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(54) **Composition comprising dried whole egg for treating hepatitis**

Zusammensetzung enthaltend getrocknetes Vollei zur Behandlung von Hepatitis

Composition a base d'oeuf entier séché pour le traitement de la hepatite

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(56) References cited:
WO-A-01/91777 **US-A- 3 607 304**
US-A- 4 902 509

- **VERDOLIVA A ET AL: "Affinity purification of immunoglobulins from chicken egg yolk using a new synthetic ligand" JOURNAL OF CHROMATOGRAPHY. BIOMEDICAL APPLICATIONS, ELSEVIER, AMSTERDAM, NL, vol. 749, no. 2, 12 January 2000 (2000-01-12), pages 233-242, XP004220588 ISSN: 0378-4347**

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Description

[0001] The present invention relates to compositions having antihepatitic activity containing components derived from natural substances, and more particularly to compositions having antihepatitic activity containing dry product of defatted whole egg of eggs of a fowl bred by feeding a feed containing dry products of certain plants.

[0002] In Japan, chronic hepatitis afflicts a huge number of persons, a majority of which is occupied by hepatitis B virus (HBV) and hepatitis C virus (HCV) carriers. Chronic hepatitis is a disease very difficult to cure. It is highly possible that after a long progress it proceeds into cirrhosis and further into hepatoma and there is no decisive specific for curing it. Methods for curing the disease currently focus on interferon (IFN) treatment contemplated to control or disinfect viruses.

[0003] However, IFN treatment has its limit and there is no other decisive therapy, so that the therapy of chronic hepatitis takes a long period of time anyhow. Accordingly, an auxiliary treatment in which glycyrrhizin formulations or Chinese galenicals such as malgranda bu-supo is used in combination has been used widely as a means for inhibiting the progress of the disease and improving the liver function injury. In particular, malgranda bu-supo, one of Chinese galenicals, causes side effects to a lesser extent than other drugs do and is relatively high in therapeutic index in respect of inhibition of inflammation. Therefore, it has come to attention.

[0004] Recently, however, the very malgranda bu-supo has come to fail to give satisfaction since its use in combination with an IFN formulation, which is the first selected drug for chronic hepatitis C, has frequently caused a side effect called interstitial pneumonia and such combined use has become a contradiction.

[0005] In any rate, under the present circumstances where no potent radical cure other than IFN treatment is present, there is little alternative therapy that supplements it. Thus, the patients suffering from chronic hepatitis desire the appearance of a novel drug for the therapy of hepatitis, which can be used daily and continuously for a long period of time with peace in mind.

[0006] Under the circumstances, challenges have been made on utilization of Chinese galenicals other than malgranda bu-supo or compositions derived from other natural substances as antihepatitic drugs. Actually, many such challenges have been described or reported in many patent publications, academic literature, and the like. There remains an ample possibility that further searches on natural substances will result in finding effective drug components.

[0007] In the case of intractable diseases such as chronic hepatitis whose therapy takes a long period of time, it is needless to say that the problems of side effects of a drug to be used and of drug tolerance in that the affected area of a patient resists the drug will become serious hindrances to the therapy.

[0008] However, it is considered that the conventional methods in which a drug component alone is isolated and purified or semi-synthesized from a particular natural substance as a therapeutic drug cannot solve the above-mentioned problems. So far as the organism (or living body) recognizes the drug as a foreign matter containing a cytotoxic factor, the problems of side effects and drug tolerance cannot be avoided to occur. Furthermore, other problems on drugs derived from natural substances include one that in many cases the component found with difficulty is poor as a resource, so that its industrial application is impossible.

[0009] Therefore, an object of the present invention is to conduct a search for natural drugs and their formulae derived from natural substances that hardly cause side effects and drug tolerance, based on quite a new idea having departed from conventional drugs such as Chinese galenicals, Chinese medicine formulae, etc. and to develop a composition that has an antihepatitic activity.

[0010] This object has been achieved by the surprising finding that a dry product of defatted whole egg (hereinafter referred to as "defatted whole egg dry product") of eggs produced by a fowl bred by feeding a feed to which a dry product of Angelica keiskei ("Ashitaba", in Japanese) (hereinafter referred to as "Angelica keiskei dry product") and optionally a dry product of Theaceae Camellia ("Tsubaki", in Japanese) (hereinafter referred to as "Theaceae Camellia dry product") has antihepatitic activity. Also, the inventors of the present invention have found that a composition containing in addition to the above-mentioned defatted whole egg dry product, the Angelica keiskei dry product and optionally the Theaceae Camellia dry product has antihepatitic activity.

[0011] That is, the inventors of the present invention have found that the composition containing the above-mentioned defatted whole egg dry product has antihepatitic activity, i.e., an activity of preventing an increase in serum transaminase level associated with liver diseases and also that the composition containing the defatted whole egg dry product, the Angelica keiskei dry product and optionally the Theaceae Camellia dry product has excellent antihepatitic activity.

