

COMPOSITION FOR SUPPRESSING FATTY LIVER AND RENAL HYPERTROPHY DUE
TO DIABETES AND METHOD FOR PRODUCING SAME

TECHNICAL FIELD

The present invention relates to a composition for suppressing fatty liver and renal hypertrophy due to diabetes and a method for producing the composition.

BACKGROUND ART

Earthworms have been used for a long time as an herbal medicine, such as an antipyretic analgesic, in East Asia, and agents for the treatment of diabetes (Patent Document 1), antihyperlipidemics (Patent Document 2), blood pressure regulators (Patent Document 3), and the like using earthworm components as an active ingredient have been proposed in recent years.

[Prior Art Documents]

[Patent Documents]

[Patent Document 1] Japanese Kokoku Patent No. 07-080778

[Patent Document 2] Japanese Kokoku Patent No. 07-080777

[Patent Document 3] Japanese Kokoku Patent No. 07-039349

DISCLOSURE OF THE INVENTION

Problems that the Invention is to Solve

As was mentioned above, earthworms are believed to have various benefits. As a result of research on additional benefits of earthworms, the present inventors finally discovered that earthworm components have a new effect of suppressing the onset of fatty liver and renal hypertrophy triggered by diabetes.

An object of the present invention is to propose a

composition that contains earthworms as an active ingredient, and thereby suppresses the fatty liver and renal hypertrophy likely to be triggered by diabetes, and a method for producing the same.

Means of Solving the Problems

The main point of the present invention is described with reference to the attached drawings.

The present invention according to a first aspect relates to a composition for suppressing fatty liver and renal hypertrophy due to diabetes characterized by containing earthworms as an active ingredient.

The present invention according to a second aspect is the composition for suppressing fatty liver and renal hypertrophy due to diabetes according to the first aspect, wherein the composition for suppressing fatty liver and renal hypertrophy due to diabetes is characterized in that the earthworms are fermented earthworms fermented by fermentation broth obtained by inoculating fruit and vegetable extract with yeast.

The present invention according to a third aspect is the composition for suppressing fatty liver and renal hypertrophy due to diabetes according to the first aspect, wherein the composition for suppressing fatty liver and renal hypertrophy due to diabetes is characterized in that the earthworms are brandling worms.

The present invention according to a fourth aspect is the composition for suppressing fatty liver and renal hypertrophy due to diabetes according to the second aspect, wherein the composition for suppressing fatty liver and renal hypertrophy due

to diabetes is characterized in that the earthworms are brandling worms.

The present invention according to a fifth aspect is the composition for suppressing fatty liver and renal hypertrophy due to diabetes of any of the first through fourth aspects, wherein the composition for suppressing fatty liver and renal hypertrophy due to diabetes is characterized by containing at least one of Panax ginseng, ants, and ginkgo leaf.

The present invention according to a sixth aspect is the composition for suppressing fatty liver and renal hypertrophy due to diabetes of any of the first through fourth aspects, wherein the composition for suppressing fatty liver and renal hypertrophy due to diabetes is characterized by containing Panax ginseng, ants, and ginkgo leaf.

The present invention according to a seventh aspect is the composition for suppressing fatty liver and renal hypertrophy due to diabetes according to the fifth aspect, wherein the composition for suppressing fatty liver and renal hypertrophy due to diabetes is characterized in that the ants are fermented ants.

The present invention according to an eighth aspect is the composition for suppressing fatty liver and renal hypertrophy due to diabetes according to the sixth aspect, wherein the composition for suppressing fatty liver and renal hypertrophy due to diabetes is characterized in that the ants are fermented ants.

The present invention according to a ninth aspect is the composition for suppressing fatty liver and renal hypertrophy due to diabetes according to the fifth aspect, wherein the

composition for suppressing fatty liver and renal hypertrophy due to diabetes is characterized in that the ginkgo leaf is fermented ginkgo leaf extract obtained by fermenting an extract extracted from ginkgo leaf.

The present invention according to a tenth aspect is the composition for suppressing fatty liver and renal hypertrophy due to diabetes according to the sixth aspect, wherein the composition for suppressing fatty liver and renal hypertrophy due to diabetes is characterized in that the ginkgo leaf is fermented ginkgo leaf extract obtained by fermenting an extract extracted from ginkgo leaf.

The present invention according to an eleventh aspect is the composition for suppressing fatty liver and renal hypertrophy due to diabetes according to the seventh aspect, wherein the composition for suppressing fatty liver and renal hypertrophy due to diabetes is characterized in that the ginkgo leaf is fermented ginkgo leaf extract obtained by fermenting an extract extracted from ginkgo leaf.

The present invention according to a twelfth aspect is the composition for suppressing fatty liver and renal hypertrophy due to diabetes according to the eighth aspect, wherein the composition for suppressing fatty liver and renal hypertrophy due to diabetes is characterized in that the ginkgo leaf is fermented ginkgo leaf extract obtained by fermenting an extract extracted from ginkgo leaf.

The present invention according to a thirteenth aspect relates to a method for producing a composition for suppressing

fatty liver and renal hypertrophy due to diabetes, wherein the method is characterized in that earthworms are mixed with a fermentation broth obtained by inoculating fruit and vegetable extract and the earthworms are fermented to yield fermented earthworms; Panax ginseng is mixed with these fermented earthworms and fermentation and drying are performed to yield a fermented mixture; and ants and ginkgo leaf are mixed with the fermented mixture to yield a composition for suppressing fatty liver and renal hypertrophy due to diabetes.

