

COMPOSITION COMPRISING EGG WHITE-CHALCANTHITE FOR PREVENTING OR TREATING CANCER

FIELD OF THE INVENTION

5 [1] The present invention relates to a composition comprising an egg white-chalcanthite for preventing or treating cancer, and more particularly, to a composition for preventing or treating cancer, which comprises only an egg white-chalcanthite prepared by mixing a chalcanthite with an egg white to remove the toxicity of the chalcanthite or comprises a mixture of the egg white-chalcanthite and a bamboo salt, and a method for preparing the
10 same.

[2]

BACKGROUND ART

[3] Cancer refers to a class of diseases which start with uncontrollable cell proliferation, then invade and destruct adjacent normal tissues and organs, can further create new growing sites
15 for cancer cells, and finally carry off individuals. Over past 10 years, to conquer cancer, there have been remarkable developments in modulating cell cycle and apoptosis and seeking new targets including oncogenes or tumor suppressor genes. Nevertheless, cancer incidence continues to increase as the civilization advances.

[4] At present, a cancer therapy depends on surgical operation, radiotherapy, and
20 chemotherapy of administering forty kinds of anti-cancer materials exhibiting potent cytotoxicity. However, most of these therapies are limited to only patients in early stage and special cancers, and thus cancer mortality is being increased continuously.

[5] Furthermore, since most of anticancer drugs are highly toxic chemicals, anti-cancer drugs with low toxicity, particularly natural product-derived anti-cancer drugs, are being developed continually.

[6]

5 [7] A chalcantite, which is a kind of sulfate minerals composed of copper sulfate, contains $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ mainly. The chalcantite is a natural mineral with a very small amount of other minerals mixed, and is a blue crystal. The chalcantite belongs to a triclinic system, is glassy-lustrous, and shows semi-transparent blue color. It is known that the chalcantite is used for emetics, insecticides, pigments, fixatives, electrolytes, etc. There is a fear of
10 toxicity because chalcantite is a crude drug from mineral sources. For this reason, there has been a limitation in making use of the chalcantite for clinical use actually, and further the fact that chalcantite can be used for anti-cancer drugs has never been known to the public.

[8]

15 [9] A Bamboo salt was first invented by Il-hoon Kim(1909~1992, pen name is In-san). The bamboo salt is prepared by synthesizing a bamboo and a salt using a roasting process. Herein, the bamboo acts as cytogenesis to generate new cells, and the salt acts as sterilization and antiseptis. Specifically, the salt filled into the bamboo is roasted several times in a furnace at a high temperature, thus removing toxic materials from the salt and
20 enhancing pharmaceutical effect.

[10] The bamboo salt has several advantageous effects such as the pharmacological effect of treating causes of inflammatory diseases by strengthening a stomach which is considered as

a basis of a human body, the blood clarification effect for clarifying blood, the effect of detoxification and elimination of body waste accumulated in a body, and the effect for converting physical constitution type from an acidic type to a weak alkaline type. In addition, it is known that the bamboo salt has three to four times stronger anti-inflammatory
5 action and sterilization ability against bacteria than the general salt, and thus such sterilization action leads to alleviation of fever in a human body.

[11]

[12] The inventors found out that the egg white-chalcanthite, in which the toxicity of chalcanthite was neutralized with the egg white, induced apoptosis of cancer cells to
10 suppress the growth of the cancer cells, and thus were applicable to a natural anti-cancer drug. Furthermore, the inventors found out that the egg white-chalcanthite was solely used or a composition comprising a mixture of the egg white-chalcanthite and the bamboo salt can be used as an anti-cancer drug, thereby completing the present invention.

[13]

15 **DECLOSURE OF THE INVENTION**

TECHNICAL PROBLEM

[14] Embodiments of the present invention are directed to a composition for preventing or treating cancer, which comprises an egg white-chalcanthite as an effective component.

[15] Embodiments of the present invention are also directed to a health functional food for
20 preventing or ameliorating cancer, which comprises an egg white-chalcanthite as an effective component.

[16] Embodiments of the present invention are also directed to a method for preparing an

egg white-chalcanthite, the method comprising the steps of: (a) heating and dehydrating a chalcanthite, which is a crude drug from mineral sources, until the entire chalcanthite turns grey; (b) cooling the dehydrated chalcanthite, and pulverizing the chalcanthite; and (c) mixing the chalcanthite of the step (b) with an egg white.

5 [17]

TECHNICAL SOLUTION

[18] In one aspect, the present invention provides a composition for preventing or treating cancer, which comprises an egg white-chalcanthite as an effective component.

[19] In the present invention, the term “egg white-chalcanthite” means a mixture of an egg
10 white and a chalcanthite, and may be prepared by roasting the chalcanthite (natural mineral mainly composed of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) to dehydrate it, pulverizing the dehydrated chalcanthite, and then mixing the pulverized chalcanthite react with the egg white to trigger the reaction therebetween. In the egg white-chalcanthite so prepared, the toxicity of the chalcanthite is neutralized by the egg white, so that the toxicity is reduced or removed and pharmaceutical
15 properties are increased.

[21] The composition of the present invention may further comprise a bamboo salt.

[22] The bamboo salt used in the present invention may be goods on the market or may be prepared personally. Here, the bamboo salt may be one that is prepared through an orthodox nine-times-melting bamboo salt fabrication method that is disclosed in books,
20 entitled ‘Universe and Miraculous Drug (1980)’ and ‘Miraculous Drug (1986)’ of Il-hoon Kim (pen name is In-san) who is known as a bamboo salt inventor, but the bamboo salt of the present invention is not limited thereto. For example, this bamboo salt fabrication

method comprises: putting a bay salt produced from the west coast in the Republic of Korea into a timber bamboo and closing the timber bamboo with an ocher stopper; laying the timber bamboos filled with the bay salt in an iron can compactly; roasting the timber bamboos using a pine tree as a firewood to thereby burn out the timber bamboos; milling a remaining salt pillar and then putting the milled salt into a new timber bamboo again; repeating the above-described processes eight times; and, in a ninth processing time, melting down the salt by increasing heating power through the addition of pine resin.

[23]

[24] The egg white-chalcanthite and the bamboo salt contained in the composition of the present invention may be powdered. Also, when the composition of the present invention comprises both the egg white-chalcanthite and the bamboo salt, the powdered egg white-chalcanthite and the bamboo salt may be mixed at various ratios ranging from 1:99 to 99:1 by weight, desirably 1:5 to 1:50 by weight, most desirably 1:5, 1:10, 1:15, 1:25, and 1:30.

[25] When the composition of the present invention is used for oral preparations, the amount of the bamboo salt powder may be equal to or larger than that of the egg white-chalcanthite powder. If a person is young, weak, old, or sick, the amount of the bamboo salt should be increased. However, if a person has a strong body, the amount of the egg white-chalcanthite may be gradually increased, and can be administered as a medicine up to a ratio of the egg white-chalcanthite to the bamboo salt ranging from 1:10 to 1:5.

[26] Furthermore, in the case where the composition is used for coating preparations that are applied to the skin of the body and used for cleaning and spraying, or used for enema, the amount of the egg white-chalcanthite may be increased, and the egg white-chalcanthite may

also be used singly.

[27]

[28] The egg white-chalcanthite composition of the present invention may have cancer-suppressing ability through improvement of activity of caspase-3.