[0012] The present invention has been accomplished based on these findings.

[0013] According to a first aspect of the present invention, the present invention relates to a composition having an antihepatitic activity, characterized by comprising a dry product of defatted whole egg of eggs of a fowl bred by feeding a feed having blended therein an additive comprising a dry product of Angelica keiskei, a dry product of brown algae, and optionally a dry product of Theaceae Camellia.

[0014] According to a second aspect of the present invention, the present invention relates to a composition according to the first aspect of the invention, wherein the fowl is a member selected from the group consisting of hens, ducks

and quails.

[0015] According to a third aspect of the present invention, the present invention relates to a composition having antihepatitic activity, characterized by comprising a dry product of defatted whole egg of the first aspect of the invention and a dry product of Angelica keiskei.

[0016] According to a fourth aspect of the present invention, the present invention relates to a composition having antihepatitic activity, characterized by comprising a dry product of defatted whole egg of the first aspect of the invention, a dry product of Angelica keiskei and a dry product of Theaceae Camellia.

[0017] According to the present invention, novel natural drugs derived from natural substances departing from conventional drugs Chinese galenicals, Chinese medicine formulae, etc. are provided. That is, compositions that contain components derived from natural substances as effective components and have antihepatitic activity are provided. In particular, the compositions of the present invention are excellent in specific antihepatitic effect to trouble in liver induced by drugs.

[0018] In addition, the compositions of the present invention are extremely weak in toxicity so that it is expected that they can be utilized not only in the therapy of hepatitis, etc. as drugs but also in preventing the above-mentioned diseases by adding them to various foods and taking them daily.

[0019] As described above, the present invention relates to compositions having antihepatitic activity derived from familiar products that have never been used conventionally as raw materials, such as Angelica keiskei and Theaceae Camellia, which are natural substances, as well as a dry product of whole egg defatted from specific eggs produced by utilizing such natural substances as feeds.

[0020] Although it is unclear as to what compound contained in the components constituting the composition of the present invention is the main body that exhibits pharmacological activity, it is believed that a plurality of compounds contained therein act in combination with each other.

[0021] Angelica keiskei used as a raw material in the present invention is an umbelliferous perennial plant known to have strong anthigenic ability and contain abundant nutritional components. It is anthigenically distributed mainly in the southern coasts of Izu peninsula and Izu islands, Japan. However, recently it is also cultivated as a vegetable or the like in Japan.

[0022] Various portions, such as leaves, stems, and roots, of Angelica keiskei may be used, with leaves and stems being preferred. There has been no report on utilization of Angelica keiskei as a component of a drug having antihepatitic activity.

[0023] The Angelica keiskei dry product used in the present invention can be obtained, for example, by cutting Angelica keiskei to a suitable size (usually 10 to 15 mm square) after optionally washing the leaves and stems of Angelica keiskei with water, freezing the cut plant pieces, and then sublimating the iced water in vacuum to dry them. The dry product is used usually after it is processed into the form of powder in consideration of convenience. This is yellowish green (young leaf color) powder. Hereinafter, the Angelica keiskei dry product is referred to as "I-01B".

[0024] Theaceae Camellia is an evergreen tall tree that is anthigenically distributed in Honshu, Shikoku, Kyushu, etc. districts in Japan and includes many kinds of variations for appreciation, etc. prepared by breeding. In the present invention, a wild type of Camellia japonica (Yabutsubaki) is preferable.

[0025] The portions of Theaceae Camellia to be used in the present invention include leaves, flowers, seeds, etc., with leaves being particularly preferable. Thus far no report has been made on utilization of leaves, etc. of Theaceae Camellia as components of drugs. In the present invention, the Theaceae Camellia dry product obtained by roasting, for example, leaves, etc. of Theaceae Camellia are used as the Theaceae Camellia dry product. As an example thereof, first, picked Theaceae Camellia leaves are dried. The drying is performed by heating them at 60 to 80°C for 3 to 5 hours. Then, the dry product is cut to a suitable size (usually, about 5 mm square) and then roasted. The roasting is performed generally by using a hot iron plate drum type roasting apparatus at 100 to 150°C for 10 to 30 minutes. For the same reason as in the case of the dry product of Angelica keiskei, usually powdered Theaceae Camellia is used. This is brown powder. Hereinafter, the Theaceae Camellia dry product is referred to as "I-01C".

[0026] The eggs produced by fowls to be used in the present invention are those eggs produced by fowls bred by feeding with a feed containing an additive comprising I-01B and a dry product of brown algae (hereinafter referred to as "brown algae dry product"), for example, dried tangle weed, as an auxiliary component, or a feed containing an additive comprising I-01B, I-01C and a brown algae dry product. Note that as basal feeds, those feeds commonly used in breeding fowls are used. For preparing feed additives, respective raw materials are blended in the following ratios: (1) I-01B : brown algae dry product = 1-4 : 1-4 (by weight ratio), preferably 1 : 1 (by weight ratio), or (2) I-01B : I-01C : brown algae dry product = 1-4 : 1-4 : 1-4 (by weight ratio), preferably 2 : 1 : 1 (by weight ratio).

