



Memorandum

Date: **SEP 05 2002**

From: Director, Division of Standards and Labeling Regulations, Office of Nutritional Products, Labeling and Dietary Supplements, HFS-820

Subject: 75-Day Premarket Notification of New Dietary Ingredients

To: Dockets Management Branch, HFA-305

New Dietary Ingredient: Monacolin 8000F

Firm: Soft Gel Technologies, Inc.

Date Received by FDA: January 8, 2002

90-Day Date: April 8, 2002

In accordance with the requirements of section 413(a) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification and related correspondence for the aforementioned new dietary ingredient should be placed on public display in docket number 95S-0316 as soon as possible since it is past the 90-day date. Thank you for your assistance.

Felicia B. Satchell
Felicia B. Satchell

Attachments

95S-0316

RPT111

**MAR 22 2002**

Yousry Naguib, Ph.D.
Soft Gel Technologies, Inc.
6982 Bandini Blvd.
Los Angeles, California 90040-3326

Dear Dr. Naguib:

This letter is in response to your notification, dated December 28, 2001, submitted to the Food and Drug Administration (FDA), making a submission for a new dietary ingredient pursuant to 21 U.S.C. 350b(a)(2) (section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act). Your letter notified FDA of your intent to market a product containing a new dietary ingredient named "Monacolin 8000F," which is derived from the fungus "*Monascus pilosus*." You forwarded supplemental information on January 8, 2002, to be included in your notification. Your notification states that you propose to market Monacolin 8000F as a capsule or tablet containing 500 mg of the ingredient to be taken once a day by adults, excluding pregnant and lactating women. FDA received and filed your complete submission on January 8, 2002.

21 U.S.C. 350b(a)(2) requires that a manufacturer or distributor of a dietary supplement that contains a new dietary ingredient submit to FDA, at least 75 days before the dietary ingredient is introduced or delivered for introduction into interstate commerce, information that is the basis on which the manufacturer or distributor has concluded that a dietary supplement containing such new dietary ingredient will reasonably be expected to be safe. FDA reviews this information to determine whether it provides an adequate basis for such a conclusion. Under 21 U.S.C. 350b(a)(2), there must be a history of use or other evidence of safety establishing that the dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe. If this requirement is not met, the dietary supplement is deemed to be adulterated under 21 U.S.C. 342(f)(1)(B) because there is inadequate information to provide reasonable assurance that the new dietary ingredient does not present a significant or unreasonable risk of illness or injury.

FDA has carefully evaluated the information in your submission. Your submission contains evidence of history of use and other information that you assert is an adequate basis to conclude that a dietary supplement product containing Monacolin 8000F will reasonably be expected to be safe. However, the agency has several significant concerns about the evidence on which you rely to support your conclusion.

First, your notification includes a translation of a protocol for preparation of a fermented rice product from a 16th century manuscript, and statements that foods containing fungi of the genus *Monascus* have a "long tradition of use" as ingredients in food in various countries and in various products. However, this information regarding historic use of *Monascus* in China

does not offer direct evidence or data to support the assumption that Monacolin 8000F has been used in, or is recognized as, a dietary ingredient. According to your submission, *Monascus* liquid extract is filtered, purified, concentrated, pasteurized and dried to produce Monacolin 8000F powder. Your submission does not provide a quantitative estimate of the typical exposure to *Monascus* in the human diet that would provide a basis to conclude that the amount of it in the typical diet is a valid basis for determining that the amount provided by recommended consumption of the concentrated Monacolin 8000F powder in a dietary supplement product extract is safe.

Second, your submission states that 1 gram of Monacolin 8000F contains approximately 8 mg of monacolin J, a compound which is structurally analogous to lovastatin. Lovastatin (trade name Mevacor and also called monacolin K) is approved by FDA as a prescription drug for treatment of hypercholesterolemia. According to your submission, "both monacolin J and lovastatin are specific inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis."

The similarity of monacolin J to lovastatin and to other HMG-CoA reductase inhibiting drugs has several implications regarding its potential health risks and the amounts and types of evidence necessary to impart a reasonable assurance of safety. Your submission includes a report of one mutation test in bacteria, one single-dose toxicity study, a summary report of one 14-day study of the effect of the "active constituent" of Monacolin 8000F on serum lipids and enzymes in rabbits, one 14-day study of serum enzymes in rats, and one 21-day human study of the effect on cholesterol levels in 7 healthy males administered Monacolin 8000F. No studies for exposures lasting longer than 21 days in animals or humans are provided.