Effects of the Invention

By having the constitution described above, the present invention makes it possible to suppress the onset of fatty liver and renal hypertrophy highly likely to be triggered by diabetes by ingesting the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph showing the results obtained by measuring the organ weights of the liver, kidney, and heart and the weight of the perirenal fat and epididymal fat in Study 1 of this example;

FIG. 2 is a graph showing the liver weight per 100 g body weight in Study 1 of this example;

FIG. 3 is an enlarged graph of the kidney weight in Figure 1;

FIG. 4 is a graph showing the proportion of total fat contained in the liver in Study 1 of this example;

FIG. 5 is a graph showing the weight of the total cholesterol contained in the liver in Study 1 of this example;

Figure 6 is a graph showing the results obtained by measuring the urinary albumin in Study 1 of this example;

Figure 7 is a graph showing the results obtained by measuring plasminogen activator inhibitor 1 (PAI-1) in Study 1 of this example;

Figure 8 is a graph showing the results obtained by measuring the euglobulin clot lysis time (ECLT) in Study 1 in this example;

Figure 9 is a graph showing the changes in the blood glucose level in Study 1 in this example;

Figure 10 is a graph showing the results obtained by measuring glycohemoglobin (HbA1c) in Study 1 in this example;

Figure 11 is a graph showing the results obtained by measuring insulin in Study 1 in this example;

Figure 12 is a graph showing the results obtained by measuring amylase in Study 1 in this example;

Figure 13 is a graph showing the results obtained by measuring uric acid in Study 1 in this example;

Figure 14 is a graph showing the results obtained by measuring glutamic oxaloacetic transferase (GOT) in Study 1 in this example;

Figure 15 is a graph showing the results obtained by measuring alkaline phosphatase (ALP) in Study 1 in this example;

Figure 16 is a graph showing the results obtained by measuring lactate dehydrogenase (LD) in Study 1 in this example;

Figure 17 is a histological image showing the results of histological examination of a kidney slice in Study 1 of this

example;

Figure 18 is a graph showing the quantified occupancy rate of mesangial matrix in the glomerulus based on the histological image of Figure 17;

Figure 19 is a histological image showing the results of histological examination of the aortic arch in Study 1 in this example;

Figure 20 is a graph quantifying the vascular wall thickness of the aortic arch based on the histological image of Figure 19;

Figure 21 is a graph showing the changes in body weight in Study 2 of this example;

Figure 22 is a graph showing the results obtained by measuring glycohemoglobin (HbA1c) in Study 2 in this example;

Figure 23 is a graph showing the results obtained by measuring insulin in Study 2 in this example;

Figure 24 is a graph showing the results obtained by measuring adiponectin in Study 2 in this example;

Figure 25 is a graph showing the results obtained by measuring glutamic oxaloacetic transferase (GOT) in Study 2 of this example;

Figure 26 is a graph showing the results obtained by measuring alkaline phosphatase (ALP) in Study 2 in this example;

Figure 27 is a graph showing the results obtained by measuring leucine aminopeptidase (LAP) in Study 2 in this example;

Figure 28 is a graph showing the results obtained by measuring the blood total cholesterol in Study 2 in this example;

Figure 29 is a graph showing the results obtained by measuring the blood urea nitrogen in Study 2 in this example;

Figure 30 is a graph showing the results obtained by measuring the creatinine clearance (Ccr) in Study 2 in this example;

Figure 31 is a graph showing the results obtained by measuring the urinary albumin in Study 2 in this example;

Figure 32 is a graph showing the results obtained by measuring the liver weight in Study 2 in this example;

Figure 33 is a graph showing the proportion of total fat contained in the liver in Study 2 in this example;

Figure 34 is a graph showing the weight of triglycerides contained in the liver in Study 2 in this example;

Figure 35 is a graph showing the weight of total cholesterol contained in the liver in Study 2 in this example;

Figure 36 is a graph showing the results obtained by measuring the euglobulin clot lysis time (ECLT) in Study 2 in this example;

Figure 37 is a graph showing the results obtained by measuring plasminogen activator inhibitor 1 (PAI-1) in Study 2 in this example; and

Figure 38 is a graph showing the occupancy rate of mesangial matrix in the glomerulus in Study 2 in this example.

BEST MODE FOR CARRYING OUT THE INVENTION

Embodiments of the present invention considered to be preferred are described briefly to illustrate the effects of the present invention.

The composition for suppressing fatty liver and renal hypertrophy due to diabetes of the present invention, by being taken orally, for example, improves lipid metabolic function lowered by diabetes or suppresses lowering of lipid metabolic function by diabetes.

It also improves microangiopathy caused by diabetes or suppresses the onset of microangiopathy due to diabetes (according to confirmation test results using Zucker diabetic fatty rats (ZDF rats) which are type II diabetes model rats).

The onset or advance of fatty liver and renal hypertrophy due to diabetes is thereby suppressed.

[Examples]

Examples of the present invention are described below with reference to the drawings.

This example relates to a composition having earthworms as an active ingredient that suppresses the onset of the fatty liver and renal hypertrophy that tend to develop as complications when one develops type II diabetes. Specifically, the composition comprises earthworms, fermentation broth, Panax ginseng (other name: notoginseng, scientific name: Panax notoginseng Berk), ants, and ginkgo leaf.

More specifically, the composition for suppressing fatty liver and renal hypertrophy due to diabetes of this example (referred to hereinafter as this composition) is obtained by obtaining fermented earthworms by fermenting earthworms by mixing earthworms with fermentation broth obtained by inoculating fruit and vegetable extract with yeast, obtaining a fermented mixture

by mixing Panax ginseng with these fermented earthworms, fermenting, and drying, and mixing ants and ginkgo leaf with this fermented mixture.