[0027] The blending amounts of the additives to the feed are not particularly limited but in the case of the additive (1), the blending amount of the additive is 1 to 20% by weight, preferably 2 to 8% by weight while in the case of the additive (2), the blending amount of the additive is 1 to 20% by weight, preferably 2 to 8% by weight.

[0028] Fowls may be bred by a conventional method except that the special additives as described above are blended to the feed. The fowls include hens, ducks, quails, etc., with hens being preferable.

5 [0029] Eggs must be collected after at least 10 days' feeding on a feed having blended therein the above-mentioned additives. The reason thereof resides in that, generally, the period during which the components contained in the feed additive move over into eggs is considered to be about 3 days in the case of water-soluble components and about 3 weeks in the case of fat-soluble components. However, according to the finding by the inventors of the present invention, a conclusion has been obtained that it is desirable that egg collection is done after feeding the feed containing the above-mentioned additive to the fowls for at least 10 days.

[0030] Moreover, although details are unclear, the inventors of the present invention have found that proteins in the above-mentioned eggs are useful for the purpose of the present invention.

10 [0031] Accordingly, the whole egg obtained from eggs collected is defatted and dried to obtain a dry product. That is, liquid egg obtained by cracking eggs is sufficiently agitated to homogenize it and then frozen by a conventional method to obtain frozen whole egg, which then is dried. The drying is performed preferably by using a microwave drier under controlling the power and heating time of the microwave drier so that the temperature of the product does not exceed a range of 80 to 90°C. This can provide a whole egg dry product in the form of chips.

15 [0032] Then, the whole egg dry chips are defatted by extracting them by distillation with an alcohol such as methanol, ethanol, etc., as an extraction solvent, and thereafter, the defatted whole egg is recovered. Note that at the time of extraction, the solvent is heated to a temperature not higher than 60°C and the extraction is completed within 1.5 hours, preferably in from 30 minutes to 1 hour. If necessary, this defatting treatment maybe repeated several times .

20 [0033] By applying a hot air drying to the defatted whole egg, a whole egg dry product is obtained. Note that it is preferred that the hot air drying is performed by using a hot air fluidized bed type drier or the like. Since powder is desirable also in the case of whole egg dry product, usually powdered whole egg dry product is used. This can be obtained by pulverizing the whole egg dry product to a suitable size by using, for example, a pulverizer such as a hammer mill. The whole egg dry product thus obtained has a pale brown color. The defatted whole egg dry product derived from eggs obtained by breeding fowls with a feed having blended therein the additive (1) is referred to as "I-01A(1)", and the defatted whole egg dry product derived from eggs obtained by breeding the fowls with a feed having blended therein the additive (2) is referred to as "I-01A(2)".

25 [0034] The composition containing, as active components, I-01A, which is the above-mentioned defatted whole egg dry product, specifically I-01A(1) or I-01A(2), is the composition having antihepatitic activity according to the first aspect of the present invention.

30 [0035] On the other hand, the composition containing, as active components, I-01A, which is the above-mentioned defatted whole egg dry product, I-01B, which is the above-mentioned Angelica keiskei dry product, and optionally I-01C, which is the above-mentioned Theaceae Camellia dry product, is a composition having antihepatitic activity according to the third or fourth embodiment of the present invention.

35 [0036] The drug compositions of the present invention are very low in toxicity. For example, in a repetitive administration of mixed feed in which a composition containing I-01A and I-01B in combination is administered to rats for 2 weeks at a mixed ratio of 30%, no toxicity was observed.

40 [0037] In the case of other compositions containing other combinations, in mouse leukemia pharmacological experiments conducted by administration at a mixed ratio of 20 to 45%, there has been observed no change suggesting toxicity in general states such as body weight, feed taking amount, amount of exercise, etc. throughout the period of 10 days before transplantation of leukemia cells and survival period after the transplantation as compared to the non-administered control group. In addition, they exhibited significant anticancer effect of 200% or more in terms of life sustaining ratio.

[0038] Therefore, the drug compositions of the present invention are excellent in safety and can be used as food additives so that daily uptake thereof can prevent diseases.

45 [0039] The drug compositions of the present invention are administered mainly by an oral route. The form of the compositions is not particularly limited and they can be prepared into capsules, granules, tablets, etc. by, for example, a conventional preparation method.