The studies included in your notification do not adequately address FDA's concerns about the potential health risks of Monacolin 8000F. A wide range of serious adverse effects are known to be caused by lovastatin and other HMG-CoA reductase inhibitors that are structurally and functionally similar to monacolin J. These serious adverse effects have been observed in numerous premarket and postmarket clinical trials and continue to be reported to postmarketing surveillance systems. Among the serious adverse effects listed in the FDA approved labeling for lovastatin (Mevacor) are the following: hepatotoxicity, pancreatitis, rhabdomyolysis, myalgia, myopathy, and hypersensitivity reactions.¹ These serious adverse effects for drugs with similar structure and pharmacological activity to monacolin J are varied, yet the studies you submitted for Monacolin 8000F are not designed to examine a wide range of possible adverse effects. In addition, these serious adverse effects occur at a frequency that may not be detected or identified in small studies such as those included in your notification. There may also be susceptible populations more likely to experience serious adverse effects to HMG-CoA reductase inhibitors. The report of a human study you submitted which enrolled seven healthy adult males may not permit extrapolation of safety to persons with other characteristics. Your submission also does not contain any information regarding the pharmacology of monacolin J. Similar HMG-CoA inhibitors, e.g., lovastatin, are known to be metabolized by the cytochrome P450 system. Exposure to drugs or other dietary supplements that inhibit the P450 cytochrome system may expose persons to higher level of Monacolin 8000F than has been studied by the sponsor or in previous historical use.

¹ Physicians' Desk Reference 2001, Medical Economics Company, Inc, 1969-1971

As several of the severe adverse effects of HMG-CoA inhibitors are dose-dependent, this is a significant concern for persons using Monacolin 8000F. As with other dietary supplements, Monacolin 8000F is likely to be used by consumers for extended periods of time. The studies included in your submission are of short duration and are, therefore, not adequate to assess the safety of monacolin J, particularly since the known serious adverse effects associated with lovastatin and other HMG-CoA reductase inhibiting drugs can occur anytime during chronic therapy. Labeling to limit the duration of use alone would not be sufficient to address these safety concerns.

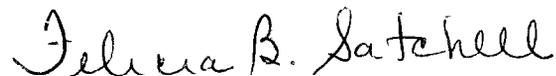
Finally, please be advised that any representation that a product is intended for the diagnosis, cure, mitigation, treatment or prevention of disease in man or animals suggests that it is a drug, as defined in 21 U.S.C. § 321(g)(1)(B), and would be subject to regulation under the drug provisions of the Federal Food, Drug and Cosmetic Act. All drugs must be approved by FDA before they can be marketed in the United States.

For the reasons discussed above, the information in your submission does not provide an adequate basis to conclude that Monacolin 8000F, when used under the conditions recommended or suggested in the labeling of your product, will reasonably be expected to be safe. Therefore, your product may be adulterated under 21 U.S.C. 342(f)(1)(B) as a dietary supplement that contains the new dietary ingredient Monacolin 8000F for which there is inadequate information to provide reasonable assurance that such ingredient does not present a significant or unreasonable risk of illness or injury. Introduction of such products into interstate commerce is prohibited under 21 U.S.C. 331(a) and (v).

Your submission will be kept confidential for 90 days from the date of receipt, and after April 8, 2002, your submission will be placed on public display at Dockets Management Branch (Docket No. 95S-0316). Commercial and confidential information in the notification will not be made available to the public. Prior to April 8, 2002, you may wish to identify in writing specifically what information you believe is proprietary in your current notification for FDA's consideration. Nevertheless, our Center's Freedom of Information Officer has the authority to make the final decision about what information in the notification should be redacted before it is posted at Dockets.

Should you have any questions concerning this matter, please contact me at (301) 436-2371.

Sincerely yours,



Felicia B. Satchell
Director
Division of Standards
and Labeling Regulations
Office of Nutritional Products, Labeling
and Dietary Supplements



JAN 7 2002

Mr. Yousry Naguib, Ph.D.
Soft Gel Technologies, Inc.
6982 Bandini Boulevard
Los Angeles, California 90040-3326

Dear Dr. Naguib:

This is to inform you that the notification dated December 13, 2001 submitted pursuant to 21 U.S.C. 350b(a)(2) was received and filed by the Food and Drug Administration (FDA) on January 8, 2002. Your notification concerns the substance "Monacolin 8000F" that you assert is a new dietary ingredient. You describe Monacolin 8000F in your notification as a fungal derivative of *Monascus pilosus*.

In accordance with 21 C.F.R § 190.6(c), FDA must acknowledge its receipt of a notification for a new dietary ingredient. For 75 days after the filing date (i.e., after March 24, 2002), you must not introduce or deliver for introduction into interstate commerce any dietary supplement that contains "Monacolin 8000F."