This composition is described in detail below.

The earthworms used in this example are brandling worms (scientific name: *Eisenia fetida*).

The fermentation broth is one obtained by inoculating a fruit and vegetable extract extracted from fruits and vegetables such as fruits and vegetables with yeast fungus (in this example, several types of yeast fungus such as opportunistic pathogens and *Candida albicans*) and fermenting. Specifically, 5 kg of bean sprouts, 1.8 kg of cabbage, 0.5 kg of Chinese cabbage, 0.5 kg of spinach, 0.5 kg of Japanese mustard spinach, 0.6 kg of lotus root, 0.5 kg of parsley, 10 kg of potatoes, 2 kg of cucumber, 0.6 kg of burdock, 45 kg of papaya, 15 kg of pineapple, 2 kg of banana, 5 kg of apple, and 1 kg of lemon were cut to a suitable size, and 81 kg of anhydrous glucose, 9 kg of hydrous glucose, and 0.2 L of yeast were added thereto, stirred and mixed, and fermented for approximately one week at room temperature (21°C). This fermented fermentation product was squeezed by a press, and the fermentation broth squeezed out was filtered (120 mesh).

Furthermore, the fruits and vegetables for obtaining the fruit and vegetable extract are not limited to the above, and fruits and vegetables other than the above can also be used.

In addition, the Panax ginseng is Panax ginseng that has been processed into a powder.

In addition, the ants are two types of ants: black ants

(scientific name: *Polyrhachis viva* Roger) and Japanese wood ants (scientific name: *Formica japonica*); specifically, these ants are fermented ants.

More specifically, these fermented ants are obtained by mixing papaya powder, powdered black ants (referred to hereinafter as black ant powder), powdered Japanese wood ants (referred to hereinafter as Japanese wood ant powder), and water, inoculating the mixture with yeast (live yeast) and fermenting, and drying and powdering the fermented product.

To explain this in even greater detail, 38.2 kg of papaya powder, 1.82 kg of black ant powder, 7.28 kg of Japanese wood ant powder, and 44 L of water (40°C) are mixed in a work room set at a room temperature of 28°C, inoculated with 7.7 g of yeast (live yeast), and fermented by standing for 24 hours. The fermented product is dried for 72 hours at 25°C, additionally dried to a water content of 10% or less at 21°C, and this product dried to a water content of 10% or less is coarsely ground by a 2 mm mesh, then ground by a 0.2 mm mesh, and powdered.

Furthermore, the method for producing the abovementioned papaya powder is to slice or mince papaya, extract the papaya extract by adding sugars thereto, add yeast fungus to this papaya extract, induce fermentation and growth of this yeast fungus and wild yeast fungus originally adhering to the papaya, separate the liquid fraction from the coculture of the abovementioned yeast fungus, wild yeast fungus, and lactic acid bacteria obtained by adding lactic acid bacteria thereto, further ferment and ripen by adding more yeast fungus and lactic acid bacteria to this liquid

fraction, and dry the product (refer to Kondo et al. Patent No. 3370302).

In addition, the ginkgo leaf is a ginkgo leaf extract extracted from ginkgo leaf, specifically, fermented ginkgo leaf extract obtained by fermenting this ginkgo leaf extract.

More specifically, this fermented ginkgo leaf extract is obtained by boiling ginkgo leaves and extracting an extract, heating and concentrating this extracted extract, adding anhydrous glucose and fermented calcium, then inoculating with yeast and fermenting, and drying and powdering the fermented product.

To explain this in even greater detail, 50 kg of ginkgo leaf and 500 L of water are placed in a kneader, heated and boiled for one hour and the extract extracted; this extract is filtered through a #350 and #500 mesh, and this filtered extracted extract is heated, concentrated to 60 L, and 17.5 kg of anhydrous glucose is admixed while the liquid temperature of this concentrated extract is high; the liquid temperature is then brought to 30°C and 5 kg of fermented calcium is added, followed by inoculation by yeast, and aerobic fermentation for 24 hours in a work room set at a room temperature of 28°C; after fermentation, the product is kept at a room temperature of 23-24°C and dried; 5 kg of brown rice and 22.5 kg of starch (flour powder starch was used in this example) are added and mixed, half the amount each time, over two days; the room temperature is lowered to 20°C the day after the end of the mixing operation, the product is dried to a water content of 5% or less, and the dried product is coarsely

ground by a 2 mm mesh, then ground and powdered by a 0.2 mm mesh.

Furthermore, the abovementioned fermented calcium was obtained by using the fermentation broth described above that had been fermented for two weeks, adding calcium powder (composed of 70 wt% Hokuriku fossil shells, 28 wt% shellfish calcium, 2 wt% fish calcium) in a proportion of 0.3 kg per liter of this fermentation broth, stirring for approximately two weeks, adding kelp extract in a proportion of 5.5 mL per liter of the fermentation broth and water in a proportion of 0.2 L per liter of fermentation broth (a mixture of the kelp extract and water was added to the fermentation broth in this example), raising the room temperature to 25°C the day after adding this kelp extract and water, lowering the room temperature again to 21°C the next day and drying to a water content of 8% or less, coarsely grinding the dried product using a 2 mm mesh, then grinding and powdering using a 0.2 mm mesh.

Next, the method for producing this composition will be described in detail. The following description is for when one kilogram of brandling worms (100 g after drying, mixing ratio 15 wt%) is used.

First, the brandling worms are cleaned and washed, then ground into granules of 2 mm or less.