50 [0040] The dosages and blending ratios of the respective components when the drug compositions of the present invention are used for humans are as follows. For example, in the case of the composition according to the first aspect of the invention, it is suitable to administer the composition such that I-01A is administered at a dosage of 0.2 to 20 g/day, preferably 1 to 5 g/day. In the case of the composition according to the third or fourth aspect of the invention containing a blend of I-01A and I-01B, it is suitable to administer the composition in a blending ratio of I-01A : I-01B = 1-10 : 0.5-5, preferably 1-2 : 0.5-1, at a dosage of 0.2 to 20 g/day, preferably 1 to 5 g/day. Also, in the case of the composition having a formulation of I-01A, I-01B and I-01C, it is suitable to administer the composition in a blending ratio of I-01A : I-01B : I-01C = 1-10 : 0.5-5 : 0.5-5, preferably 1-2 : 0.5-1 : 0.5-1 at a dosage of 0.2 to 20 g/day, preferably 1 to 5 g/day.

55 [0041] The blending ratios and dosages of the respective components described above are merely exemplary and since the components are not toxic, the blending ratios and dosages are not particularly limited to the above-mentioned values and various preparations and formula patterns of dosage in consideration of conditions such as site of disease,

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progress of disease, state of disease, sex, age, etc. as appropriate are applicable.

[0042] Furthermore, also when the drug compositions of the present invention are used as food additives, there is no fear of any toxicity or side effects, so that the addition amounts to foods, etc. may be determined in consideration of the above-mentioned preparations and formula patterns of dosage, etc.

EXAMPLES

[0043] Hereinafter, the present invention will be described in detail by examples. However, the present invention should not be considered as being limited thereto.

Production Example 1

Production of Ashitaba dry product

[0044]

(1) Leaves and stems of Angelica keiskei (Ashitaba) were cut from cultivation field of Angelica keiskei to obtain 1,960 kg of a raw material. The length of the obtained stems was adjusted to 70 to 80 cm. Then, the raw material was washed with tap water. The component analysis of (raw) leaves of Angelica keiskei gave results as shown in Table 1.

The washed raw material was cut to about 10 to 15 mm square with a cutter.

(2) The cut raw material was placed on a tray and preliminarily frozen. Furthermore, after freezing the cut raw material in a freeze-drier, the freeze-drier was evacuated to sublimate the frozen water.

[0045] The obtained freeze-dried product was pulverized in a pulverizer to a size of about 100 meshes. Thus, 238.5 kg of Angelica keiskei dry product powder I-01B was obtained.

Table 1

General Components Composition	
Item	Content*
Water	88.6g
Protein	3.3g
Lipids	0.1g
Ash	1.3g
Carbohydrates (sugars)	5.2g
Carbohydrates (fibers)	1.5g
Energy	33kcal
Sodium	60mg
Potassium	540mg
Substantial amount of salt	0.2g

*Quoted from "Fourth Edition, Japan Standard Tables of Food Composition (1982)"

Production Example 2

Production of Camellia dry product

[0046]

(1) Raw leaves of Camellia japonica (Yabutsubaki, wild type) were picked to obtain 476 kg of a raw material. The raw material was washed with tap water. Then, the raw material was heated at 70°C for 5 hours to obtain a dry product, which then was roasted on a hot iron plate at 135°C for 15 minutes to obtain a roasted dry product.

(2) The above-mentioned roasted dry product was pulverized by a pulverizer to a size of about 30 meshes to obtain 116.7 kg of Theaceae Camellia dry product powder I-01C.

[0047] The component analysis of the Theaceae Camellia dry product powder I-01C was performed. That is, general

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components composition, amino acid composition and fatty acid composition in 100 g of edible portion were examined. The results obtained are shown in Tables 2, 3, and 4, respectively.

Table 2

General Components Composition	
Item	Content
Water	5.4g
Protein	9.0g
Lipids	3.9g
Ash	5.9g
Carbohydrates	75.6g
Energy	374kcal
Sodium	129mg
Substantial amount of salt	0.3g
Tannic acid	5200mg

Table 3

Amino Acid Composition			
Item	Content (mg)	Item	Content (mg)
Arginine	356	Glycine	470
Lysine	414	Proline	392
Histidine	163	Glutamic acid	858
Phenylalanine	399	Serine	387
Tyrosine	226	Threonine	357
Leucine	668	Aspartic acid	626
Isoleucine	298	Cystine	7
Methionine	48	Hydroxyproline	94
Valine	384	γ -aminobutric acid	52
Alanine	456		

Table 4

Fatty Acid Composition	
Item	Content (g)
Palmitic acid	0.30
Palmitoleic acid	0.06
Stearic acid	0.03
Oleic acid	0.10
Linoleic acid	0.08
Linolenic acid	0.20

Production Example 3

Production of Egg (Part 1)

[0048]

(1) Preparation of feed additive

The Angelica keiskei dry product I-01B obtained in Production Example 1 and commercially available cut tangle weed (dry product) were mixed in a weight ratio of 1 : 1 to prepare a feed additive.

