against dry stress of cornea cells and preventing and improving dry eye (Figs. 12 and 13).

[0134]

Example 8: Effect of suppressing inflammation

The transcription factor "NFκB" is involved in a variety of different inflammatory responses, and when cells are stimulated by cytokines such as TNF-α, inactive NFκB is activated, eliciting an inflammatory response. Expression of NFκ-B has been reported to accelerate many diseases such as allergic disease and cancer (Kagaku to Seibutsu, Vol. 47, No.9, P.602-604(2009)). The test substance was confirmed to have an effect of suppressing inflammation by suppression of TNF-α induced NFκ-B activation. As the NFκ-B activity measurement system, a reporter gene was introduced into the target cells and TNFα stimulation-induced NFκB transcription activity was measured based on luciferase activity. The final concentrations of the test substances were selenoneine (partially purified): 0.4 mg/mL (HEK293 cells), selenoneine (purified): 100 nM.

[0135]

HEK293 cells (Riken BioResource Center, RBC1637, Lot. 20) were precultured in basal medium (MEM, 10% FBS, 1% NEAA, antibiotic-containing (MEM NEAA: Gibco, Cat. No. 11140-050, FBS: Biowest, Cat. No. S1820, Lot No. 516536, antibiotic: Nacalai Tesque, Cat. No. 26253-84)), with a T75 flask in a CO_2 incubator (5% CO_2, 37°C), until reaching the necessary cell count. For subculturing of the cells, trypsin/EDTA solution (Nacalai Tesque, Cat. No. 32777-44) was used for detachment from the flask, and after neutralizing the trypsin with test medium, centrifugal separation was performed and the cells were collected. The cells were then resuspended in test medium and used as a cell suspension.

[0136]

HEK293 cells were seeded in a 96-well plate at 1.5×10^4 cells/0.1 mL/well and cultured for 1 day in a CO_2 incubator (5% CO_2, 37°C). Next, a DNA vector (pGL4.32[luc2P/NF-kB-RE/Hygro] vector (Promega, Cat. No. E8491)) was introduced into the cells using a transfection reagent (Roche, Cat. No. 06366244001), and on the following day the medium was exchanged with test medium (0.1 mL/well) containing the added test substance (selenoneine-containing fish extract final concentration: 0.4 mg/mL or selenoneine final concentration: 100 nM), after which culturing was carried out for 2 hours at 37°C, 5% CO_2. There was added 5 μL (per well) of 200 ng/mL TNF-α medium (prepared by adding 10 μL/mL TNF-α (Sigma, Cat. No. T0157-10UG) stock to the test medium), to a TNF-α final concentration of 10 ng/mL. Culturing was then carried out for 5 hours at 37°C, 5% CO_2, activating the NF-κB by TNF-α stimulation and promoting induction of the luciferase gene. After 5 hours of culturing, 30 μL of culture

supernatant was removed out from each well. A luciferase assay reagent (Promega, Cat. No. E2610) was added at 70 μL to each well (total 150 μL of culture supernatant), and the mixture was gently mixed and allowed to stand for 2 minutes. Next, the relative light emission in each well (RLU: Relative Light Units) was measured using a luminescence measuring plate reader (luminometer: Thermo Fisher Scientific, Varioscan Flash, Model No. 5250040). The positive control used was 200 mM ammonium pyrrolidine dithiocarbamate (APDC) (Sigma, Cat. No. P8765-1G).

[0137]

Both selenoneine and selenoneine-containing fish extract significantly suppressed NFκ-B activation induced by addition of TNF-α. Inflammatory response associated with diseases such as allergy, arthritis, rheumatism and cancer, as well as chronic inflammation, were suppressed (Fig. 14).

[0138]

[Table 6]

Table 6. NF-κB activity in each test group

NF-κB activity (relative light emission)	Control (TNF-α-free)	Control	Selenoneine-containing fish extract	Selenoneine	Positive control
Average	86100.28	8244312	7212094	6589564	65632.28

[0139]

The specific embodiments in the present disclosure have been described herein as examples, but it will be understood that various modifications may be implemented without deviating from the spirit and scope of the present disclosure. The disclosure is therefore not restricted in any way other than by the descriptions in the claims. The right of the claims extends to their full scope and equivalents.