Specifically, the cleaned, washed brandling worms and water (3.5 L of water is added per kilogram of brandling worm weight) are placed in a mixer, and ground by mixer for 40-80 minutes to obtain "brandling worm paste." Furthermore, since there is a risk that the active ingredient brandling worms will denature and

break down if the liquid temperature rises and the elevated liquid temperature exceeds 30°C during grinding, the temperature of the water added should be 15°C or less so that the temperature does not exceed 30°C during grinding.

Next, 215 mL of fermentation broth (mixing ratio 20 wt%) and 125 mL of lactic acid bacteria are mixed with this ground brandling worm paste and allowed to stand for 17-21 hours at 25°C, and fermented brandling worms are obtained by fermenting. Furthermore, K-1 bacteria or *Leuconostoc mesenteroides*, which have a good bacteriostatic effect, were used in this example, but the type of lactic acid bacteria used is not particularly restricted as long as it suppresses the growth of *Escherichia coli* and coliform bacteria and is capable of killing these in the process of fermentation and drying.

Next, 180 g of Panax ginseng powder (mixing ratio: 27 wt%) is mixed with these fermented brandling worms, and dried until moisture disappears while fermenting this mixed first mixture.

Specifically, the first mixture is fermented and dried for 48-72 hours while ventilating and maintaining a room temperature of 23°C (*E. coli* and coliform bacteria are killed by the action of the lactic acid bacteria admixed during preparation of the fermented earthworms in this fermentation/drying step). After this fermentation/drying has been completed, this first mixture is dried to a water content of 5% or less by ventilating and drying for approximately two weeks while maintaining a room temperature of 20-28°C and humidity of 18% or less.

Next, 20 g of powdered, fermented ants (mixing ratio 3 wt%)

and 233 g of fermented ginkgo leaf extract (mixing ratio 35 wt%) are mixed with this dried first mixture, and a second mixture is obtained.

Finally, this second mixture is coarsely ground by a 2 mm mesh, then ground by 0.2 mm mesh and powdered, and this powder is housed in capsules and the operation completed. Furthermore, the final form is not limited to capsule form; it may be as is as a powder, or may be made into tablets.

In addition, each of the components (materials) were mixed in the above-stated mixing ratios in this example, but the ratios can be varied as appropriate as long as they are within the ranges of 12-18 wt% of fermented brandling worms, 22-32 wt% of Panax ginseng powder, 2-4 wt% of fermented ants, 28-42 wt% of fermented ginkgo leaf extract, and 16-24 wt% of fermentation broth.

The effects of this composition will be described next.

In this example, effect confirmation tests were conducted twice in Study 1 and Study 2. Study 1 simply confirmed the effects of this composition. Study 2 confirmed the effects of the earthworm (brandling worm) component and other components that could not be confirmed in Study 1 and the effects on early symptoms. Study 1 will be described in detail first.

<<Study 1>>

This study using Zucker diabetic fatty (ZDF) rats, which are type II diabetes model rats, compared differences in the condition of ZDF rats given normal feed not containing this composition discussed above and ZDF rats given feed containing

this composition, and confirmed the effects of this composition.

Specifically, 20 seven-week-old male ZDF rats were used divided into two groups of 10 each so that the average body weight and blood glucose level would be basically the same. One group was designated as a control group (abbreviated hereinafter as group C) given normal feed (control feed) not containing this composition; the other was designated as an *Eisenia fetida* group (abbreviated hereinafter as group EF) given feed containing this composition.

The normal feed given to group C was a feed that conformed to AIN-93G, adjusted to use casein as a protein source. For the feed given to group EF, 5% (weight) of the cornstarch contained in the normal feed given to group C was substituted by this composition.

In addition, the feeding method involved giving the respective feeds to the respective groups by pair feeding for 12 weeks (from 7 to 19 weeks of age). Furthermore, water was provided ad libitum, and the animals were fasted for 18 hours prior to measurement to measure the fasting blood glucose level once a week.

The blood glucose level, glycohemoglobin (abbreviated hereinafter as HbA1c), insulin, organ weights (kidney, liver, heart, perirenal fat, epididymal fat), euglobulin clot lysis time (abbreviated hereinafter as ECLT), and blood components of each of the ZDF rats of groups C and EF reared under the above conditions were measured, and kidney slices and the aortic arch were examined histologically. Group C and group EF were compared,

and the effects were confirmed.

Figures 1-3 are graphs showing the results obtained by measuring the organ weights of the kidney, liver, and heart and the weight of the perirenal fat and epididymal fat.

In measurement of the weights of these organs, as shown in Figure 1, there were significant differences relating to the kidney and liver (t-test, $p < 0.01$) (there were no significant differences between group C and group EF relating to the heart, perirenal fat, and epididymal fat).

More particularly, as is evident from the results obtained by measuring the liver weight shown in Figure 2 and the results obtained by measuring the kidney weight shown in Figure 3, there were significant differences (t-test, $p < 0.01$) in each between group C and group EF, and the results showed that both the liver weight and kidney weight were lower in group EF than in group C.

Figure 4 is a graph showing the weight of total lipid contained in the liver. Figure 5 is a graph showing the weight of total cholesterol (T-cho) contained in the liver.

In this measurement of the weight of the total lipid and total cholesterol contained in the liver, there were significant differences (t-test, $p < 0.01$) in both the total lipid and total cholesterol between group C and group EF, and the results showed that the total lipid and total cholesterol values were both lower in group EF than in group C. Furthermore, as a reference, the total lipid in the liver is approximately 4% and the total cholesterol approximately 0.4% in normal rats (healthy rats) relative to the total weight of the liver.