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(2) Preparation of feed

To a basal feed (trade name: Kumiai Blended Feed for Adult Chicken SELECT, produced by Asahi Industries Co., Ltd.; composition, 61% of grains, 20% of plant oil cakes, 5% of animal-derived feed, 4% of chaff and bran, 10% of miscellaneous) was blended 2% by weight of the feed additive obtained as described in (1) above to prepare a feed.

(3) Feeding and egg collection

A thousand (1,000) egg-laying hens which were all 300 days old were given the feed as described in (2) above and allowed to freely take it. Egg-laying ratio was good and no difference in egg-laying ratio from that of normal egg-laying hens fed under the same conditions was observed. After 10 days from the start of feeding, eggs were continuously collected for 23 days to obtain 1,200 kg of eggs.

(4) Component analysis of the egg obtained as described in (3) above was performed. That is, the general components composition and amino acid composition in 100 g of edible portion were examined. The results obtained are shown in Tables 5 and 6, respectively.

Table 5

General Components Composition		
Item	Egg of the present invention	Control egg*
Water	74.9g	74.7g
Protein	12.8g	12.3g
Lipids	10.1g	11.2g
Ash	1.0g	0.9g
Carbohydrates	1.2g	0.9g
Energy	147kcal	162kcal
Sodium	147mg	130mg
Cholesterol	471mg	470mg
Iodine	0.4mg	(0.02mg)**

*Quoted from "Fourth Edition, Japan Standard Tables of Food Composition (1982)"

** Calculated value

Table 6

Amino acid Composition (content in grams)					
Item	Egg of the present invention	Control egg*	Item	Egg of the present invention	Control egg*
Arginine	0.84	0.78	Alanine	0.74	0.70
Lysine	0.97	0.89	Glycine	0.44	0.41
Histidine	0.34	0.31	Proline	0.49	0.47
Phenylalanine	0.72	0.64	Glutamic acid	1.71	1.60
Tyrosine	0.58	0.50	Serine	0.99	0.84
Leucine	1.15	1.10	Threonine	0.63	0.57
Isoleucine	0.69	0.68	Aspartic acid	1.34	1.30
Methionine	0.46	0.40	Tryptophan	0.19	0.19
Valine	0.86	0.83	Cystine	0.36	0.32

*Quoted from "Revised Japan Standard Tables of Food Composition (1986)"

Production Example 4

Production of egg (Part 2)

[0049]

(1) Preparation of feed additive

The Angelica keiskei dry product I-01B obtained in Production Example 1, the Theaceae Camellia dry product

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I-01C obtained in Production Example 2, and commercially available cut tangle weed (dry product) were mixed in a weight ratio of 2 : 1 : 1 to obtain a feed additive.

(2) Preparation of feed

To a basal feed (trade name: Kumiai Blended Feed for Adult Chicken SELECT, produced by Asahi Industries Co., Ltd.; composition, 61% of grains, 20% of plant oil cake, 5% of animal-derived feed, 4% of chaff and bran, 10% of miscellaneous) was blended 8% by weight of the feed additive obtained as described in (1) above to prepare a feed.

(3) Feeding and egg collection

Seven hundred (700) egg-laying hens which were all 180 days old were given the feed as described in (2) above and allowed to freely take it. Egg-laying ratio was good and no difference in egg-laying ratio from that of normal egg-laying hens fed under the same conditions was observed. After 10 days from the start of feeding, eggs were continuously collected for 35 days to obtain 1,150 kg of eggs.

(4) Component analysis of the egg obtained as described in (3) above was performed. That is, the general components composition and amino acid composition in 100 g of edible portion were examined. The results obtained are shown in Tables 7 and 8, respectively.

Table 7

General Components Composition		
Item	Egg of the present invention	Control Egg *
Water	75.6g	74.7g
Protein	13.2g	12.3g
Lipids	8.6g	11.2g
Ash	0.8g	0.9g
Carbohydrates	1.8g	0.9g
Energy	137kcal	162kcal
Sodium	132mg	130mg
Cholesterol	412mg	470mg
Iodine	0.18mg	(0.02mg)**

*Quoted from "Fourth Edition, Japan Standard Tables of Food Composition (1982)"

** Calculated value

Table 8

Amino Acid Composition (content in grams)					
Item	Egg of the present invention	Control egg*	Item	Egg of the present invention	Control egg*
Arginine	0.86	0.78	Alanine	0.78	0.70
Lysine	0.97	0.89	Glycine	0.49	0.41
Histidine	0.30	0.31	Proline	0.50	0.47
Phenylalanine	0.73	0.64	Glutamic acid	1.78	1.60
Tyrosine	0.50	0.50	Serine	0.96	0.84
Leucine	1.14	1.10	Threonine	0.57	0.57
Isoleucine	0.56	0.68	Aspartic acid	0.99	1.30
Methionine	0.43	0.40	Tryptophan	0.08	0.19
Valine	0.70	0.83	Cystine	0.26	0.32

*Quoted from "Revised Japan Standard Tables of Food Composition (1986)"

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Production Example 5

Production of defatted whole egg dry product (Part 1)

5 **[0050]**

(1) Freezing of whole egg

1,160 kg of whole egg obtained in Production Example 3 was cracked, and thus obtained liquid whole egg was sufficiently agitated and then frozen to obtain 938.9 kg of frozen whole egg.