Please note that acceptance of this notification for filing is a procedural matter and thus, does not constitute a finding by FDA that the new dietary ingredient or supplement that contains the new dietary ingredient is safe or is not adulterated under 21 U.S.C. 342. As another procedural matter, your notification will be kept confidential for 90 days after the filing date. After April 18, 2002, the notification will be placed on public display at FDA's Docket Management Branch (Dockets) in docket number 95S-0316. However, any trade secret or otherwise confidential commercial information in the notification will not be disclosed to the public.

Prior to March 28, 2002, you are welcome to identify in writing for us any information in your notification that you believe is proprietary or confidential. Our Freedom of Information

Page 2 – Dr. Yousry Naguib

Office will consider this when making the final decision about what information should be redacted from the notification before it is sent to Dockets.

Please contact us at (301) 436-1443, if you have any questions concerning this matter.

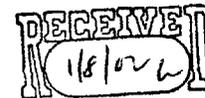
Sincerely yours,

A handwritten signature in black ink, appearing to read "Gary Coody". The signature is fluid and cursive, with a large initial "G" and a long, sweeping underline.

Gary Coody, R.Ph.
Acting Team Leader
Dietary Supplements Team
Division of Standards
and Labeling Regulations
Office of Nutritional Products, Labeling
and Dietary Supplements
Center for Food Safety
and Applied Nutrition

SOFT GEL

TECHNOLOGIES, INC.™



January 8, 2002

Gary Coody, R.Ph.
Acting Team Leader for Dietary Supplements
Office of Nutritional Products, Labeling and Dietary Supplements
Center for Food Safety and Applied Nutrition
U. S. Food and Drug Administration

Dear Dr. Coody:

Pursuant to your request for additional information regarding our submission for Monacolin 8000F as a new dietary supplement ingredient:

- The authors of the Latin binomial name "*Monascus pilosus*" are Hawksworth DL and Pitt JI. This information is given in a paper entitled: "A new taxonomy for *Monascus* species based on cultural and microscopical characters"; published in Aust. J. Bot. Volume 31, pages 51-61, year 1983
- Monacolin 800F is a dietary ingredient for use in dietary supplements. No claims will be made on this product, and will be labeled for adults only. Monacolin 8000F is not intended for use by pregnant or lactating women or children and the product will be labeled accordingly.

Please let me know should you need further information.

Sincerely,

Yousry Naguib

Yousry Naguib, Ph.D.

Soft Gel Technologies, Inc.

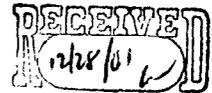
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78756

SOFT GEL

TECHNOLOGIES, INC.™



December 13, 2001

Office of Nutritional Products (HFS-820)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, SW
Washington, DC 20204

Pursuant to Section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 350b(a)(2)) dated December 30, 1998, concerning the marketing of a new dietary ingredient, Soft Gel Technologies, Inc. (SGTI) located at 6982 Bandini Boulevard, Los Angeles, CA 90040 wishes to notify the Food and Drug Administration that SGTI will market a new dietary ingredient, Monacolin 8000F, a fungal derived from Monascus. Accordingly, enclosed please find two copies of this notification.

The dietary supplement which contains Monacolin 8000F, will consist of 500 mg of Monacolin 8000F in a capsule or tablet which will be suggested to be taken one time per day and no claims will be made on this product.

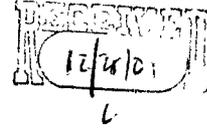
Attached please find the scientific studies, which establish that this dietary ingredient, when used as suggested in the labeling of dietary supplement, is reasonably expected to be safe. This information includes:

- (1) Development, manufacturing, chemistry and analytical methods
- (2) Safety studies
- (3) Clinical Research Study
- (4) Differentiation table verses other products
- (5) Cholesterol lowering effect of monacolin powder 8000F

Sincerely yours,

Yousry Naguib

Yousry Naguib, Ph.D.



MONACOLIN POWDER 8000F

MARUZEN PHARMACEUTICALS CO., LTD.
14703-10 MUKAIHIGASHI ONOMICHI CITY
HIROSHIMA 722-0062 JAPAN
TEL: 81-848-44-2200 FAX: 81-848-44-6851

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(1) Development, manufacturing, chemistry and analytical method

1. Introduction Monascus and Monacolin

MONACOLIN POWDER 8000 F is a powdered preparation used as the ingredient of dietary supplements in Japan.

Monacolin is the fungal derivative from Monascus, a fungus has a long tradition of use as food stuff in China, Japan, Korea, Thailand, India, Phillipine.