5 [29] In specific embodiments of the present invention, in the case of treating liver cancer cells (HepG2), colon cancer cells (SW480), breast cancer cells (MCF-7), lung cancer cells (NCI-H460) with the egg white-chalcanthite, it can be observed that the suppression of cell proliferation is concentration-dependent. Furthermore, in the case of treating liver cancer cells and lung cancer cells with the egg white-chalcanthite, it can be observed that nuclear
10 fragmentation and chromatin condensation occur because apoptosis is induced. In addition, the cancer-suppressing ability of the egg white-chalcanthite composition is observed at a protein level, and resultantly it can be confirmed that the activation of caspase-3 protein induces apoptosis so that the composition containing the egg white-chalcanthite suppresses the growth of cancer cells.

15 [30]

[31] The egg white-chalcanthite composition of the present invention may be used for preventing or treating cancer. In the present disclosure, the term “preventing” means every practice that suppresses the development of diseases and suspends the onset of diseases by administering the composition, and the term “treating” means every practice that improves
20 the diseases or changes the conditions favorably by administering the composition.

[32] The composition of the present invention may be applicable to most of cancers, for example, liver cancer, breast cancer, lung cancer, colon cancer, stomach cancer, pancreatic

cancer, uterine cancer, prostate cancer, bone cancer, glioma, leukemia, etc. Desirably, the composition of the present invention may be applied to cancers of liver, colon, breast and lung, but is not limited thereto.

[33]

5 [34] The composition comprising the egg white-chalcanthite of the present invention as an effective component may be used as a pharmaceutical composition by further comprising pharmaceutically acceptable carriers. In this case, the composition can be prepared together with the carriers. Also, the composition of the present invention may be used as a single-agent or may be used as a complex agent with other effective components improving
10 drug efficacy.

[35] In the present invention, the term “pharmaceutically acceptable carriers” refers carriers or diluents that do not inhibit biological activities and characteristics of administered chemicals without stimulating living organisms. The pharmaceutically acceptable carrier in a composition prepared in liquid solution may comprise carriers available for a living body,
15 for example, saline, sterilized water, linger, buffered saline, albumin injection, dextrose solution, malto dextrine solution, glycerol, ethanol, and a mixture containing at least one of them. If necessary, typical additives such as antioxidant, buffering agent and bacteriostatic agent may be added to the carrier. Also, it is possible to prepare formulations for injection use (e.g., aqueous solution, suspension, emulsion, or the like), pills, capsules, granules, or
20 tablets, by further adding diluents, dispersing agents, surfactants, binding agents, and lubricants.

[36] The composition of the present invention may be applicable to any formulation

containing it as an effective component, and formed in oral or parenteral formulation. The pharmaceutical formulation of the present invention may include shapes suitable for oral, rectal, nasal, topical, subcutaneous, vaginal or parenteral administration, for inhalation, or for insufflation. Herein, the parenteral administration may include intramuscular, subcutaneous and intravenous administration.

[37] The formation for oral administration including the composition of the present invention may be prepared in the form of, for example, tablet, troche, lozenge, water-soluble or lipophilic suspension, formulated powders or granule, emulsion, hard or soft capsule, syrup, elixir, or the like. To prepare the composition in the form of tablets and capsules, the composition may include binders (e.g., lactose, saccharose, sorbitol, manitol, starch, amylopectin, cellulose, or gelatin), excipients (e.g. dicalcium phosphate), disintegrants (e.g., corn starch, or sweet potato starch), and lubricants (e.g., magnesium stearate, calcium stearate, sodium stearyl fumarate, or polyethylene glycol wax). In the case of capsule formations, liquid carriers such as fatty oil may be further added besides the above-described substances.

[38] The formation for parenteral administration comprising the composition of the present invention may be prepared in the form of injection type such as subcutaneous injection, intravenous injection and muscular injection, in the form of suppository injection type, or in the form of spray type such as aerosols that can be inhaled through a respiratory tract. To prepare the formations for injection use, the composition of the present invention is mixed with stabilizer or buffering agent in water to thereby fabricate solution or suspension, and the fabricated solution or suspension can be prepared for unit dose of ampules or vials. For

suppository injection, the formation may be prepared in the form of compositions for rectal administration such as enema or suppository including typical suppository base, e.g., cocoa butter or other glycerides. In the case of preparing the spray-type formations such as aerosols, propellants may be mixed with additives such that water-dispersed condensed
5 material or moisturized powder is dispersed.

[39]

[40] In another aspect, the present invention provides a method of preventing or treating cancer using a composition comprising an egg white-chalcanthite.

[41] In the present invention, the treating of diseases may comprise administering a
10 pharmaceutical composition comprising an egg white-chalcanthite. In the present invention, the term “administering” means introducing the pharmaceutical composition of the present invention to patients through appropriate methods.

[42] The composition of the present invention can be administered through a variety of administration routes, for example, an oral administration route or a parenteral
15 administration route only if the composition can reach a target organ. To be specific, the composition may be administered through general methods, for example, oral, rectal, topical, intravenous, intrapenitoneal, intramuscular, intraarterial, transdermal, intranasal, inhalation, or intraocular administration, or intradermal route. Desirably, the composition may be administered through oral or cutaneous administration.

20 [43] The treating method of the present invention comprises administering the composition of the present invention with pharmaceutically acceptable dose. It is obvious to a person with ordinary skill in the art that total one-day dosage may be determined by a doctor within

the scope of proper medical judgment. The therapeutically effective dose for a specific patient may be differently determined according to various factors or pseudo-factors well-known in the medical field, for example, the kind and extent of reaction to be achieved, whether or not other formulations are used depending on circumstances, specific compositions, age of the patient, body weight, general health conditions, gender, diet, administration route, and distribution ratio of composition, treatment duration, drugs used together or simultaneously with the specific compositions, and so forth. Therefore, the one-day effective dose of the pharmaceutical composition suitable for the objective of the present invention may be determined in consideration of the aforesaid. Most desirably, the one-day effective dose of the pharmaceutical composition may be administered in a range of 0.1 g to 30 g based on the weight of the egg white-chalcanthite powder. However, there is no limitation in effective dose when the composition is used as external preparations such as enema, cutaneous use, and the like.

[44]

[45] Furthermore, the treating method of the present invention can be applied to any animal in which cancer may be developed. Herein, the animal may include not only humans and primates, but also livestock such as cows, pigs, sheep, horses, dogs, and cats.

[46] Particularly, in oral administration of the composition of the present invention, it is possible to swallow the composition with water, with water in which ginger and licorice root are mixed and decocted, with water containing the extract of the root bark of *Ulmus davidiana* var, or with saliva. As an intake method, there is a method of taking the capsule containing the composition through the above-described methods as well as a method of

directly taking the composition. In this case, a method of taking the capsule containing the composition with salvia is better than a method of taking the capsule with water or beverage. The composition may be administered such that it is mixed into food and beverage in a small amount. For example, the mixture may be added into rice paste or roasted garlic, or the egg
5 white-chalcanthite powder may also be added to bamboo salt soybean.

[47] In specific embodiments of the present invention, the egg white-chalcanthite of the present invention was administered to cancer patients together with the bamboo salt, and anti-cancer effect was then observed. Resultantly, it can be observed that the administration of the egg white-chalcanthite with the bamboo salt is effective for treating
10 breast cancer, leukemia, stomach cancer, colon cancer, lung cancer, bone cancer, dysplasia of cervix uteri, thyroid cancer, etc.

[48]

[49] In another aspect, the present invention provides a health functional food for preventing or ameliorating cancer, which comprises an egg white-chalcanthite as an effective
15 component.