10 (2) Drying treatment

The frozen whole egg as described in (1) above was dried by microwave to obtain dry whole egg chips . When performing the drying, the temperature of the product was controlled so as to be kept at no higher than 90°C.

(3) Defatting treatment

15 The dry whole egg chips as described in (2) above were refluxed with ethanol heated at 60°C to extract lipids. The extraction time for lipids was set to 1 hour per time and the defatting was performed 2 times. Thereafter, the solvent and lipids were recovered by distillation to obtain the objective defatted extract.

(4) Drying treatment

The defatted extract obtained as described in (3) above was subjected to hot air drying with a hot air fluidized bed type drier to obtain a defatted whole egg dry product.

20 (5) Pulverizing treatment

The defatted whole egg dry product obtained as described in (4) above was pulverized to a size of about 100 meshes by using a hammer mill to obtain 111.3 kg of the objective defatted whole egg dry product powder I-01A(1).

25 (6) Component analysis of the defatted whole egg dry product powder I-01A(1) obtained as described in (5) above was performed. That is, the general components composition and amino acid composition in 100 g of edible portion were examined. The results obtained are shown in Tables 9 and 10, respectively.

Table 9

General Components Composition		
Item	I-01A(1)	Control dried whole egg*
Water	5.4g	3.2g
Protein	86.9g	47.2g
Lipids	0.8g	41.7g
Ash	3.6g	3.8g
Carbohydrates	3.3g	4.1g
Energy	366kcal	611kcal
Sodium	569mg	500mg
Substantial amount of salt	1.4g	1.3g

*Quoted from "Fourth Edition, Japan Standard Tables of Food Composition (1982)"

Table 10

Amino Acid Composition (content in milligrams)			
Item	I-01A(1)	Item	I-01A(1)
Arginine	4774	Alanine	4626
Lysine	4955	Glycine	2832
Histidine	1808	Proline	2903
Phenylalanine	4086	Glutamic acid	11172
Tyrosine	2895	Serine	6422
Leucine	6762	Threonine	3797
Isoleucine	3585	Aspartic acid	8416
Methionine	2499	Tryptophan	51
Valine	4410	Cystine	1549

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Production Example 6

Production of defatted whole egg dry product (Part 2)

5 **[0051]**

(1) Freezing of whole egg

1,090 kg of whole egg obtained in Production Example 4 was cracked, and thus obtained liquid whole egg was sufficiently agitated and then frozen to obtain 890 kg of frozen whole egg.

10 (2) Drying treatment

The frozen whole egg as described in (1) above was dried by microwave to obtain dry whole egg chips . When performing the drying, the temperature of the product was controlled so as to be kept at no higher than 90°C.

(3) Defatting treatment

15 The dry whole egg chips as described in (2) above were refluxed with ethanol heated at 60°C to extract lipids. The extraction time for lipids was set to 1 hour per time and the defatting was performed 2 times. Thereafter, the solvent and lipids were recovered by distillation to obtain the objective defatted extract.

(4) Drying treatment

The defatted extract obtained as described in (3) above was subjected to hot air drying with a hot air fluidized bed type drier to obtain a defatted whole egg dry product.

20 (5) Pulverizing treatment

The defatted whole egg dry product obtained as described in (4) above was pulverized to a size of about 100 meshes by using a hammer mill to obtain 104.38 kg of the objective defatted whole egg dry product powder I-01A(2).

25 (6) Component analysis of the defatted whole egg dry product powder I-01A(2) obtained as described in (5) above was performed. That is, the general components composition and amino acid composition in 100 g of edible portion were examined. The results obtained are shown in Tables 11 and 12, respectively.

Table 11

General Components Composition		
Item	I-01A(2)	Control dried whole egg*
Water	5.3g	3.2g
Protein	82.7g	47.2g
Lipids	3.5g	41.7g
Ash	3.8g	3.8g
Carbohydrates	4.7g	4.1g
Energy	381 kcal	611 kcal
Sodium	685mg	500mg
Substantial amount of salt	1.7g	1.3g

*Quoted from "Fourth Edition, Japan Standard Tables of Food Composition (1982)"

Table 12

Amino Acid Composition (content in milligrams)			
Item	I-01A(2)	Item	I-01A(2)
Arginine	4672	Alanine	4536
Lysine	4813	Glycine	2773
Histidine	1868	Proline	2811
Phenylalanine	3981	Glutamic acid	10860
Tyrosine	2670	Serine	6215
Leucine	6571	Threonine	3699
Isoleucine	3512	Aspartic acid	8218
Methionine	2295	Tryptophan	63
Valine	4280	Cystine	1541

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Example 1

[0052] Using the defatted whole egg dry product powder I-01A(1) produced in Production Example 5 above, the in vivo inhibitory effect on carbon tetrachloride-induced liver injury was examined.