Especially in the Southern region of China, Monascus has been used for more than 2,000 years as food stuff and 1,590 was probably the first time when monograph of Li Shih-Chun where he mentions the application of Monascus. The copy of this writing is attached in Exhibit ①.

Further it has been consumed in Germany and the other European countries for many years due to the popularity of Asian cookery.

Now it is used in meat products, poultry, fish, fermented fish pasta, surimi, mirin, Hoi-sin sauce, miso-pasta, fermented tofu (sufu), ketchup, rice-wine (shaoxin), chocolates, brandy, liqueur, rice-wine vinegar, pickles, soya-sauce, soja pasta, ice cream, pasta, snacks, cereals, marmalade, beverages and etc.

Scientific research reports the biological effects, especially the reduction of cholesterol, of HDL-cholesterol and Triglyceride blood values.

In 1980 a Japanese scientist Endo isolated metabolite from Monascus which reduced by rats the artificially induced hyperlipoprotein anemia.

Then the capsules made from Monacolin comes as a dietic product on the Japanese market and nowadays Monacolin has become popular in this field.

Exhibit ① :

**"Monascus" from *General Explanation of Compendium of
Materia Medica* (Translated)**

Interpretation (Mr. Li Shih Chen put it)

The way of making Monascus began in modern times. About 270 liters of non glutinous rice are washed and soaked in water for one night to make into steamed rice. 1.5 kg of seed malt are put in the rice at 15 spots, and after they are rubbed by hands until spread uniformly, the mixture is gathered at one spot. It is wrapped firmly with cloth, and when it becomes hot, it is spread with the cloth off. As soon as it is warm, it is gathered to be wrapped with cloth. In the afternoon on the following day, the mixture is separated into 3 heaps, and after a while, into 5 heaps. In a short period of time, these heaps are gathered into one, and after a while it is divided into 15 pieces. When these are warm, they are gathered into one again. This work is repeated several times. On the third day, clean water is put into a big bucket. The malted rice is placed in a bamboo basket, which is swatter for 5 to 6 minutes. After finishing soaking it all, it is gathered at one spot again. This work is done another time. On the fourth day, the above work is done once again, where if there is any of malted rice not floating, the work is repeated again until all the rice floats. When the malted rice is all in a state of floating, this work is all over. Thereafter, the rice is taken out to dry in the sun. When the malted rice becomes fermented to the core, it is referred to as *sheng huang* (literally raw yellow) and it is used for *sake* brewing and pickling fish and meat with salt alike. Brightly deep red, it is good to look at. If the rice is not fermented to its core, it is not good in quality.

Flavor

The malt is featured by sweetness and warmth besides being not toxic. (Mr. Wujui put it). If *sake* is brewed using red malt, it has nature of being dry and hot.

Main effect

It helps digestion and promotes blood circulation alike. And it helps the spleen work as well as strengthens the stomach. Besides curing dysentery and rota virus enteritis, it sharpens one's appetite (Mr. Chenshih). The malt is brewed into *sake*, which furthers blood circulation and helps other medicines have effect. And it is not merely good for dizziness and ear ringing but actually cures a bruise (Mr. Wujui). If a woman drinks *sake* to which ground red malt is added, she undergoes a good effect in menstrual pain and residual bad blood after childbirth (Mr. Li Shih Chen).

Invention (Mr. Li Shih Chen put it)

A human eats water and grain, which in turn enter the stomach. They are digested with gastric juice etc. in the stomach. And it is full of essence, which changes red and spreads into the internal organs and veins. This is a conduct of blood. This workmanship is an extremely subtle conduct of nature. In making red malt, when exposed to moisture and heat, boiled rice turns red, which is a genuine color. The color does not change even in a long period of time, this is because humans make skillful use of nature. For this reason, red malt helps the spleen and the stomach work, thus furthering blood circulation. This is because both seek for the same spirit.

2. Production Flow Chart of MONACOLIN POWDER 8000 F



MONACOLIN POWDER 8000 F

3. Specification of MONACOLIN POWDER 8000 F
Please refer to attached specification and MSDS.

July, 1998

SPECIFICATIONS AND TEST METHOD FOR MONACOLIN POWDER 8000F

1. Essence

This is a powdered preparation of Monascus Extract obtained through the filtration, concentration and sterilization of cultivated *Monascus pilosus* the bacteria of which is separated and removed, and blended with dextrin as a filler.