[50] The health functional food of the present invention may further comprise bamboo salt besides the egg white-chalcanthite.

[51] The composition of the present invention may be prepared by further containing food additives, which are sitologically acceptable, and may be available for a health functional
20 food for preventing or ameliorating cancer. The health functional food of the present invention may include formations such as tablets, capsules, pills and liquid.

[52] Foods into which the composition of the present invention can be added may include,

for example, various kinds of groceries, beverage, gum, tea, vitamin complex, health functional food.

[53] The term “health functional food” defined in the present disclosure means foods manufactured and processed by using raw materials and components having useful functions to a human body. The term “functional” means that intake of food is directed to controlling nutriment on the structure and function of a human body or achieving the useful effect for preservation of health such as physiological action.

[54] The composition of the present invention may be added to foods and beverages for the purpose of preventing or ameliorating cancer. Here, the amount of the composition added into the food or beverage may be in a range of 0.01 wt% to 10 wt% of the total food weight. For example, when the total volume of the health beverage is 100 ml, the composition may be added in an amount of 0.01 g to 5 g, desirably 0.5 g to 1 g.

[55]

[56] In another aspect, the present invention provides a method for preparing an egg white-chalcanthite, the method comprising the steps of: (a) heating and dehydrating a chalcanthite, which is a crude drug from mineral sources, until the entire chalcanthite turns grey; (b) cooling the dehydrated chalcanthite, and pulverizing the chalcanthite; and (c) mixing the chalcanthite of the step (b) with an egg white.

[57] Herebelow, the method for preparing the egg white-chalcanthite according to the present invention will be described in each step.

[58] In the step (a), a chalcanthite (natural mineral containing $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) is heated and then dehydrated. The heating of the chalcanthite may be performed using a typical method

that is well-known in the art. Preferably, the chalcantite is put into a caldron, and then roasted with gas fire, wood fire or charcoal fire. During heating, the chalcantite may be turned upside down to uniformly receive heat while carefully watching a change in color at every 3 to 5 hours. Although the appropriate heating duration may vary with one-time use
5 amount, it may be in the range of 10 to 24 hours. The chalcantite may be heated until whole the chalcantite turn grey to be in a state of dehydrated chalcantite.

[59]

[60] In the step (b), the chalcantite heated and dehydrated in the step (a) is cooled, and then pulverized. The dehydrated chalcantite should be cooled until heat is completely released.

10 Here, the moisture content of the chalcantite may range from 0 % to 5 %. After the dehydrated chalcantite is completely cooled, the chalcantite is pulverized finely. Thereafter, the pulverized chalcantite may be put into a plastic bag or an airtight container so as to prevent moisture from being absorbed thereinto, and then kept in a dry place.

[61]

15 [62] In the step (c), the chalcantite powder prepared in the step (b) is mixed with an egg white to trigger the reaction between the chalcantite and the egg white, which reduces or removes the toxicity of the chalcantite and increases pharmaceutical properties.

[63] Herein, an egg should be separated into an egg yolk and an egg white, and then only the egg white is used in the present invention. The egg may be a homegrown egg, preferably

20 Korean ogol chicken egg. The dehydrated chalcantite powder prepared in the step (b) is mixed with the egg white. At this time, the mixing ratio may be set such that 600 g of the chalcantite powder may be mixed with 140 to 400 g of egg white that is obtained from 7 to

20 eggs. While watching a mixed state, the amount of egg white may be adjusted and the chalcantite and the egg white may uniformly be mixed using a tool, e.g., wood spatula, which rarely participates in the reaction. In addition, the mixing may be performed in a vessel that is inactive in chemical reaction, for example, an earthen vessel, a ceramic ware,
5 or an elvan vessel. During the mixing, special attention should be paid because much heat is generated due to reaction heat.

[64] Furthermore, if the amount of egg white is too small during the mixing, it is difficult to neutralize the toxicity of the dehydrated chalcantite powder. On the contrary, if the amount of egg white is too great, the reaction heat is very weak or not generated, making it
10 difficult to achieve the mixing effect sufficiently. Thus, the amount of egg white should be adjusted carefully. After the chalcantite and the egg white are sufficiently mixed, the mixture may be cooled until the reaction heat is completely released.

[65]

[66] Moreover, the method for preparing the chalcantite according to the present invention
15 may further comprise, after the step (c), (d) cooling the mixture of the step (c), and then pulverizing the cooled mixture after the step (c).

[67] In the step (d), the mixture prepared in the step (c) is cooled until the heat is completely released, and the chalcantite with the egg white mixed is pulverized finely to form egg white-chalcantite powders. Consequently, by pulverizing the egg white-chalcantite, the
20 egg white-chalcantite can be effectively used for pharmaceutical or sitological preparations, and further its reaction area is increased, thus maximizing therapeutic activity.

[68] In the egg white-chalcantite prepared through the above method, the toxicity of the

chalcantite is removed but pharmaceutical properties are enhanced, and therefore it is applicable to a pharmaceutical or sitological composition for preventing or treating cancer.

[69]

ADVANTAGEOUS EFFECTS

5 [70] The composition comprising an egg white-chalcantite according to the present invention was prepared in the form wherein a toxicity of chalcantite is removed and a pharmaceutical activity of the prepared composition is maximized, thus exhibiting an excellent anticancer activity. Therefore, it can be used as a composition for preventing or treating cancer.

10 [71]

BRIEF DESCRIPTION OF THE DRAWINGS

[72] Fig. 1 illustrates a test procedure of a microorganism reverse mutation test.

[73] Figs. 2 to 4 and 5 illustrate results of MTT assay on each cell after liver cancer cells (HepG2), lung cancer cells (NCI-H460), colon cancer cells (SW480), and breast cancer cells
15 (MCF-7) are treated with a roasted chalcantite (IS3), an egg white-chalcantite (IS4), and a raw chalcantite (IS5), respectively.

[74] Figs. 6 and 7 illustrate results of DAPI stained cells after NC-H460 and HepG2 cells are treated with a roasted chalcantite (IS3) and an egg white-chalcantite (IS4) having concentrations of 50 $\mu\text{g/ml}$, respectively.

20 [75] Fig. 8 illustrates results of a western blot test to observe the protein expression degree of cells after NCI-H460 cells are treated with an egg white-chalcantite (IS4) having various concentrations.

[76]

EXAMPLES

[77] Hereinafter, a composition comprising an egg white-chalcanthite for preventing or treating cancer according to the present invention will be described in detail with reference
5 to the accompanying drawings. However, below-described embodiments are merely provided for illustration of the present invention, not limiting to the present invention.

[78]

[79] **Example 1. Prepare test material (raw chalcanthite, roasted chalcanthite and egg white-chalcanthite)**

10 [80] A roasted chalcanthite obtained by roasting and dehydrating a raw chalcanthite and an egg white-chalcanthite obtained by processing a roasted chalcanthite with an egg white were prepared. In this test, the raw chalcanthite, the roasted chalcanthite and the egg white-chalcanthite were utilized.

[81] First, the roasted chalcanthite is prepared by heating and dehydrating the raw
15 chalcanthite. To be specific, when the raw chalcanthite is roasted for 24 hours, the raw chalcanthite turns grey because it is dehydrated. Such a dehydrated chalcanthite is referred to as 'roasted chalcanthite'. After the roasted chalcanthite is completely cooled, it is finely pulverized.