[0053] Carbon tetrachloride was formulated as a 50% olive oil solution and the solution was intraperitoneally administered to rats at a dosage of 1.5 ml/kg body weight to prepare liver injured model rats.

[0054] As the test substance, suspensions of the defatted whole egg dry product powder I-01A(1) in 0.5% carboxymethylcellulose sodium (produced by Kanto Kagaku Kogyo Co., Ltd.) solutions in a concentration of 0.8 mg/ml or 80 mg/ml were prepared. Tests were performed by forcibly administering the suspensions to the laboratory animals (SD rats (Cri:IGS) of 6 weeks old) by an oral route.

[0055] The tests were performed on the laboratory animals divided into groups (8 animals per group) depending on the dosage of the test substance and the number of times of administration. That is, the number of times of administration for two groups on which the dosages were 16 mg/kg and 1,600 mg/kg, respectively, was set to three and separately, the number of times of administration for one group on which the dosage was 1,600 mg/kg was set to 1.

[0056] For the test lot with the number of times of administration being 3, administrations were performed in total three times consisting of two times being 2 days before the administration of carbon tetrachloride and one time being 1 hour after the administration of carbon tetrachloride. For the test lot with the number of times of administration being one, the administration was performed only once 1 hour after the administration of carbon tetrachloride. One group without administration of the test substance was served as a control group.

[0057] After 24 hours from the administration of the test substance, blood was extracted from the abdominal aorta and then centrifuged at 3,000 rpm for 15 minutes to obtain serum. The serum was measured on GOT and GPT by using an Autoanalyzer (produced by Toshiba Medical Systems Co., Ltd.) to examine the inhibitory effect on liver injury. The results obtained are shown in Table 13.

Table 13

Results of Measurement of GOT and GPT			
Lot		GOT(IU/L)	GPT(IU/L)
Control group		2692.6	851.6
I-01A(1) - administered group	16mg/kg x 3 times	1664.0	521.5
	1600mg/kg x 3 times	2100.9	648.6
	1600mg/kg x 1 time	1480.4	523.5

Example 2

[0058] Using a 11:4 (weight ratio) mixture of the defatted whole egg dry product powder I-01A(1) produced in Production Example 5 above and the Angelica keiskei dry product I-01B produced in Production Example 1 above, the in vivo inhibitory effect on carbon tetrachloride-induced liver injury was examined.

[0059] Similarly to Example 1, carbon tetrachloride was formulated as a 10% olive oil solution, and the solution was intraperitoneally administered to mice at a dosage of 5 ml/kg body weight on day 6 of the administration of the test substance to prepare liver injured model mice.

[0060] As the test substance, a composition containing a mixture of the defatted whole egg dry product powder, I-01A(1), and the Angelica keiskei dry product, I-01B, (I-01A(1) : I-01B = 11 : 4 (ratio by weight)) was blended in a powdered basal feed (trade name: CE2, produced by CLEA Japan, Inc.) at a mixing ratio of 0.5% or 5%. The tests were performed by allowing laboratory animals (CDF₁ mice (Cri:CDF₁) of 6 weeks old) to freely take this composition via oral administration. The number of days of administration was set to seven days.

[0061] The tests were performed on the laboratory animals divided into two groups, one administered with the feed with the mixing ratio of the test substance being 0.5% and the other administered with the feed with the mixing ratio of the test substance being 5% (8 animals per group). Also, one group administered with no test substance was used as a control group.

[0062] After one day from the administration of carbon tetrachloride, blood was extracted from the postcava of the mice and then centrifuged at 3,000 rpm for 15 minutes to obtain serum. The serum was measured on GOT and GPT by using an Autoanalyzer (produced by Toshiba Medical Systems Co., Ltd.) to examine the inhibitory effect on liver injury. The results obtained are shown in Table 14.

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Table 14

Results of Measurement of GOT and GPT			
Lot		GOT(IU/L)	GPT(IU/L)
Control group		4931.0	13742.0
I-01A(1):I-01B administered group	0.5%-Feed mixing ratio	4831.0	13521.9
	5%-Feed mixing ratio	2198.9	7015.4

Example 3

[0063] Using a composition I-01A(2) I-01B: I-01C prepared by adding the Theaceae Camellia dry product, I-01C, produced in Production Example 2 to a mixture of the defatted whole egg dry product powder, I-01A(2), produced in Production Example 6 above and the Angelica keiskei dry product, I-01B, produced in Production Example 1 above, and I-01A(2) alone as test substances, the in vivo inhibitory effect on carbon tetrachloride-induced liver injury was examined.