2. Composition

3. Specifications and Test Method

Item	Specifications	Test Method
Description	Pale red powder, having a slight characteristic odor.	JSFA-GTP* General Provisions
Identification Monacolin J	Positive	HPLC Method
Purity Test (1) Heavy metals (as Pb)	Not more than 20 $\mu\text{g/g}$	JSFA-GTP General Test Method
(2) Arsenic (as As ₂ O ₃)	Not more than 2 $\mu\text{g/g}$	JSFA-GTP General Test Method
Loss on Drying	Not more than 10 %	JSFA-GTP General Test Method
Ignition Residue	Not more than 10 %	JSFA-GTP General Test Method
Monacolin J	7000 ~ 8000 mg/Kg	HPLC Method

*JSFA-GTP - The Japanese Standards of Food Additives - General Test Procedure

MONACOLIN POWDER 8000F

Test method of analysis

DESCRIPTION

Method : Japan Pharmacopoeia - General Test Procedure

The test of color is carried out by placing 1 g of the solid medicine on a sheet of white paper or in a watch glass placed on white paper. The test of odor shall be carried out by placing 1 g of the solid medicine in a beaker.

IDENTIFICATION

Method : According to the method of "Monacolin J" item

PURITY TEST

(1) Heavy metals

Method : Japan Pharmacopoeia - General Test Procedure

Proceed with 1.0 g of sample according to Method 2 and perform the test.

(2) Arsenic

Method : Japan Pharmacopoeia - General Test Procedure

Take 1.0g of sample, prepare the test solution according to Method 3, and perform the test using Apparatus B.

LOSS ON DRYING

Method: Japan Pharmacopoeia - General Test Procedure

Weigh accurately a weighing bottle that has dried for 30 minutes. Take the sample within the range of $\pm 10\%$ of the amount directed, then weigh it accurately. Place the loaded bottle in a drying chamber and dry. When it is dried by heating, the temperature is within the range of $\pm 2^\circ\text{C}$ of that directed, and after drying allow the bottle to cool in a desiccator (silica gel) before weighing.

IGNITION RESIDUE

Method : Japan Pharmacopoeia - General Test Procedure

Previously ignite a crucible of platinum, quartz or porcelain to constant weight between 450°C and 550°C , and weigh accurately after cooling.

Take the sample within the range of $\pm 10\%$ of the amount directed, transfer into the above ignited container, weigh it accurately. Moisten the sample with a few drops of sulfuric acid, then heat slowly at a temperature as low as practicable until the sample is almost incinerated or volatilized and cool

it. Moisten again with little the sample is amount of sulfuric acid, heat gently until white fumes are evolved no longer, and ignite between 450°C and 550°C until the residue is completely incinerated. Cool the crucible and reweigh accurately. Use a desiccator (silica gel) for the cooling procedure.

MONACOLIN J

Method : HPLC

Accurately weigh approximately 0.5 g of sample and add 20 ml of the 50 v/v % ethanol and 10 ml of the 0.5 (mol/l) potassium hydroxide solution. Cool after the 30 minutes of recirculation, add 50 v/v % ethanol to make exactly 50 ml. Transfer 5 ml of this prepared solution and add 50 v/v % ethanol to make exactly 50 ml. Use this as the test solution.

Separately, accurately weigh about 5 mg of the monacolin J (lactone) and dissolve in 50 v/v % ethanol to make exactly 50 ml. Transfer 5 ml of the prepared solution and add 2 ml of the potassium hydroxide solution to make exactly 50 ml. Use this solution as the standard solution.

Analyze the test solution and the standard solution by HPLC under the following condition in order to determine the amounts of monacolin J in the sample.

HPLC Condition

Column : YMCJ sphere ODS - H80 (4.6 I.D. x 150 mm)
Mobile Phase : Acetonitrile / 0.1 % phosphoric acid aqueous solution (45 : 55)
Detector : UV 237 nm
Flow Rate : 1.0 ml / min.
Column temperature : 42° C

Method for calculation of the ingredient

Content of monacolin J (mg / kg) =

$$\frac{M_t}{M_s} \times \frac{A_s}{A_t} \times 10^6$$

M_t : Peak area of monacolin J in the test sample solution

M_s : Peak area of monacolin J in the standard sample solution

A_s : Amount of the standard sample weighed (mg)

A_t : Amount of the test sample weighed (mg)

SAFETY DATA SHEET

EC-Legislation 91/155/EEC

Product name : MONACOLIN POWDER 8000F

MARUZEN PHARMACEUTICALS CO., LTD.
14703-10 Mukaihigashi Onomichi
Hiroshima 722 Japan
TEL 81-848-44-2200
FAX 81-848-44-6851

1. CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

Product Name : MONACOLIN POWDER 8000F
Common Chemical Name : MONACOLIN
Product Code (Supplier) :
INCI Name : NA

2. COMPOSITION / INFORMATION ON INGREDIENTS

Information on hazardous Ingredients

Chemical name:	%compound	EINECS No.	CAS Number	EC Number	Symbol
		_____	_____	_____	_____

3. HAZARD IDENTIFICATION

Environmental Hazards : Nothing known about yet.
Human Health Hazards : Nothing known about yet.