[82] The egg white-chalcanthite is prepared by mixing and reacting the roasted chalcanthite
20 powder with egg white. To be specific, 600 g of the roasted chalcanthite powder is mixed with 260 g of egg white that is separated from 13 homegrown eggs, and then reacts with moisture contained in the egg white to generate a great amount of heat (reaction heat). As a

result, the chalcantite turns green and the powders are agglomerated, which is referred to as 'egg white-chalcantite'.

[83]

[84] To use the roasted chalcantite (IS3), the egg white-chalcantite (IS4) and the raw
 5 chalcantite (IS5) as test materials, respectively, each was weighed to 100 mg by a balance, and then dissolved in D.D.W to prepare a mixture with total volume of 1 ml. After the test materials were fully dissolved, a supernatant was filtered with 0.8- μ m syringe filter by centrifuging the mixture at 600 rpm.

[85]

10 [86] Table 1

	Test material		Total vol.	Particulars	Filter size	Remarks
Dissolved in D. W	IS3	Roasted chalcantite	100 mg/ml	Dissolved by 97%(about 30 μ g precipitated)	0.8 μ m filter	After filtering, supernatant is used after being rotated at 6,000 rpm for 10 min.
	IS4	Egg white-chalcantite	100 mg/ml	Very small amount of white powder remains	0.8 μ m filter	
	IS5	Raw chalcantite	100 mg/ml	Dissolved	0.8 μ m filter	

[87]

[88] **Example 2. Microorganism reverse mutation test for genetic toxic test**

[89] To measure genetic toxic degrees of the roasted chalcantite and the egg white-

chalcantite, a microorganism reverse mutation test that can detect a cell mutagen was performed.

[90] The principle of this test is to make use of histidine-requiring strains that are not alive without histidine. This test is performed for detecting histidine recovery, which is original strain property, caused by mutagen in a mutant test. In the test, if the number of strains exceeds two times the number of untreated group (negative control group) or increases depending on the concentration of the test material, the test result is determined positive, and it is concluded as a mutagen.

[91] To carry out the microorganism reverse mutation test, the test was conducted on test standard strains, salmonella typimurium TA98, TA100, TA1535 and TA1537 as pre-culture. A tolerance test was performed first, and thereafter two strains, i.e., salmonella typimurium TA100 and TA102, were selected as test strains among the strains exhibiting tolerance against the chalcantite. The salmonella typimurium TA100 is a test strain upon the reversion of guanine and cytosine in a gene, and the salmonella typimurium TA102 is a test strain upon the reversion of adenine and thymine.

[92]

[93] When an antibacterial test was performed on cells using the egg white-chalcantite (5,000 $\mu\text{g}/\text{plate}$) and the roasted chalcantite (2,500 $\mu\text{g}/\text{plate}$), it was observed that the growth of the strains were suppressed. Therefore, the genetic toxic (reverse mutation) test was performed at below-described concentrations where the strain growth was not suppressed (see FIG. 1).

[94] The egg white-chalcantite having the concentrations of 1,250, 625, 313, 156, and 78

$\mu\text{g}/\text{plate}$ are used as a treated group while setting the maximum concentration to 2,500 $\mu\text{g}/\text{plate}$. Also, the roasted chalcantite having the concentrations of 625, 313, 156, and 78 $\mu\text{g}/\text{plate}$ are also used as the treated group while setting the maximum concentration to 1,250 $\mu\text{g}/\text{plate}$.

5 [95] As a positive control group, sodium azide with the concentration of 1 $\mu\text{g}/\text{plate}$ (for treating salmonella typhimurium TA100) and mitomycin C with the concentration of 1 $\mu\text{g}/\text{plate}$ (Salmonella typhimurium TA102) were used. As a negative control group, distilled water was used.

[96] The respective test materials, positive control group chemicals, distilled water were
10 treated on a plate, and thereafter the number of revertant strains was measured. The results are as follows.

[97]

[98] Table 2

Egg white-chalcantite, Strain : TA100								
	Positive control	Negative control	Treated					
Concentration ($\mu\text{g}/\text{plate}$)	1	0	2,500	1,250	625	313	156	78
Number	1,136	194	241	195	157	142	159	186
	1,421	211	205	221	184	173	162	162
Average	1,279	202	223	208	171	158	161	174

[99] Table 3

Roasted chalcantinite, Strain : TA100							
	Positive control	Negative control	Treated				
Concentration (µg/plate)	1	0	1,250	625	313	156	78
Number	1,662	237	192	186	193	192	173
	1,456	201	235	179	182	193	169
Average	1,559	219	214	183	188	193	171

[100] Table 4

Egg white-chalcantinite, Strain : TA102								
	Positive control	Negative control	Treated					
Concentration (µg/plate)	1	0	2,500	1,250	625	313	156	78
Number	>1,000	5	3	4	2	1	0	2
	>1,000	3	5	3	2	0	3	1

[101] Table 5

Roasted chalcantinite, Strain : TA102							
	Positive control	Negative control	Treated				
Concentration (µg/plate)	1	0	1,250	625	313	156	78

Number	>1,000	2	2	4	1	3	0
	>1,000	3	1	0	3	4	2

[102]

[103] In the case of the salmonella typimurium TA100, the number of strains in the group treated with the egg white-chalcanthite and roasted chalcanthite were remarkably smaller than that of the positive control group and even similar to that of the negative control group
5 at the maximum concentration not inducing the growth suppression of microorganisms. Furthermore, the number of colonies was not increased depending on the concentration. Therefore, it was determined that the egg white-chalcanthite and the roasted chalcanthite were not mutagens.

[104] Also, in the case of the salmonella typimurium TA102, colonies rarely appeared in the
10 group treated with the egg white-chalcanthite and roasted chalcanthite at the maximum concentration not inducing the growth suppression of microorganisms. That is, the number of colonies in the group treated with the egg white-chalcanthite and roasted chalcanthite was remarkably smaller than that of the positive control group, and even similar to that of the negative control group. In addition, the number of colonies was not increased depending
15 on the concentration. Consequently, it was determined that the egg white-chalcanthite and the roasted chalcanthite were not mutagens.

[105]

[106] **Example 3. Cancer cell culture**

20 [107] 10% FBS, 100 U/ml penicillin and 100 U/ml streptomycin were put into RPMI 1640

medium (containing L-glutamine) and lung cancer cells (NCI-H460) were cultured in 5% CO₂ incubator.

[108] Colon cancer cells (SW480) and breast cancer cells (MCF-7) were cultured under the same conditions as the lung cancers (NCI-H460). 10% FBS, 100 U/ml penicillin and 100 U/ml streptomycin were put into DMEM medium (containing L-glutamine) and liver cancer cells (HepG2) were cultured in 5% CO₂ culture medium.

[109] The NCI-H460, MCF-7, SW480 and HepG2 cells were obtained from Korean Cell Line Bank (KLCB) and then used.

[110]

10 [111] **Example 4. Measurement of cell viability through MTT assay**

[112] To investigate the effects of the roasted chalcantite (IS3), egg white-chalcantite (IS4) and raw chalcantite (IS5) on the growth of cancer cells, these materials were treated onto the cancer cells at various concentrations to measure cell viability through MTT (3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide) assay thereby assaying the suppression of the cancer cell growth.

[113] The MTT assay was performed on four cancer cells, i.e., liver cancer cell (HepG2), colon cancer cell (SW480), breast cancer cell (MCF-7) and lung cancer cell (NCI-H460). First, each cell was inoculated into a 96-well plate at a concentration of 1×10^5 cells/ml by 100 μl , and the well plate was then cultured for 24 hours in 5% CO₂ incubator at 37°C.