When the ratio of total lipid and total cholesterol to liver weight was calculated in group C and group EF based on the results obtained by measuring the liver weight shown in Figure 2, the results obtained by measuring the total lipid weight shown in Figure 4, and the results obtained by measuring the total cholesterol weight shown in Figure 5, the ratios of both the total lipid and total cholesterol to the liver weight were higher in group C than in normal rats, but the ratios of the total lipid and total cholesterol to the liver weight were the same in group EF as in normal rats.

Based on this result, the reason that the liver weight of the ZDF rats in group EF was significantly lower than the liver weight of the ZDF rats in group C was believed to be this difference in the total lipid and total cholesterol in the liver.

Specifically, it is believed that the accumulation of lipid in the liver was suppressed, total lipid and total cholesterol in the liver did not increase, and the liver weight presented basically the same value as in normal rats in the ZDF rats of group EF because lipid metabolism was carried out in a close to normal state as the lipid metabolic function that had been lowered by diabetes was improved by taking this composition or because lipid metabolism was carried out normally by suppressing the lowering of lipid metabolic function due to diabetes by taking this composition.

Figure 6 is a graph showing the results obtained by measuring the urinary albumin.

In measurement of the urinary albumin, there was no

significant difference between group C and group EF from the start of the study (7 weeks of age) up to week 6 (13 weeks of age), but there was a significant difference (t-test, $p < 0.05$) between group C and group EF in week 8 (15 weeks of age) after the start of the study. The results showed the urinary albumin value to be lower in group EF than in group C. The significant difference again disappeared from week 10 (17 weeks of age) after the start of the study. Nonetheless, the urinary albumin value was lower in group EF than in group C from week 4 after the start of the study to the end of the study, and a tendency was seen for albumin excretion to be suppressed.

Generally speaking, diabetic nephropathy often develops as a complication as diabetes progresses. It is known that albumin, which inherently scarcely enters the urine, leaks as this diabetic nephropathy develops, and is effective in the evaluation of early diabetic nephropathy. This leakage of albumin into the urine is believed to be caused by microangiopathy due to diabetes.

Specifically, the urinary albumin level is believed to have been lower in the ZDF rats of group EF than group C due to enhanced fibrinolytic capacity and a beneficial effect on microangiopathy caused by taking this composition.

The reason that the kidney weight of the ZDF rats of group EF that took this composition became lower than the kidney weight of the ZDF rats of group C that did not take this composition is believed to be because symptoms of renal hypertrophy were improved or suppressed since the onset of microangiopathy due to

diabetes was suppressed by enhanced fibrinolytic capacity and renal function did not decline.

Figure 7 is a graph showing the results obtained by measuring plasminogen activator inhibitor 1 (abbreviated hereinafter as PAI-1) in the blood.

In this measurement of PAI-1, there was a significant difference (t-test, $p < 0.05$) between group C and group EF, and the results showed the PAI-1 value to be lower in group EF than in group C.

The amount of PAI-1 released increases as lipid metabolism is reduced by diabetes and fat cells become hypertrophic in association with the accumulation of visceral fat due to this decline in lipid metabolism. The fibrinolytic capacity is known to decrease and thrombus formation to be facilitated when the amount of PAI-1 released increases.

Specifically, these PAI-1 measurement results support that this composition improves lipid metabolism that has been reduced by diabetes and suppresses a decline in fibrinolytic capacity triggered by diabetes.

Figure 8 is a graph showing the results obtained by measuring ECLT. Furthermore, in this ECLT measurement, healthy rats that do not develop diabetes other than the ZDF rats of group C and group EF, specifically SD rats, were used, and the ECLT values of group C and group EF were compared with the ECLT value of these SD rats, taking the ECLT value of SD rats as a benchmark.

ECLT evaluates the fibrinolytic capacity by adding thrombin,

a blood coagulation factor, to a euglobulin fraction, creating a thrombus artificially, and measuring the time until this coagulation dissolves. The longer the ECLT, the greater the decline in fibrinolytic capacity.

In this study, as shown in Figure 8, there was a significant difference (t-test, $p < 0.01$) between group C and group EF, and the results showed that ECLT presented a lower value in group EF that was given this composition than in group C. Additionally, the ECLT value of the ZDF rats of group EF given this composition could be confirmed to be the same as the ECLT value of healthy SD rats.

These results obtained by measuring ECLT were able to confirm the possibility that this composition has the effect of improving the fibrinolytic capacity that has been decreased by diabetes or the effect of suppressing a decrease in fibrinolytic capacity due to diabetes.

Specifically, the onset of thrombosis as a complication poses a problem, generally speaking, when diabetes develops. However, it could be confirmed that this composition possibly actualizes the effect of suppressing the onset of thrombosis due to diabetes.

Figure 9 is a graph showing the changes in the fasting blood glucose level. Figure 10 is a graph showing the results obtained by measuring HbA1c.

Since HbA1c generally tends to be formed when the blood glucose level is high, it increases conspicuously as the blood glucose level rises when diabetes develops.

In this study, neither the blood glucose level nor HbA1c differed significantly between group C and group EF, and both the blood glucose level and HbA1c presented high values in both groups. Furthermore, the HbA1c value of ordinary ZDF rats is approximately 9.4% at 18 weeks of age (refer to Charles River Japan data). In this study, the results showed values slightly lower than this, but there was judged to be no problem with the HbA1c value by confirming that a hyperglycemic state was maintained based on the results obtained by measuring the blood glucose level.

Figure 11 is a graph showing the results obtained by measuring insulin.

ZDF rats secrete excess insulin in the early stages of diabetes, but the secretion capacity is known to disappear around the age of 18 weeks, and the insulin concentration drops.

In this study, as shown in Figure 11, the insulin secretion capacity tended to be higher in group EF that took this composition, but the results found no significant difference between group C and group EF.