4. FIRST AID MEASURES

Effects and Symptoms :

Ingestion : No adverse effects known.
Inhalation : No adverse effects known.
Skin Contact : No adverse effects known.
Eye Contact : No adverse effects known.

First Aid Measures

Ingestion : Not a direct hazard.
Inhalation : Not a direct hazard. If you feel unwell seek medical advice immediately.
Skin Contact : Not a direct hazard. Flush with lots of water.
Eye Contact : Rinse thoroughly with a lot of water for some minutes. Call a doctor if necessary.

5. FIRE FIGHTING MEASURES

Extinguishing Media

Suitable : Water, dry fire extinguisher, waterspraying-jet, alcohol-resistant foam and carbon dioxide.

Not suitable : NA

Special Firefighting Procedures : NA

Unusual Fire / Explosion Hazards : NA

Hazardous Thermal (de)composition Products : NA.

Protection of Firefighters : According to size of fire.

6. ACCIDENTAL RELEASE MEASURES

Personal Precautions : Do not inhale the dust. Ventilate the room with sufficient fresh air.

Environmental Precautions : Do not empty into drains.

Methods of Cleaning Up : Collect mechanical and dispose according to regulation.

Rince the remaining material with lots of water.

7. HANDLING AND STORAGE

Handling : Do not breath dust

Storage : Keep container tightly closed and dry.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Respiratory System Protection : Dust mask with filter for fine dust.

Skin and Body Protection : If necessary.

Hand Protection : Wear suitable gloves..

Eye Protection : Recommendable.

9. PHYSICAL AND CHEMICAL PROPERTIES

Physical State : powder

Color : dark red.

Odor : slightly characteristic odor

Solubility : water . soluble

10. STABILITY AND REACTIVITY

Conditions and avoid : Not expected if stored and handled properly.

Materials to avoid : NA

Hazardous Decomposition Products : NA

11.TOXICOLOGICAL INFORMATION

ACUTE TOXICITY : LD₅₀=not less than 2,000mg/kg (mouse, o.a.)

Skin Irritation : Not an irritant.

Eye Irritation : Slightly irritant.

Sensitation : Not a sensitizer.

12.ECOLOGICAL INFORMATION

13.DISPOSAL CONSIDERATIONS

Methods of Disposal : Take notice of national special regulations. Suitable incineration plant.

14.TRANSPORT INFORMATION

Comments : No dangerous material according to the above mentioned regulations.

15.REGULATORY INFORMATION

Label Name :MONACOLIN POWDER 8000F

16.OTHER INFORMATION

HISTORY

Date of Issue :JULY 9.1998

SDS prepared by :MARUZEN PHARMACEUTICALS CO.,LTD

Authorisation :T. Yokota

The statements made here are supposed to describe the product with regard to necessary safety precautions.
They do not guarantee special characteristic and are made to the best of our current knowledge.

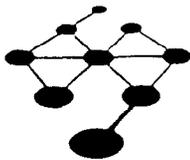
4. Physical Studies of MONACOLIN POWDER 8000 F

MONACOLIN POWDER 8000 F contains 0.8 % of Monacolin J.

Residual amount of Monacolin J after exposed to each test condition measured by HPLC.

Test Item	Result
Chronic stability	Stored at 40 °C for 3 months in a bottle. (This condition is equivalent to the storage for 1.5 years at room temperature.) Residual content of Monacolin J after 3 months was 94.4 %.
Heat stability	0.2 % of MONACOLIN POWDER 8000F aqueous solution was heated at various conditions. 1) 100 °C for 30 minutes at pH 4 Residual content of Monacolin J was 89.17 %. 2) 115 °C for 15 minutes at pH 4 Residual content of Monacolin J was 82.88 %. 3) 100 °C for 30 minutes at pH 7 Residual content of Monacolin J was 99.93 %. 4) 115 °C for 15 minutes at pH 7 Residual content of Monacolin J was 94.45 %.
Light stability	0.2 % of MONACOLIN POWDER 8000F McIlvaine Buffer solution was exposed to UV Auto Fade Meter for 60 minutes. 1) pH 3 : Residual content of Monacolin J was 29.35 %. 2) pH 4 : Residual content of Monacolin J was 47.95 %. 3) pH 5 : Residual content of Monacolin J was 79.61 %. 4) pH 6 : Residual content of Monacolin J was 81.61 %. 5) pH 7 : Residual content of Monacolin J was 82.18 %.