20 Thereafter, 100 μl of each test material (IS3, IS4 and IS5) was put into wells at the concentration of 0, 3.125, 6.25, 12.5, 25, and 50 $\mu\text{g/ml}$, respectively, and then treated for 24 hours.

[114] MTT (thiazolyl blue, SIGMA Co.) with the concentration of 2 mg/ml was prepared, and added to the wells by 15 μl to react with the test materials for 3 to 4 hours. 115 μl of the test material was removed from each well to leave only 30 μl of a violet material, and then 150 μl of dimethyl sulfoxide (DMSO) is added. Afterwards, the resultant was
5 sufficiently mixed for 10 minutes in a microplate mixer to dissolve precipitates, and then optical density (OD) was measured with the absorbance of 540 nm in a micro-reader. All the test results were compensated by the absorbance measured in the well in which cells were not cultured.

[115] In all samples, the growth suppression effect appears as the concentration increases.
10 Especially, it can be observed that the growth of cancer cells is most effectively suppressed when the cancer cells are treated with the egg white-chalcanthite (IS4) (See Figs. 2 to 5)

[116]

[117] **Example 5. Cell apoptosis assay using DAPI staining**

[118] To observe how the roasted chalcanthite (IS3), egg white-chalcanthite (IS4) and raw
15 chalcanthite (IS5) suppress cell growth by inducing apoptosis of cancer cells (NCI-H460 and HepG2), the cancer cells were treated with each test material. Then, the cancer cells were stained with DAPI, and cell types were observed through a fluorescence microscope. Specific test procedure is as follows.

[119] 400 μl of each cell (1×10^5 cell/ml) was put in a 8-well chamber slide and cultured for
20 24 hours. Thereafter, the cultured cells were treated with the test material having the concentration of 50 $\mu\text{g/ml}$ and made to react for 24 hours. After the reaction, the medium is discarded and the cells react with 500 μl of 75 mM KCL. This expands the cells to

make it easy to observe nucleus. After making cold-ice state by mixing acetic acid and methanol at a ratio of 1:3, this was dispensed in a volume of 500 μl to react for 5 minutes, thereby immobilizing the cells. This procedure was repeated twice. After immobilized, the resultant is sufficiently dried in air, and 100 μl of DAPI stain solution was dropped to
5 stain the resultant for 10 minutes, and thereafter washed with PBS. A cover glass was covered with glycerol and the resultant cells were observed through a fluorescence microscope (x100, or x200). Among three hundred cells counted, the cells exhibiting nuclear fragmentation and chromatin condensation were detected and observed according to the morphologic criteria of apoptosis.

10 [120]

[121] It has been reported that apoptosis, which is programmed cell death, accompanies cell shrinkage, chromatin condensation, DNA fragmentation, mitochondria dysfunction, caspase protease activation. DAPI is a blue fluorescent stain, and has characteristic that fluorescence increases when it bonds with minor grooves in which AT clusters of DNA exist.

15 In virtue of this characteristic, it is possible to visibly observe the degree of DNA fragmentation by simply checking fragmented and condensed apoptotic bodies through a microscope.

[122] DAPI staining was performed on H460 and HepG2 cells, which had been treated with the roasted chalcantite (I3) and the egg white-chalcantite (I4) having at the concentration
20 of 50 $\mu\text{g/ml}$. In comparison with the control group (group untreated with the chalcantite), nuclear fragmentation and chromatin condensation could be observed in all the cells. Particularly, it was possible to observe many apoptotic bodies in the egg white-chalcantite

(IS4) (See Figs. 6 and 7).

[123]

[124] **Example 6. Detection of apoptosis-related protein expression by western blot test**

5 [125] The control degree of expression of apoptosis-related protein, e.g., caspase-3, bax and bcl-2 was observed in order to observe the effect of the egg white-chalcanthite upon cancer cells at a protein level.

[126] Bax functions to induce apoptosis by promoting the secretion of cytochrome C while moving from cytosol to mitochondria. In contrast, it is known that bcl-2 acts as an
10 important signal transferring system that transfers or receives situations in or outside cells, and inhibits the movement of bax to mitochondria to thereby suppress apoptosis (Nomura et al., 1999; Murphy et al., 2000).

[127] Caspase-3 is most directly associated with apoptosis, and acts at an initial stage of apoptosis. Further, caspase-3 has an active form of heterodimer of 17 kDa and 19 kDa
15 derived by the separation of 35 kDa proenzyme (Fernandes-Alnemri et al., 1994), and amplifies initial signals of caspase-8 and caspase-9.

[128] The apoptosis is classified into an internal path and an external path, which induce apoptosis through caspase-3 activity. In order for caspase-3 to be observed in an active form, the expression degree of procaspase-3 of inactive 35kDa should be reduced relatively
20 or a protein having a molecular weight of 17kDa and 19kDa which is an active form thereof should be detected (Kang et al., 2002; Ahn et al., 2004).

[129]

[130] A western blot test was performed to observe whether the egg white-chalcanthite (IS4) controls the expression of proteins (e.g., caspase-3, bax and bcl-2) that has been known to induce apoptosis. Through this test, it was possible to know a path through which the egg white-chalcanthite induces apoptosis in cells. Specific test procedure is as follows.

5 [131] Lung cancer cells (NCI-H460) were treated with the egg white-chalcanthite (IS4) at concentrations of 0, 25, 50 and 100 $\mu\text{g/ml}$, respectively, and then cultured for 24 hours at 37°C. Thereafter, the cells are collected using a scraper, the collected cells were centrifuged at 1,000 rpm together with a medium containing them, a supernatant was removed, and then washed twice with 2 ml cold PBS. 50 to 100 μl of lysis buffer is
10 added to the resultant and sufficiently mixed, and then dissolved for 2 hours at 4°C. Herein, the lysis buffer is composed of 50 mM Tris pH 8.0, 150 mM NaCl, 0.02% sodium azide, 0.2% SDS, 100 $\mu\text{g/ml}$ of PMSF (phenylmethylsulfonyl fluoride), 50 $\mu\text{l/ml}$ of aprotinin, 1% igapel 630 (or NP-40), 100 mM NaF, 0.5% sodium deoxychoate, 0.5 mM EDTA (Ethylendiamineetraacetic acid--Sigma E-4884), and 0.1 mM EGTA (Ethylene glycol-bis(β -
15 aminoethylether) N,N,N',N'-tetraacetic acid--sigma E-4378). After the reaction, the test material is put into a 1.5-ml tube, then stirred for 30 seconds, and centrifuged for 1 hour at 23,000 g at 4°C. Only centrifuged supernatant was taken. The protein amount of a final test material was measured using a bio-rad protein kit. Lysis buffer and 5X sample buffer are mixed with the measured protein to make the protein amount equal, and then the
20 resultant was boiled for 5 minutes in a 100°C heat block. After that, a test material is collected by centrifuging the resultant for a while. After preparing a separating gel (12.5%) and a stacking gel (5%), an electrophoresis is performed and the gel is then transferred.

Transferred gel was dipped in a staining solution (Coomassie blue staining solution) for 10 minutes, and brought into a destaining solution to observe remaining proteins. A transferred membrane was washed with TBS-T solution and a bit of moisture was then removed. Thereafter, the membrane is blocked for 2 hours with about 5% skim milk
5 diluted with TBS-T solution, and washed several times with TBS-T solution. After reacting with a primary antibody (bax, bcl-2, cleaved caspase-3; cellsignaling) and secondary antibody (anti-rabbit) followed by the reaction in ECL solution for 1 minute, a film was placed on a cassette, and was observed through photographing and developing.