It is understood based on these blood glucose level, HbA1c, and insulin measurement results that this composition does not act directly on a mechanism to improve the blood glucose level.

Specifically, this composition does not suppress the onset of fatty liver and renal hypertrophy due to diabetes by improving the symptoms of hyperglycemia, but is believed to act directly on the liver and kidneys and to improve the lipid metabolic function and renal function lowered by diabetes or to suppress a decline

in the lipid metabolic function of the liver and a decline in renal function due to diabetes.

Additionally in this study, 24 parameters, total protein (TP), albumin (Alb), zinc sulfate turbidity test (ZTT), thymol turbidity test (TTT), total bilirubin (T-BIL), glutamic oxaloacetic transferase (GOT), glutamic pyruvic transferase (GPT), alkaline phosphatase (ALP), lactate dehydrogenase (LD), gamma-glutamic acid (γ -GT), leucine aminopeptidase (LAP), cholinesterase (CHE), creatine kinase (CK), acid phosphatase (ACP), amylase (AMY), uric acid (UA), urea nitrogen (BUN), creatinine (Cre), serum iron (Fe), total cholesterol (T-cho), triglycerides (TG), HDL cholesterol (HDL), arteriosclerotic index (AI), and LDL cholesterol (LDL), were measured by blood testing and analyzed.

Two parameters, amylase and uric acid, among these 24 differed significantly (t-test, $p < 0.01$) between group C and group EF.

Amylase is a parameter that tends to be high in diabetes and renal failure. In this study, as shown in Figure 12, group EF presented a significantly low value, and it can be hypothesized based on the result that this composition suppresses or delays the advance of the onset of diabetic nephropathy or improves the symptoms of diabetic nephropathy.

The uric acid value is a parameter that tends to be high in metabolic syndrome and the like. In this study, as shown in Figure 13, group EF presented a significantly low value. This result is believed to show the possibility that this composition

has some type of effect on the uric acid metabolic system.

In addition, as shown in Figures 14-16, three parameters, glutamic oxaloacetic transferase (GOT), alkaline phosphatase (ALP), and lactate dehydrogenase (LD), presented better values in group EF even though there was no significant difference. This result suggests the possibility that this composition suppresses a decline in liver function or the possibility that it improves reduced liver function.

Figure 17 is a histological image showing the results obtained by histological examination of a kidney slice. Figure 18 is a graph obtained by quantifying this histological image.

In histological examination of this kidney slice, as shown in Figure 17, suppression of an increase in mesangial matrix was observed in group EF that took this composition.

In addition, there was a significant difference (t-test, $p < 0.05$) between group C and group EF as a result of comparing the quantified histological images examined here. The proportion of glomerular area occupied by mesangial matrix was lower in group EF than in group C, that is, the results showed an increase in mesangial matrix to be suppressed.

This mesangial matrix plays the role of supporting the glomerulus in the normal kidney. In diabetes, however, the mesangial matrix is known to become hypertrophic for reasons such as hyperglycemia and to press on the capillaries of the glomerulus in the surrounding area. Exactly this tendency was seen in group C that did not take this composition, but this increase in mesangial matrix was suppressed and no symptoms of

pressure on the glomerular capillaries could be seen in group EF that took this composition.

Capillaries pressed upon by the mesangial matrix also undergo narrowing of the lumen (blood flow path), leading to poor blood flow. However, since inflowing blood is filtered in the glomerulus, poor blood flow lowers the filtration function. There are also holes in the walls of the capillaries through which blood is filtered that play the role of a filter. However, thickening of the walls makes the pores coarse, allowing large amounts of protein to leak, and resulting in proteinuria.

In this study, albuminuria was suppressed in group EF because hypertrophy of the mesangial matrix was suppressed.

Figure 19 is a histological image showing the results of histological examination of the aortic arch. Figure 20 is a graph obtained by quantifying the arterial arch vascular wall thickness based on this histological image.

In this histological examination of the aortic arch, as shown in Figure 19, a significant tendency to suppression of hypertrophy of the vascular walls of the aortic arch could be observed in group EF that took this composition.

In addition, the vascular walls were thinner in group EF than in group C, and even though there was no significant difference, hypertrophy could be confirmed to be suppressed by a numerically significant trend as a result of comparing the quantified histological images examined here.

Study 2 will be described in detail next.

<<Study 2>>

The purpose of this study is to clarify the effects of the earthworm (brandling worm) component and the effects of components other than the earthworm (brandling worm) component in this composition as well as to confirm the effects on early symptoms by setting a short study period.

Specifically, ZDF rats were used as in Study 1, and differences in the conditions of ZDF rats given normal feed not containing this composition, ZDF rats given feed containing this composition, and ZDF rats given feed containing an earthworm-free composition in which earthworms (brandling worms) were not added in this composition were compared. The effects of the earthworm component and components other than the earthworm component in this composition were clarified. Furthermore, the earthworm-free composition simply had the earthworm (brandling worm) component removed from this composition, and the mixture ratios of the other components were the same as in this composition.

Specifically, seven-week-old male ZDF rats were used in the same way as in Study 1. They were assigned to group C given normal feed (control feed) not containing this composition, group EF given feed containing this composition, and a non-*Eisenia fetida* group (abbreviated hereinafter as group NE) given feed containing the earthworm-free composition so that the body weight and blood glucose levels would be basically the same in each group.

The feed given to group C and group EF was the same as in Study 1. The feed given to group NE was feed in which the earthworm-free composition was substituted for 4.25% (weight) of

the cornstarch contained in the normal feed given to group C.

The feeding method was the same in all regards as in Study 1 except that the study period was set at 10 weeks (from 7 to 17 weeks of age).