[0140]

Example 9: Examining effect of improving impaired glucose tolerance

It has previously been reported that the expression level of the selenoprotein P (SELENOP) gene in human liver is correlated with insulin resistance, that treatment of hepatocytes and muscle cells with purified SELENOP inhibits insulin signals and induces abnormal glucose metabolism, and that conversely, knockout of the SELENOP gene and knockdown by RNAi improves systemic insulin sensitivity and glucose tolerance in mice (Misu H., et al., Cell Metabolism, 12, 483-495(2010)). It has additionally been reported that 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3) is a positive regulating factor for the glycolytic pathway and insulin signal transduction system, with inhibition of PFKFB3 with an inhibitor resulting in inhibition of insulin-stimulated uptake of glucose and translocation

of the glucose transporter (GLUT4) to the cell membrane, and inhibition of the Akt signal transduction system, and conversely that the Akt signal transduction system is activated in cells with overexpression of the PFKFB3 gene (Trefely S., et al., J. Biol. Chem., 290, 25834-25846(2015)).

[0141]

In this Example, the effect of selenoneine-containing fish extract on expression levels of the SELENOP gene and PFKFB3 gene was verified. Normal human fibroblasts (Kurabo Industries, Ltd., KF-4109) were increased to a usable cell count using dedicated medium [DMEM (Nacalai Tesque, Cat. No. 08456-65), 10% FBS (Biowest, Cat. No. S1820, Lot No. 516536), 1% Penicillin-Streptomycin Mixed Solution (Nacalai Tesque, Cat. No. 26253-84)] in a CO_2 incubator (5% CO_2, 37°C), and the cells were seeded in a plate with a diameter of 8 cm at 8 × 10^5 cells/15 mL and then cultured. On the following day (80% confluence), the medium was exchanged with 15 mL of medium containing or not containing selenoneine-containing fish extract (1 mg/mL), and culturing was continued for 24 hours. Upon completion of culturing, the cells were detached from the wells using a 2.5 g/l-Trypsin/1 mmol/l-EDTA solution Nacalai Tesque, Cat. No. 32777-44). Specifically, the culture supernatant was removed from each well, 1 × PBS (Nissui Pharmaceutical Co., Ltd., Code 05913) was added at 0.3 mL/well, and after rinsing the cells to remove the PBS, 50 μL of 2.5 g/l-Trypsin/1 mmol/l-EDTA solution was added and reaction was conducted at 37°C. A 450 μL portion of PBS containing 10% FBS was then added to each well to terminate the trypsin reaction. The detached cell solution was recovered and centrifugation was performed at 500 × g or 10 minutes at room temperature, the supernatant was removed and the cell portion (precipitate) was collected. Recovery of the total RNA from the cells was carried out using an RNeasy Micro Kit (Qiagen, Cat. No. 74004), and RNA concentration and purity were measured with a spectrophotometer and Bioanalyzer (Agilent Technologies, Agilent 2100). The subsequent procedure was carried out using Affymetrix products such as a GeneChip WT Terminal Labeling and Controls Kit following the instruction manual, for total RNA sample amplification, fluorescent labeling and quality checking. The prepared sample was then hybridized to a DNA microarray (Affymetrix, GeneChip Human Genome U133 Plus 2.0 Array) using a GeneChip Scanner 3000 7G System (Affymetrix), and rinsed and stained, and after hybridization, the DNA microarray was scanned and the image file was converted to numerical data which were processed for background subtraction, normalization and expression ratio calculation.

[0142]

The selenoneine-containing fish extract suppressed the SELENOP gene expression level to less than 1/3 compared to no addition (control) while conversely increasing the PFKFB3 gene expression level more than two-fold, thus demonstrating that the selenium-containing

composition of the invention has an effect of improving impaired glucose tolerance (Table 7).