[132]

10 [133] As a result of the treatment of H460 cells with the egg white-chalcanthite (IS4), the expression of cleaved caspase-3 tends to increase at 25 $\mu\text{g/ml}$ and 50 $\mu\text{g/ml}$, but tends to slightly decrease at 100 $\mu\text{g/ml}$. It is considered that this is because the test material with higher concentrations exhibit toxicity. The expression of bcl-2 slightly increases but the expression of bax decreases in the group treated with the egg white-chalcanthite (IS4) (see
15 Fig. 4).

[134] From these results, it can be confirmed that the egg white-chalcanthite, which is a mainly effective component of the inventive composition, activates caspase-3 protein in cancer cells to thereby induce apoptosis. Also, it can be appreciated that the apoptosis is induced by activating caspase-3 protein through a path differing from a bax/bcl-2 path.

20 [135]

[136] **Example 7. Identification of anticancer effect**

[137] Human-derived lung cancer cell (NCI-H460) was subcutaneously transplanted to a front leg of nude mouse to form a solid cancer up to a certain size. The egg white-chalcanthite (IS4) exhibiting the best apoptosis ability from a human-derived lung cancer cell in an *in vivo* experiment was subcutaneously administered for four weeks and the

5 research on anticancer effect of egg white-chalcanthite was carried out. That is, the assay of anticancer effect was carried out by measuring a size of solid cancer using digital calipers twice per one week and identifying a secular change along with a body weight. At a date of final measurement, the inhibition rate of solid cancer growth was calculated and compared.

At a date of completion of experiment, the weight and solid volume of the solid cancer

10 extracted were measured using a plethysmometer. The individual exhibiting a central necrosis of solid cancer through one-day condition observation during the test, and the individual having the solid cancer volume of greater than 1500mm³ as the result of

measurement of respective solid cancer size were indentified. The individual corresponding to one of the two conditions was considered as a death individual. The mean survival day of

15 each group was calculated. After completion of the test, the percent increase in life span was calculated as compared with a positive control group. Internal organ abnormal was visually identified upon autopsy after completion of the test. The blood collection was then carried out to analyze ALP, CRE, BUN, TG, ALT, AST, Ca level, GLU and total cholesterol in the blood. The organ (heart, spleen, testis) was extracted to measure the weight of organ.

20 [138]

[139] <7-1> Material and Method

[140] 1. Preparation of cancer cell to transplant

[141] Human lung carcinoma (NCL-H460) cells to transplant was cultured in 95% CO₂, 37°C incubator (MCO-20AIC, Sanyo) wherein 100 U/ml penicillin, 100g/ml streptomycin, 10% heated fetal calf serum-containing RPMI-1640 (Gibco BRL) were put in 260 ml tissue flasks. Once four cells were adhered, they were washed twice with Hanks balanced salt solution (HBSS) (Gibco BRL), washed with 0.2% trypsin in HBSS and used.

[142]

[143] 2. Medical inspection and acclimation

When a test animal was obtained, a veterinary inspection on a general health condition of all animals was carried out. The healthy animals suitable to perform the test were selected, and the acclimation was made for one week to adapt to the environment.

[145]

[146] 3. Group Classification

[147] After the acclimation, a tumor lump was subcutaneously transplanted to the healthy passage group to extract a cultured tumor lump. The blood vessel and fat layer distributed on the surface of the tumor lump were removed. Only fresh tumor tissue was selected and subcutaneously transplanted to the non-treated animal. When the individual tumor volume after transplant reaches 100 mm³, the body weight and tumor size were measured, and the group classification was made by a random method.

[148]

[149] 4. Individual identification

[150] An individual identification card describing a test type, a test number, a test material, a group number, an individual number, a sex, an administration dose, a test period and a responsible tester was attached to a breeding box. The individual identification was performed by using a tail marking. The treatment group and administration dose were shown in Table 6 below.

[151] Table 6

Group		Administration Dose	N
G1	NCI-460 alone	NCI-H460 tumor cell alone	7
G2	IS4	45mg/kg + NCI-H460 tumor	7
G3	IS4	90mg/kg + NCI-H460 tumor	7

[152]

[153] 5. Administration Method

[154] The test material was dissolved in a physiological saline and orally administered using zonde once a day for 4 weeks.

[155]

[156] 6. Evaluation method

5 [157] 1) Measurement of body weight

[158] In order to observe a change of body weight during the test period, the body weight was measured per 7 days after initiation of test.

[159] 2) Preparation of tumor-containing model

10 [160] 1×10^7 cells/nude mouse/100 μl was injected to obtain a tumor strain which was then sub-cultured in a passage group. The subculture was repeated three times to obtain a tumor lump which the property of original solid cancer was recovered. The tumor lump was transplanted to five passage groups.

15 [161] The animal containing a tumor of the step of rapidly growing due to a sufficient blood supply before generation of a central necrosis of tumor was sacrificed. The contour moiety in which a rapid division was mainly made was cut at a certain size (3x3x3mm) to prepare a tumor fragment. The tumor fragment was raised to the end of trocar, and the front side of left hind limb of animal was excised to about 4mm through which the prepared trocar was inserted and the end was placed at a body side section of the rear of left front leg. The trocar was removed while slightly and rapidly rotating 360-degree. The tumor fragment was placed
20 at a desired position. The excised portion was disinfected. The position of tumor was confirmed with touching a skin. The growth of tumor was observed over twice for one week. After transplant, the groups were classified into those which the volume reaches 100 mm³, and the test material was then administered.

[162] 3) Anti-cancer test (mean tumor volume)

25 [163] The long axis and short axis of the tumor were measured with calipers twice for one week, and the body weight was then measured. If 28 days lapsed after initial administration of drug, the test was completed and the test materials were checked to determine the effect of suppressing a solid cancer due to the test material. The solid cancer volume and inhibition rate after lapse of time were calculated according to the following
30 equation:

[164] $V (\text{Mean tumor volume}) = (A \times B^2)/2$

(A= long axis length, B = short axis length)

[165] $IR (\text{inhibition rate}) = [CV-TV/TV] \times 100$

[166] (CV = Control group tumor volume, TV = Treatment group tumor volume)

[167] 4) Mean survival time & percent increase in life span (%ILS)

[168] The individual exhibiting the central necrosis of solid cancer through one-day condition observation of test groups during the test, and the individual having the solid cancer volume of greater than 1500mm³ as the result of measurement of respective solid cancer size, were indentified. The individual corresponding to one of the two conditions was considered as a death individual. The percent increase in life span was calculated according to the following equation:

[169] $\%ILS = [T-C]/C \times 100$

[170] (where, C and T are mean survival day of mice in control and treatment groups)

[171]

[172] 7. Inspection of blood lipid level and general blood

[173] The blood was collected through aorta abdominalis upon autopsy to obtain a serum. Alanine phosphatase (ALP), creatinine (CRE), Ca, glucose (GLU), alanine transferase (ALT) and AST.UN were analyzed by using blood biochemistry automatic analyzer (HITACHI, Tokyo, Japan).

[174]

[175] 8. Histopathological finding

[176] Upon completion of the test, the bleeding and killing were made to extract liver, kidney and spleen. Their weights were then measured and the visual finding was observed. The tissues were set in 10% neutral formalin solution. The tissue fragment with thickness of 4 μm was prepared by the paraffin mediated process, stained with Hematoxylin & Eosin and then observed by an optical microscope.