The changes in body weight, HbA1c, insulin, adiponectin, liver function markers (in the blood), blood total cholesterol, liver lipids, ECLT, PAI-1, and mesangial matrix ratio were measured in each ZDF rat of group C, group EF, and group NE raised under the above conditions. Group C, group EF, and group NE were compared, and the effects on early symptoms were confirmed together with clarifying the effects of the earthworm (brandling worm) component and the effects of components other than earthworms in this composition.

Figure 21 is a graph showing the changes in body weight. As shown in Figure 21, there was virtually no weight gain from week 5 (12 weeks of age) in group C, and significant differences (LSD test, $p < 0.01$) from group EF and group NE were found from week 6 (13 weeks of age) onward, resulting in a significant difference in a decline in growth (no significant difference between group EF and group NE).

Figure 22 is a graph showing the results obtained by measuring HbA1c. Although there was no significant difference between group C and group EF in Study 1, significant differences (LSD test, $p < 0.05$) were found between group C and group EF and between group C and group NE in this study, resulting from significant suppression of the HbA1c value in group EF and group NE (no significant difference between group EF and group NE).

This is thought to be because this study looked at the HbA1c value in the early stages of diabetes.

In addition, since the HbA1c value was suppressed even in group NE that did not include an earthworm (brandling worm) component, it was confirmed that a component other than the earthworm (brandling worm) component has the effect of suppressing the HbA1c value.

Figure 23 is a graph showing the results obtained by measuring insulin. As in Study 1, there was no significant difference between group C and group EF, and similarly no significant differences between group C and group NE and between group EF and group NE.

Figure 24 is a graph showing the results obtained by measuring adiponectin (new parameter not measured in Study 1). As shown in Figure 24, there were significant differences between group C and groups EF and NE (LSD test, $p < 0.05$), and the results showed significantly high adiponectin values in group EF and group NE (no significant difference between group EF and group NE).

This adiponectin is a beneficial adipokine known to decrease in diabetes. It is related to insulin resistance and the like and has a blood glucose-lowering effect. A decrease in adiponectin is also said to trigger arteriosclerosis.

Since the adiponectin value was high even in group NE that did not include the earthworm (brandling worm) component, this confirmed that a component other than the earthworm (brandling worm) component has the effect of suppressing decreases in

adiponectin. Furthermore, the effect of suppressing the HbA1c value described above is believed to be the result of an improvement in insulin resistance brought about by this suppression of a decrease in adiponectin.

Figures 25-27 are graphs showing the results obtained by measuring liver function markers (in the blood). Specifically, Figure 25 is a graph showing the results obtained by measuring glutamic oxaloacetic transferase (GOT), Figure 26 those on alkaline phosphatase (ALP), Figure 27 those on leucine aminopeptidase (LAP), and Figure 28 those on the blood total cholesterol.

One of these liver function markers, leucine aminopeptidase, is a parameter known to rise in liver disease and known to rise as well in fatty liver. The blood total cholesterol is a parameter known to rise due to the advance of diabetes.

There were no significant differences in any of these parameters (significant trends were seen in glutamic oxaloacetic transferase and alkaline phosphatase) in Study 1. In this study, however, as shown in Figures 25-28, there were significant differences (LSD test, $p < 0.05$) between group C and groups EF and NE.

Specifically, the elevation of the liver function markers glutamic oxaloacetic transferase, alkaline phosphatase, and leucine aminopeptidase was significantly suppressed in this study, and the elevation of the blood total cholesterol was significantly suppressed, suggesting suppression of lowering of liver function and improved lipid metabolism in group EF and

group NE (no significant difference between group EF and group NE).

In addition, since each parameter differed significantly from group C even in group NE that did not include the earthworm (brandling worm) component, this confirmed that a component other than the earthworm (brandling worm) component has the effect of suppressing a decline in liver function and the effect of improving lipid metabolism.

Figure 29 and 30 are graphs showing the results obtained by measuring kidney function markers. Specifically, Figure 29 is a graph showing the results obtained by measuring the blood urea nitrogen (BUN), and Figure 30 those on the creatinine clearance (Ccr).

These kidney function markers, blood urea nitrogen and creatinine clearance, are both parameters used as indicators of kidney function in diabetes. The blood urea nitrogen is known to increase as the filtration function of the kidneys declines. The creatinine clearance represents the filtration capacity of the kidneys and is a parameter known to decrease as diabetic nephropathy advances.

There were no significant differences in the results obtained by measuring the blood urea nitrogen in Study 1. In this study, however, there were significant differences (LSD test, $p < 0.05$) between group C and groups EF and NE. Although there were no significant differences, there were significant trends between group C and groups EF and NE in the creatinine clearance.

Specifically, in this study, elevation of the kidney

function marker blood urea nitrogen was significantly suppressed and a decrease in creatinine clearance tended to be suppressed in group EF and group NE, suggesting a suppressive effect on decreases in kidney function in group EF and group NE (no significant difference between group EF and group NE).

Figure 31 is a graph showing the results obtained by measuring the urinary albumin.

A significant difference was seen eight weeks after the start of the study in Study 1. In this study, however, a significant difference (LSD test, $p < 0.05$) arose between group C and groups EF and NE from week 6 (13 weeks of age) after the start of the study. In the end (ten weeks after the start of the study), significant differences (LSD test, $p < 0.05$) were found between all groups.

Specifically, the amount of leaked urinary albumin was significantly suppressed in group EF in comparison to group C, and the amount of leaked urinary albumin was also significantly suppressed in group NE in comparison to group EF. This confirmed that a component other than the earthworm (brandling worm) component has the effect of suppressing leakage of urinary albumin.