[0064] That is, a mixed feed prepared by blending a composition containing I-01A(2), I-01B, and I-01C in a weight ratio of 65:35:5 or I-01A(2) on its own in a basal feed was orally administered to laboratory animals (Cri:CD(SD)IGS rats, 6 weeks old) by allowing them to freely take it. Note that, as the basal feed was used a powdered feed (trade name: CRF, produced by Oriental Yeast Industry Co., Ltd) each with a mixing ratio of 5%.

[0065] The groups administered with the test substances were two groups described above and one group administered with no test substance was used as a control group. The tests were performed with the number of animals in each group being 8.

[0066] Carbon tetrachloride was formulated as a 50% olive oil solution, and the solution was administered to the dorsal hypodermis of rats at a dosage of 2.5 ml/kg body weight on day 7 of the administration of the test substance to prepare liver injured model rats.

[0067] After 24 hours from the administration of carbon tetrachloride, blood was extracted from the cervical vein and then centrifuged at 3,000 rpm for 15 minutes to obtain serum. The serum was measured on GOT and GPT by using an Autoanalyzer (7150 auto-analyzing apparatus, produced by Hitachi, Ltd.) to examine the inhibitory effect on liver injury. The results obtained are shown in Table 15.

Table 15

Results of Measurement of GOT and GPT			
Lot		GOT(IU/L)	GPT(IU/L)
Control group		295.6	72.5
I-01A(2) -administered group	5%-Feed mixing ratio	203.4	53.9
I-01A(2):I-01B:I-01C -administered group	5%-Feed mixing ratio	146.3	42.2

Example 4

[0068] Tests were carried out using the same composition and in the same manner as in Example 3 except that instead of the composition containing I-01A(2):I-01B:I-01C in a weight ratio of 65:35:5, a composition containing I-01A(1):I-01B:I-01C in a weight ratio of 65:35:5 was used. Results showed antihepatitic activity as seen in Example 3.

Claims

1. A composition having an antihepatitic activity comprising a dry product of defatted whole egg of eggs of a fowl bred by feeding a feed having blended therein an additive comprising a dry product of Angelica keiskei, a dry product of brown algae, and optionally a dry product of Theaceae Camellia.
2. The composition according to claim 1, wherein the fowl is a member selected from the group consisting of hens, ducks and quails
3. The composition according to claim 1 or 2, further comprising a dry product of Angelica keiskei.

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4. The composition according to claim 1 or 2, further comprising a dry product of Angelica keiskei and a dry product of Theaceae Camellia.
5. The composition according to any of claims 1 to 4 for use as pharmaceuticals.
6. Use of a composition according to any of claims 1 to 4 for the preparation of a pharmaceutical composition having an antihepatitic activity.

Patentansprüche

1. Zusammensetzung mit einer antihepatitischen Aktivität, umfassend ein Trockenprodukt von entfettetem Vollei aus Eiern von Geflügel, das gezüchtet wird durch Fütterung eines Futters, das mit einem Zusatz vermischt wurde, umfassend ein Trockenprodukt von Angelica keiskei, ein Trockenprodukt von Braunalgen und gegebenenfalls ein Trockenprodukt von Theaceae Camellia.
2. Zusammensetzung nach Anspruch 1, wobei das Geflügel ausgewählt ist aus Hennen, Enten und Wachteln.
3. Zusammensetzung nach Anspruch 1 oder 2, ferner umfassend ein Trockenprodukt von Angelica keiskei.
4. Zusammensetzung nach Anspruch 1 oder 2, ferner umfassend ein Trockenprodukt von Angelica keiskei und ein Trockenprodukt von Theaceae Camellia.
5. Zusammensetzung nach einem der Ansprüche 1 bis 4 zur Verwendung als Arzneimittel.
6. Verwendung einer Zusammensetzung nach einem der Ansprüche 1 bis 4 zur Herstellung eines Arzneimittels mit einer antihepatitischen Aktivität.

Revendications

1. Composition ayant une activité anti-hépatite comprenant un produit sec d'oeuf entier dégraissé provenant d'oeufs d'une volaille élevée en l'alimentant avec un aliment dans lequel est mélangé un additif comprenant un produit sec d'Angelica keiskei, un produit sec d'algue brune, et éventuellement un produit sec de Theaceae Camellia.
2. Composition selon la revendication 1, dans laquelle la volaille est un membre sélectionné dans le groupe constitué des poules, des canes et des cailles.
3. Composition selon la revendication 1 ou 2, comprenant en outre un produit sec d'Angelica keiskei.
4. Composition selon la revendication 1 ou 2, comprenant en outre un produit sec d'Angelica keiskei et un produit sec de Theaceae Camellia.
5. Composition selon l'une quelconque des revendications 1 à 4 destinée à une utilisation pharmaceutique.
6. Utilisation d'une composition selon l'une quelconque des revendications 1 à 4 pour la préparation d'un médicament ayant une activité anti-hépatite.