[0143]

[Table 7]

Table 7. Effect of improving impaired glucose tolerance

Gene name		Non-added (control)	Selenoneine-containing fish extract (1 mg/mL)
SELENOP	Normalized expression level	893.9	270.2
	Relative value (%)	100.0	30.2
PFKFB3	Normalized expression level	408.8	1071.6
	Relative value (%)	100.0	262.1

[0144]

Example 10: Examining effect of improving lipid metabolism disorder

The compound 27-hydroxycholesterol (27HC) is a cholesterol metabolite abundantly present in the blood, and it promotes development of arteriosclerosis and provokes inflammation of blood vessels. In the body, 27HC is catabolized by cytochrome P450 family 7 subfamily B member 1 (CYP7B1) (Umetani M. et al., Cell Metabolism, 20, 172-182(2014)). In actual CYP7B1 gene knockout mice, the concentration of 27HC in the blood and tissues increases by 4- to 5-fold compared to the wild type (Umetani M., ibid). In this Example, the effect of selenoneine-containing fish extract on expression levels of the CYP7B1 gene was verified. Cells and samples were prepared by the same method as Example 9, and the data were collected and analyzed. The selenoneine-containing fish extract suppressed the CYP7B1 gene expression level to more than 8-fold compared to no addition (control), thus demonstrating that the selenium-containing composition of the invention has an effect of improving lipid metabolism disorder (Table 8).

[0145]

[Table 8]

Table 8. Effect of improving lipid metabolism disorder

Gene name		Non-added (control)	Selenoneine-containing fish extract (1 mg/mL)
CYP7B1	Normalized expression level	8.0	64.2
	Relative value (%)	100.0	802.5

INDUSTRIAL APPLICABILITY

[0146]

The selenium-containing composition of the invention can be applied for the novel use of reducing ischemia reperfusion injury and as an agent to counter metabolic syndrome. A novel

use for selenoneine concentrate extracted from fishery processing residues has been found, making it possible to increase the value of unused marine products. It becomes possible to commercialize new food materials and functional materials for health-oriented consumers.

[0147]

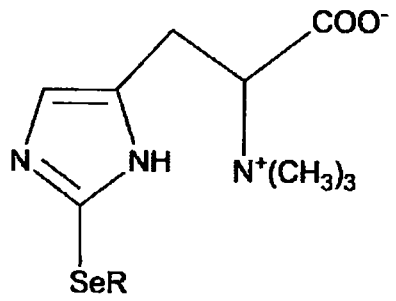
All of the publications and patent literature cited herein are incorporated into the present specification in their entirety as reference. The specific embodiments of the invention were explained in the present specification for the purpose of example, and it will be easily appreciated by a person skilled in the art that various modifications may be employed such as are not outside of the spirit and scope of the invention.

CLAIMS

[Claim 1]

A composition for reduction of oxidative stress, suppression of oxidized lipids, prevention of ischemic disease, improvement of metabolic syndrome, improvement of impaired glucose tolerance, protection from and improvement of damage associated with metabolic syndrome including abnormal lipid metabolism, suppression of carcinogenesis, suppression of general immunity disorders, suppression of vascular endothelial damage, suppression of arteriosclerosis, prevention of cognitive disorder, improvement of brain function, protection of corneal epithelial cells, improvement of dry eye or suppression of inflammation, the composition comprising an organic selenium compound represented by formula I:

[Chemical Formula 1]



I

wherein

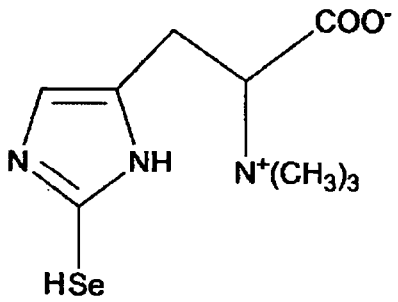
R is hydrogen or an ergothionyl, glutathionyl or cysteinyl group,

as an active ingredient.