[177]

[178] 9. Statistical analysis

[179] Levenet's test was performed to compare the homology of dispersion about each test result. In a case where the dispersion has a homology, one-way analysis of variance (ANOVA) was performed. If the significance was observed, Dunnett's t-test was performed to find out a test group having a significant difference as compared with a control group ($p < 0.05$ or $p < 0.01$).

[180]

[181] <7-2> Result and Consideration

[182] 1. Change of body weight

[183] For a change of body weight and a change of uptake amount of feed and beverage during the test, the initial administration weight and feed uptake amount in the IS4 administration group were reduced as compared with NCI-H460 cell alone group, but they were recovered to a normal after one week (Figures 9, 10 and 11). During the test, the matters that need to note between groups were not shown.

[184]

[185] 2. Change of tumor (solid tumor) volume

[186] The solid cancer volume during the test was measured twice for one week by using calipers. As a result, the tumor volume of IS4 45,90mg/kg administration group was more concentration-dependently suppressed than that of NCI-H460 cell alone group (Figure 12). In checking the time-lapse change of the groups, the NCI-H460 tumor growth was suppressed from 12 days after administration of IS4, and from 15 days after the administration it has shown a solid cancer inhibition tendency as compared with NCI-H460 cell alone group. In numerically checking the solid cancer inhibition tendency, it has been shown that, at 22 days of the last half test, IS4 45 mg/kg group is 2221.74mm³ and IS45, 90 mg/kg group is 1517.07mm³, whereas NCI-H460 cell alone group is 2806.47mm³.

[187]

[188] 3. Measurement of tumor (solid cancer) volume inhibition rate

[189] In checking the analysis result of the growth inhibition rate based 100% of the solid cancer volume of NCI-H460 cell alone group, the test material administration groups has shown a volume dependent inhibition tendency from 8 days to 25 days (IS4 45 mg/kg I.R 82.47% < IS4 90 mg/kg I.R 59.99% at 22 day). IS4 90mg/kg administration group has shown to reduce the solid cancer growth inhibition after 22 day-administration (Table 7).

[190] Table 7

treat group	day								
	0	4	8	12	15	19	22	26	29
NCI-H460 cell alone	100	100	100	100	100	100	100	100	100
IS4 45 mg/kg	99.99	76.19	72.11	79.55	75.44	85.92	82.47	79.32	73.73

IS4 90 mg/kg	92.76	75.26	78.71	75.41	68.23	68.97	59.99	69.37	66.06
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[191]

[192] 4. Measurement of tumor (solid tumor) weight and volume

[193] In checking the weight of solid cancer extracted at the completion date of test and the volume measurement result according to plethysmometer, the solid cancer weight and volume of NCI-H460 cell alone were 2.52 ± 0.75 g and 4.67 ± 1.18 cm³, whereas those of IS4 45mg/kg group were reduced to 2.24 ± 0.78 g and 4.03 ± 1.28 cm³, and those of IS4 90mg/kg group were 2.17 ± 0.60 g, 3.79 ± 0.85 cm³, showing a significant reduction ($p < 0.05$) (Table 8).

[194] Table 8

	NCI-H460 tumor alone	IS4 45mg/kg	IS4 90mg/kg
Tumor weight (g)	2.52 ± 0.75	2.24 ± 0.75	2.17 ± 0.60
Tumor volume(cm ³)	4.67 ± 1.18	4.03 ± 1.28	3.79 ± 0.85

[195]

[196] 5. Measurement of mean survival time and percent increase in life span

[197] The individual exhibiting a central necrosis of solid cancer through one-day condition observation of test groups during the test period, and the individual having the solid cancer volume of greater than 1500mm³ as the result of measurement of respective solid cancer size, were indentified. The individual corresponding to one of the two conditions was considered as a death individual and the percent increase in life span was calculated. The solid cancer weight and volume of NCI-H460 cell alone group were 18.29 ± 3.59 day and IS4 45mg/kg administration group was 20.71 ± 2.61 day, thus the life span has been increased to 10.68%. Also, the IS4 90mg/kg administration group is 22.33 ± 2.44 day and the lift span has been increased to 22.08% (Table 9, Figure 13).

[198] Table 9

Treatment	Mean survival time (day)	%ILS
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NCI-H460 tumor only	18.29 ± 3.59	0
IS4 45mg/kg	20.71 ± 2.61	10.68
IS4 90 mg/kg	22.33 ± 2.44*	22.08
*Significant difference from positive control (NCI-H460 cell only) group at p < 0.05		

[199]

[200] 6. Histopathological finding (optical microscopy) and organ weight measurement

[201] In identifying the organ weight change upon autopsy, there was no considerable change in other organ weight except that the relative weight of liver of IS4 administration group (45mg/kg) was significantly reduced ($p < 0.01$) as compared with NCI-H460 cell alone group. In reviewing detailed results, the organ weight upon autopsy has shown 1.76 ± 0.07 g for NCI-H460 cell alone group and 1.54 ± 0.07 g for IS4 45 mg/kg group; this proves a significant reduction ($p < 0.01$). However, IS4 90 mg/kg group has been reduced to 1.69 ± 0.10 g, but a significance was not shown (Table 10). Even in a histopathological aspect, a significant finding between groups has not been shown (Figure 14).

[202] Table 10

Treatment	Absolute organ weights			
	Body weight (g)	Liver (g)	Spleen (g)	Heart (g)
NCI-H460 cell alone	26.64 ± 1.61	1.76 ± 0.07	0.33 ± 0.04	0.15 ± 0.01
IS4 45 mg/kg	25.37 ± 0.88	1.54 ± 0.07*	0.31 ± 0.04	0.15 ± 0.01
IS4 90 mg/kg	26.13 ± 0.90	1.69 ± 0.10	0.31 ± 0.08	0.15 ± 0.01
Treatment	Absolute organ weights			
	Kidney (g)		Testis (g)	
	L	R	L	R
NCI-H460 cell alone	0.22 ± 0.06	0.21 ± 0.05	0.11 ± 0.06	0.11 ± 0.06

IS4 45 mg/kg	0.22 ± 0.03	0.22 ± 0.02	0.09 ± 0.01	0.09 ± 0.01
IS4 90 mg/kg	0.23 ± 0.01	0.23 ± 0.01	0.09 ± 0.01	0.09 ± 0.01
Treatment	Relative organ weights			
	Body weight (g)	Liver (g)	Spleen (g)	Heart (g)
NCI-H460 cell alone	26.64 ± 1.61	6.61 ± 0.22	1.27 ± 0.22	0.58 ± 0.03
IS4 45 mg/kg	25.37 ± 0.88	6.07 ± 0.13*	1.24 ± 0.19	0.58 ± 0.05
IS4 90 mg/kg	26.13 ± 0.90	6.47 ± 0.24	1.18 ± 0.28	0.59 ± 0.03
Treatment	Relative organ weights			
	Kidney (g)		Testis (g)	
	L	R	L	R
NCI-H460 cell alone	0.82 ± 0.22	0.80 ± 0.19	0.42 ± 0.21	0.42 ± 0.20
IS4 45 mg/kg	0.82 ± 0.10	0.87 ± 0.08	0.35 ± 0.04	0.36 ± 0.04
IS4 90 mg/kg	0.89 ± 0.04	0.90 ± 0.06	0.34 ± 0.03	0.35 ± 0.03
*Significant difference from positive control(NCI-H460 cell only) group at $p<0.05$.				