Figures 32-35 are graphs showing the results of measurements relating to liver lipids. Figure 32 is a graph showing the results obtained by measuring the liver weight, Figure 33 the results obtained by measuring the liver total lipid, Figure 34 the results obtained by measuring the weight of triglycerides in the liver, and Figure 35 the weight of total cholesterol in the

liver.

In measurement of the liver weight, there were significant differences (LSD test, $p < 0.05$) between group C and groups EF and NE (no significant difference between group EF and group NE). In measurement of the liver total lipid, there were significant differences between groups C and NE and group EF (LSD test, $p < 0.05$) (no significant difference between group C and group NE).

In measurement of the triglyceride weight in the liver as well, there were significant differences (LSD test, $p < 0.05$) between groups C and NE and group EF (no significant difference between group C and group NE). In measurement of the total cholesterol weight, there were significant differences (LSD test, $p < 0.05$) between each group.

Specifically, the total cholesterol value was significantly lower in group NE than in group C, and the total cholesterol value was also significantly lower in group EF than in group NE.

This made it possible to confirm that the earthworm (brandling worm) component suppresses accumulation of lipids in the liver.

Figure 36 is a graph showing the results obtained by measuring ECLT. Figure 37 is a graph showing the results obtained by measuring PAI-1.

As in Study 1, there was a significant difference (LSD test, $p < 0.05$) between group C and group EF in the ECLT measurement results in this study as well. The ECLT value was lower in group EF than in group C. Furthermore, there were no significant differences between group NE and either group C or group EF.

As for PAI-1, as in Study 1, there was a significant difference (LSD test, $p<0.01$) between group C and group EF, and the PAI-1 value was lower in group EF than in group C. As regards group NE as well, as in group EF, there was a significant difference (LSD test, $p<0.01$) from group C, and the PAI-1 value was lower than in group C (no significant difference between group EF and group NE).

These results of measuring ECLT and PAI-1 made it possible to confirm that the earthworm (brandling worm) component has the effect of significantly suppressing the decrease in fibrinolytic function in diabetes and to confirm that a component other than the earthworm (brandling worm) component has the effect of suppressing a fibrinolysis inhibitor (PAI-1).

Figure 38 is a graph showing the mesangial matrix occupancy rate in the glomerulus.

As in Study 1, there was a significant difference (LSD test, $p<0.05$) between group C and group EF. The proportion of the glomerular area occupied by mesangial matrix was less in group EF than in group C. In other words, the results showed an increase in mesangial matrix to be suppressed.

In addition, in this study, there was a significant difference (LSD test, $p<0.05$) between group C and group NE, and also a significant difference (LSD test, $p<0.05$) between group NE and group EF. In other words, the increase in mesangial matrix was most suppressed in group EF, followed by the suppression of the increase in mesangial matrix in group NE. This made it possible to confirm that the earthworm component contributes

greatly to suppressing changes in the histological structure of the kidneys.

As above, it was possible to confirm based on the results of Study 1 and Study 2 described above that this composition has effects (improvement of lipid metabolism, suppression of the advance of diabetic nephropathy) that suppress complications of diabetes.

In addition, it could be confirmed that the effect of the earthworm (brandling worm) component in this composition is significant in improvement of lipid metabolism in the liver, enhancement of fibrinolytic function, and suppression of changes in the histological structure of the kidneys, and that the effect of components other than the earthworm (brandling worm) component (Panax ginseng, fermented ants, fermented ginkgo leaf extract, fermentation broth) is significant in the improvement of kidney function.

Thus, this composition is an unprecedented innovation that makes it possible to suppress fatty liver and renal hypertrophy due to type II diabetes through the action of each of the components, earthworms (brandling worms), Panax ginseng, fermented ants, fermented ginkgo leaf extract, and fermentation broth.

Moreover, in addition to the effects described above, this composition also demonstrates an anti-inflammatory effect and analgesic effect through the action of the fermented ants and can also be expected to alleviate various diseases arising from diabetes.

Furthermore, the present invention is not limited to this example, and the specific constitution of each of the constituent elements can be designed as is appropriate.

CLAIMS

[Claim 1]

A composition for suppressing fatty liver and renal hypertrophy due to diabetes characterized by containing as active ingredients fermented earthworms fermented using a fermentation broth obtained by inoculating fruit and vegetable extract with yeast, Panax ginseng, fermented fermented ants, and fermented ginkgo leaf extract obtained by fermenting an extract extracted from ginkgo leaf.

[Claim 2]

The composition for suppressing fatty liver and renal hypertrophy due to diabetes according to Claim 1 wherein the composition for suppressing fatty liver and renal hypertrophy due to diabetes is characterized in that the fermented earthworms are brandling worms.

[Claim 3]

A method for producing a composition for suppressing fatty liver and renal hypertrophy due to diabetes, characterized in that a composition for suppressing fatty liver and renal hypertrophy due to diabetes is obtained by mixing earthworms into a fermentation broth obtained by inoculating fruit and vegetable extract with yeast and fermenting the earthworms to obtain fermented earthworms; mixing Panax ginseng with these fermented earthworms and fermenting and drying the resulting mixture to obtain a fermented mixture; and mixing fermented fermented ants and fermented ginkgo leaf extract obtained by fermenting an extract extracted from ginkgo leaf with this fermented mixture.

[Claim 4]

The method for producing a composition for suppressing fatty liver and renal hypertrophy due to diabetes according to Claim 3 wherein the method for producing a composition for suppressing fatty liver and renal hypertrophy due to diabetes is characterized in that the earthworms are brandling worms.