[Claim 2]

The composition according to [1] above, wherein the organic selenium compound is 3-(2-hydroseleno-1H-imidazol-5-yl)-2-(trimethylammonio)propanoate represented by formula II:

[Chemical Formula 2]

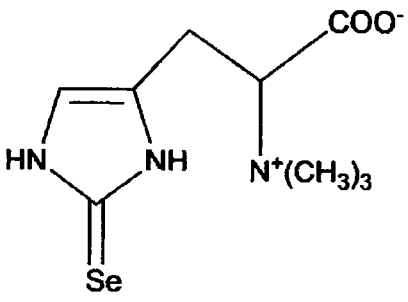


II

[Claim 3]

A composition for reduction of oxidative stress, suppression of oxidized lipids, prevention of ischemic disease, improvement of metabolic syndrome, improvement of impaired glucose tolerance, protection from and improvement of damage associated with metabolic syndrome including abnormal lipid metabolism, suppression of carcinogenesis, suppression of general immunity disorders, suppression of vascular endothelial damage, suppression of arteriosclerosis, prevention of cognitive disorder, improvement of brain function, protection of corneal epithelial cells, improvement of dry eye or suppression of inflammation, the composition comprising an organic selenium compound represented by formula III:

[Chemical Formula 3]



III

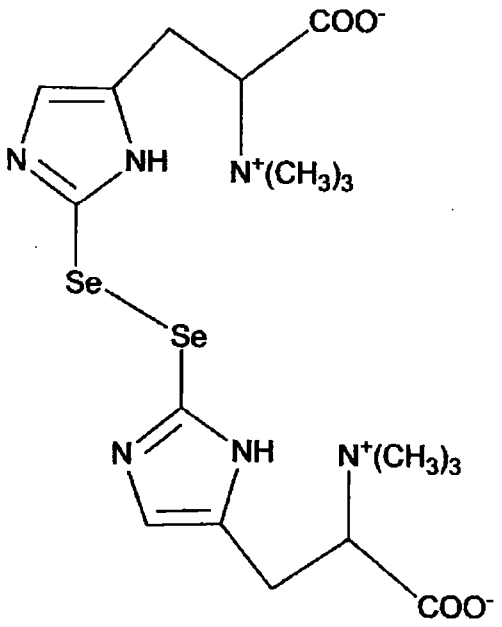
as an active ingredient.

[Claim 4]

A composition for reduction of oxidative stress, suppression of oxidized lipids, prevention of ischemic disease, improvement of metabolic syndrome, improvement of impaired glucose tolerance, protection from and improvement of damage associated with metabolic

syndrome including abnormal lipid metabolism, suppression of carcinogenesis, suppression of general immunity disorders, suppression of vascular endothelial damage, suppression of arteriosclerosis, prevention of cognitive disorder, improvement of brain function, protection of corneal epithelial cells, improvement of dry eye or suppression of inflammation, the composition comprising an organic selenium compound represented by formula IV:

[Chemical Formula 4]



IV

as an active ingredient.

[Claim 5]

A food composition for reduction of oxidative stress, suppression of oxidized lipids, prevention of ischemic disease, improvement of metabolic syndrome, improvement of impaired glucose tolerance, protection from and improvement of damage associated with metabolic syndrome including abnormal lipid metabolism, suppression of carcinogenesis, suppression of general immunity disorders, suppression of vascular endothelial damage, suppression of arteriosclerosis, prevention of cognitive disorder, improvement of brain function, protection of corneal epithelial cells, improvement of dry eye or suppression of inflammation, containing a composition according to any one of [1] to [4] above.

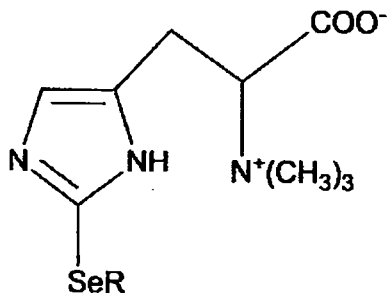
[Claim 6]

A food containing a composition according to any one of [1] to [4] above.