[203]

[204] 7. Biochemical analysis of blood

[205] In checking the result of the blood biochemical analysis upon autopsy, blood ALP, CA, CRE, ALT, AST levels have not shown a significant difference due to the administration of test material.

[206] On the other hand, blood UN levels have shown a significant reduction in all the test material administration groups (IS4 45mg/kg, IS4 90mg/kg, $p < 0.01$). Also, PHOS levels of IS4 45mg/kg group was 7.79 ± 0.82 mg/dl, showing a significant difference as compared with 9.27 ± 0.99 mg/dl of NCI-H460 cell alone group (IS4 45mg/kg group, $p < 0.05$).

5

[207] Table 11

Group	ALP	CA	CRE	ALT	AST	PHOS	UN
	IU/U	mg/dl	mg/dl	IU/L	IU/L	mg/dl	mg/dl
NCI-H460	$67.62 \pm$	$10.18 \pm$	$0.35 \pm$	$184.08 \pm$	$25.75 \pm$	$9.27 \pm$	$29.38 \pm$
cell only	7.27	0.44	0.06	43.85	4.62	0.99	3.68
IS4 45	$70.57 \pm$	$10.10 \pm$	$0.30 \pm$	$175.19 \pm$	$24.10 \pm$	$7.79 \pm$	$21.23 \pm$
mg/kg	13.61	0.79	0.06	53.17	3.51	0.82*	2.29**
IS4 90	$67.93 \pm$	$10.40 \pm$	$0.35 \pm$	$160.50 \pm$	$30.25 \pm$	$8.35 \pm$	$21.58 \pm$
mg/kg	4.45	0.43	0.06	36.66	11.91	0.75	1.63*

Results are mean $\pm$ S. D. * Significant difference from positive control (NCI-H460 cell alone) group at $p < 0.05$. ** Significant difference from positive control (NCI-H460 cell alone) group at $p < 0.01$.

[208]

[209] **Example 7. Treatment cases**

10

[210] An egg white-chalcanthite bamboo salt was prepared by mixing egg white-chalcanthite and bamboo salt powder at a weight ratio of 1:20, and thereafter a powder mixture of the egg

white-chalcanthite and the bamboo salt was put into a capsule and then provided to patients.

10 to 20 capsules were administered per one day by swallowing the capsules one by one with saliva or drinking water (one-day administration dose is 5 to 10 g in total on the basis of an adult, or 1 to 3 capsules per 10 kg in body weight). The patients took the capsules over 2 to 10 times at intervals of 1 to 2 hours. If necessary, herbal decoction and Sari-Jang for recovering energy was administered together with the egg white-chalcanthite bamboo salt.

[211]

[212] <8-1> Case 1. Treatment of dysplasia of cervix uteri

[213] Patient information (surname: Ko, age: 35, gender: female): She was afflicted with dysplasia of cervix uteri, has taken antituberculosis drugs for 2 years, and had hepatitis B virus carriers. In this state, she took herbal decoction, egg white-chalcanthite bamboo salt, and Sari-Jang for 3 months, and further egg white-chalcanthite bamboo salt water was injected into the uterus. After that, an inspection was carried out, and she was diagnosed that most of cells became normal and only nucleus was slightly enlarged. Thereafter, she relapsed into the disease due to the interruption of administration. However, she was re-treated (that is, took the herbal decoction and egg white-chalcanthite bamboo salt, and the egg white-chalcanthite bamboo salt is injected), and resultantly was perfectly cured after 1 month.

[214] <8-2> Case 2. Treatment of colon cancer, lung cancer, and bone cancer

[215] Patient information (surname: Chun, age: 57, gender: male): He was diagnosed with colon cancer, lung cancer, and bone cancer (cervical vertebrae cancer). After he had received operations for excising colon cancer and lung cancer (when an inspection had been

carried out due to urination problem at that time, a bladder is normal but a portion of a bladder had been excised because it had pressed down the nerve), the cancer metastasized to the vertebrae. The vertebrae was then destructed by the growth of cancer cells, and thus nerves around a rib are pressed down so that he suffered from severe pain. In spite of 6-
5 times chemical therapy, his conditions was not improved at all but the cancer cells remained intact. There was no therapeutic possibility, and he could not help withstanding the pain with an anodyne everyday because he was too weak to receive chemical therapy. However, in this situation, herbal decoction, egg white-chalcanthite bamboo salt, and Sari-Jang were administered to him for 5 months. After that, he underwent a medical examination, and the
10 examination result proved that he was perfectly cured.

[216] <8-3> Case 3. Treatment of thyroid cancer

[217] Patient information (surname: Kim, age: 46, gender: male): He was treated for acute hepatitis 12 years ago, and currently his liver is calcified. After being diagnosed with thyroid cancer, he took herbal decoction, egg white-chalcanthite bamboo salt, and Sari-Jang
15 without chemical therapy. One month later, CT inspection result demonstrated that the size of the cancer was reduced to 4.6 mm from 5.1 mm. After additional administration for 6 months, he was perfectly cured.

[218]

INDUSTRIAL APPLCABLITY

20 [219] According to the present invention, since the toxicity of the chalcanthite is removed but the pharmaceutical activity of the chalcanthite is maximized in the composition comprising the egg white-chalcanthite, the composition comprising the egg white-

chalcantite exhibits excellent anti-cancer activity. Consequently, the composition of the present invention can be usefully applied to pharmaceutical preparations for preventing or treating cancer, and also be applicable to health functional foods.

WHAT IS CLAIMED IS:

1. A composition for preventing or treating cancer, comprising an egg white-chalcanthite as an effective component.

5

2. The composition of claim 1, further comprising a bamboo salt.

3. The composition of claim 1 or 2, wherein the egg white-chalcanthite and the bamboo salt are powdered.

10

4. The composition of claim 2, wherein the composition comprises the egg white-chalcanthite and the bamboo salt at a ratio of approximately 1:5 to approximately 1:50 by weight.

15

5. The composition of claim 1, wherein the egg white-chalcanthite is prepared by roasting the chalcanthite, dehydrating the roasted chalcanthite, pulverizing the dehydrated chalcanthite, and mixing the pulverized chalcanthite with an egg white.

20

6. The composition of claim 1 or 2, further comprising a pharmaceutically acceptable carrier.

7. The composition of claim 1 or 2, wherein the cancer is selected from the group consisting of liver cancer, breast cancer, lung cancer, colon cancer, stomach cancer, pancreatic cancer, uterine cancer, prostate cancer, bone cancer, glioma, and leukemia.

25

8. The composition of claim 1, wherein the composition has a cancer-suppressing ability through improvement of caspase-3 activity.

9. A health functional food for preventing or ameliorating cancer, comprising an egg white-chalcanthite as an effective component.

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10. The health functional food of claim 9, further comprising a bamboo salt.

11. A method for preparing an egg white-chalcanthite, the method comprising the steps of:

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(a) heating and dehydrating a chalcanthite, which is a crude drug from mineral sources, until the entire chalcanthite turns grey;

(b) cooling the dehydrated chalcanthite, and pulverizing the chalcanthite; and

(c) mixing the chalcanthite of the step (b) with an egg white.

12. The method of claim 11, further comprising, after the step (c), cooling the mixture (d) of the step (c), and pulverizing the mixture.

5 13. The method of claim 11, wherein, in the step (c), 600 g of the roasted chalcantite powder is mixed with 140 to 400 g of egg white obtained from 7 to 20 eggs.

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