(2) Safety studies



Japan
Food
Research
Laboratories

Japan Food Research Laboratories

AUTHORIZED BY THE JAPANESE GOVERNMENT

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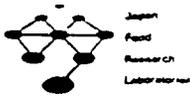
REPORT

No. OS59030612-002

April 14, 1998

Test of MONACOLIN POWDER 8000F for acute oral toxicity in mice

Received: March 13, 1996



I, the undersigned, hereby declare that the work described in this report was performed under my supervision, as a Study Director, and that the report provides a true and accurate record of the results obtained.

This is a translation of the original report, No. OS59030612(April 23, 1996) written in Japanese. The translation was done as faithfully as possible to our knowledge.

Study Director


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Department of Environmental Safety Research
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April 16, 1998
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Test of MONACOLIN POWDER 8000F for acute oral toxicity in mice

1. Abstract

The test sample, MONACOLIN POWDER 8000F, was tested for the acute oral toxicity in mice in accordance with OECD Guidelines for the Testing of Chemicals (1987).

Oral administration of MONACOLIN POWDER 8000F at a dose of 2,000 mg/kg b.w. caused neither abnormalities nor death in any mice.

Consequently, we concluded that the lethal dose of MONACOLIN POWDER 8000F was higher than 2,000 mg/kg b.w. in both male and female mice.

2. Test sample

MONACOLIN POWDER 8000F

Character: dark red powder

3. Test period

From March 21 to April 23, 1996

4. Preparation of test solution

The test sample was suspended in purified water to make a 100 mg/mL test solution.

5. Experimental animals

Male and female mice of the ICR strain, purchased from Japan SLC, Inc., were used. The mice were obtained at an age of four weeks and acclimated to the laboratory conditions for a week. They were housed in plastic cages (five animals per cage) under the standard laboratory conditions (temperature: $23 \pm 2^\circ\text{C}$, light-dark cycle: 12/12 hours) and given F-2 diet [Funabashi Farm Co., Ltd.] and tap water *ad libitum*.

6 Procedures

Ten each of male and female mice were allocated to each group. The mice were not fed for about four hours prior to the administration, and then each was weighed. To the mice of the experimental group, the test sample suspended in purified water was administered orally with a stomach tube at a dose of 2,000 mg/kg. To the control group, purified water alone was administered at a volume of 0.6 mL each in males and 0.5 mL each in females in the same manner as described above. ~

Clinical observations were made frequently on the day of administration and once a day during the following period. The mice were each weighed weekly and the mean body weight values of the experimental and the control groups were statistically analyzed by the t-test ($p=0.05$). At the end of the test period (14 days), all mice were sacrificed for necropsy.

7. Results

1) Deaths of animals and mortalities

No mouse of either males or females died throughout the experimental period.

2) Clinical observations

No abnormalities were observed in either males or females throughout the experimental period

3) Body weight (Tables 1 and 2)

No significant difference in the body weight gain between the experimental and the control groups was detected in either males or females.

4) Necropsy

No remarkable changes were found in any organ of either males or females.

8. Discussion

The acute oral toxicity of MONACOLIN POWDER 8000F in mice was tested in accordance with OECD Guidelines for the Testing of Chemicals (1987).

The guidelines recommend that if compound-related mortality is produced at a dose of 2,000 mg/kg, a full study may need to be considered. In this test, oral administration of 2,000 mg/kg of the test sample caused neither death nor abnormalities at necropsy in any mouse

Consequently, we concluded that the lethal dose of the test sample was higher than 2,000 mg/kg

Table 1. Body-weight changes after oral administration of the test sample (Male)

Group	Body-weight (g)		
	Pre-administration	7 days	14 days
Experimental group	29.4 ± 1.0 (10)	33.7 ± 1.5 (10)	37.8 ± 2.0 (10)
Control group	29.7 ± 0.9 (10)	34.2 ± 1.2 (10)	38.1 ± 1.9 (10)

Values are mean ± SD.

Values in parentheses show the number of animals.

Table 2 Body-weight changes after oral administration of the test sample (Female)

Group	Body-weight (g)		
	Pre-administration	7 days	14 days
Experimental group	23.9 ± 0.7 (10)	26.2 ± 1.6 (10)	29.4 ± 2.0 (10)
Control group	23.6 ± 0.6 (10)	26.2 ± 1.2 (10)	29.6 ± 1.7 (10)

Values are mean ± SD.

Values in parentheses show the number of animals.