[Claim 7]

A medical composition for reduction of oxidative stress, suppression of oxidized lipids, prevention of ischemic disease, improvement of metabolic syndrome, improvement of impaired glucose tolerance, protection from and improvement of damage associated with metabolic syndrome including abnormal lipid metabolism, suppression of carcinogenesis, suppression of general immunity disorders, suppression of vascular endothelial damage, suppression of arteriosclerosis, prevention of cognitive disorder, improvement of brain function, protection of corneal epithelial cells, improvement of dry eye or suppression of inflammation, the medical composition comprising an organic selenium compound represented by formula I:

[Chemical Formula 5]



I

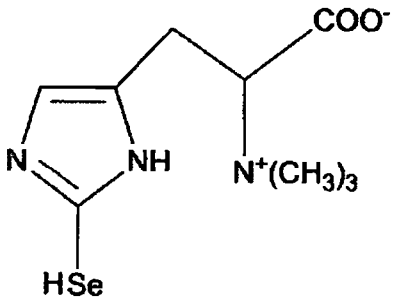
wherein

R is hydrogen or an ergothionyl, glutathionyl or cysteinyl group,

as an active ingredient.

[Claim 8] The medical composition according to claim 7, wherein the organic selenium compound is 3-(2-hydroseleno-1H-imidazol-5-yl)-2-(trimethylammonio)propanoate represented by formula II:

[Chemical Formula 6]

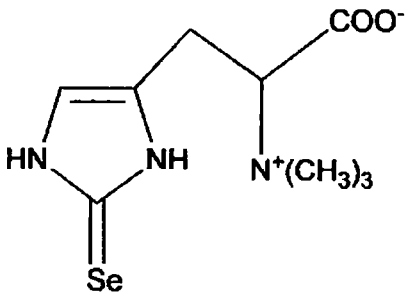


II

[Claim 9]

A medical composition for reduction of oxidative stress, suppression of oxidized lipids, prevention of ischemic disease, improvement of metabolic syndrome, improvement of impaired glucose tolerance, protection from and improvement of damage associated with metabolic syndrome including abnormal lipid metabolism, suppression of carcinogenesis, suppression of general immunity disorders, suppression of vascular endothelial damage, suppression of arteriosclerosis, prevention of cognitive disorder, improvement of brain function, protection of corneal epithelial cells, improvement of dry eye or suppression of inflammation, the medical composition comprising an organic selenium compound represented by formula III:

[Chemical Formula 7]



III

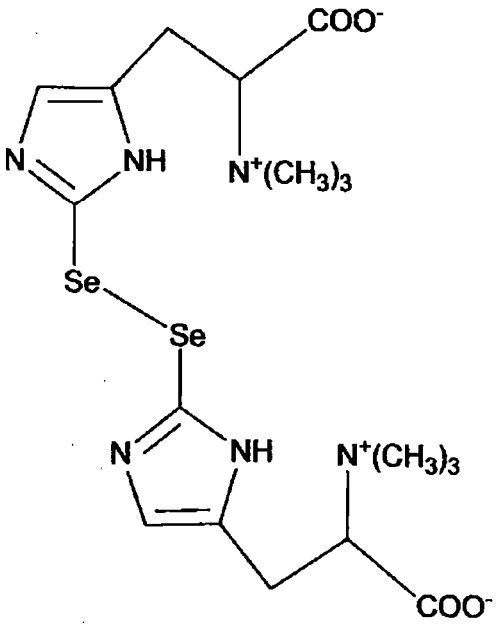
as an active ingredient.

[Claim 10]

A medical composition for reduction of oxidative stress, suppression of oxidized lipids, prevention of ischemic disease, improvement of metabolic syndrome, improvement of impaired glucose tolerance, protection from and improvement of damage associated with metabolic

syndrome including abnormal lipid metabolism, suppression of carcinogenesis, suppression of general immunity disorders, suppression of vascular endothelial damage, suppression of arteriosclerosis, prevention of cognitive disorder, improvement of brain function, protection of corneal epithelial cells, improvement of dry eye or suppression of inflammation, the composition comprising an organic selenium compound represented by formula IV:

[Chemical Formula 8]



IV

as an active ingredient.