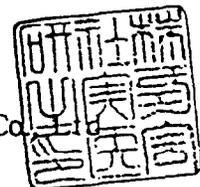
Final Report

Title: Bacterial reverse mutation test of Monacolin Powder 8000F

PROJECT No. H-97642

Date of reporting: March 18, 1998

Nippon Experimental Medical Research Institute Co



3303-58 Ohaza Ohdo, Agatsuma machi, Agatsuma gun,
Gunma, Japan

STATEMENT OF COMPLIANCE

Title: Bacterial reverse mutation test of Monacolin Powder 8000F

PROJECT No. H-97642

I, the undersigned, hereby declare that the work described in this report was performed in compliance with the GLP Standard for Safety Studies on Drugs, Ministry of Health and Welfare (Ordinance No. 21: March 26, 1997).

Further, declare that this is the exact English version of the original report that written in Japanese language and there is no difference in the contents of this report to that of the original (Japanese) report which provides a true and accurate record of the results.



Date: March 18, 1998

Golam Sarwar, Ph.D.

Study Director (translator)

Nippon Experimental Medical Research Institute Co., Ltd.

Summary

The mutagenicity of Monacolin Powder 8000F was examined at the doses of 312.5, 625, 1250, 2500 and 5000 $\mu\text{g}/0.1\text{ml}/\text{plate}$ using the strains of *Salmonella typhimurium* (TA 98, TA 100, TA 1535, and TA 1537) and the strain of *Escherichia coli* (WP2uvrA) in direct and metabolic activation systems.

As a result, the numbers of revertant colonies of the test substance treated plates were almost similar to that of the corresponding negative control plates of each strain. Bactericidal effect was not noted at any one of the tested doses in any strain. Further, the numbers of revertant colonies of negative and positive controls were within the range of background.

Based on the findings of this study condition, Monacolin Powder 8000F was judged as non-mutagen.

Results

The results are shown in Appendices 1 & 2 and Figs. 1 & 2.

A dose selection test was conducted to find out a dose at which the test substance inhibited bacterial growth and the doses at which precipitations occurred. As a result, bacterial growth inhibition and precipitations of the test substance were not noted and this findings were irrespective of metabolic activation system.

Due to such findings, the doses of 312.5, 625, 1250, 2500 and 5000 $\mu\text{g}/0.1\text{ml}/\text{plate}$ were selected and conducted the mutagenicity test in direct and metabolic activation system. As a result, none of the tested doses caused any significant difference in the number of revertant colonies in any tester strain compared to the negative control of each tester strain.

Further, the numbers of revertant colonies in all positive controls were significantly increased compared to their respective negative controls.

Discussion

The mutagenicity of Monacolin Powder 8000F was examined on the basis of bacterial reverse mutation test using the tester strains of TA 98, TA 100, TA 1535, TA 1537 of *Salmonella typhimurium* and the strain of WP2uvrA of *Escherichia coli* in direct and metabolic activation system.

As a result, the numbers of revertant colonies in the tester strains were not increased dose dependently and not became 2-fold compared to corresponding negative control in any systems.

The numbers of revertant colonies in all negative and positive controls were within the range of background data which indicated that the study was conducted appropriately.

Based on the findings of this experimental condition, Monacolin Powder 8000F was judged as non-mutagen.

References

- 1) Maron, D.M., and B.N. Ames: Revised methods for the *Salmonella* mutagenicity test, *Mutation Res.*, 113, 173 - 215, 1983.

Appendix 1. Reverse mutation test of Monacolin Powder 8000F in *S. typhimurium* and *E. coli* (Dose determination test)

With (+) or Without (-) S9 mix	Test substance concentration (μ g/plate)	Number of revertants (number of colonies/plate) ^{a)}				
		Base-pair substitution type			Frameshift type	
		TA 100	TA 1535	WP 2 u v r A	TA 98	TA 1537
S9 mix (-)	Solvent control	152 149 (151)	14 12 (13)	37 32 (35)	25 20 (23)	11 8 (10)
	5	145 153 (149)	13 12 (13)	38 38 (38)	21 24 (23)	9 6 (8)
	10	142 144 (143)	12 17 (15)	34 41 (38)	23 25 (24)	8 6 (7)
	50	159 150 (155)	15 14 (15)	33 37 (35)	23 27 (25)	10 7 (9)
	100	147 138 (143)	16 14 (15)	34 38 (36)	28 29 (29)	8 8 (8)
	500	139 131 (135)	16 15 (16)	35 33 (34)	21 27 (24)	6 4 (5)
	1000	152 137 (145)	10 14 (12)	36 32 (34)	20 25 (23)	5 4 (5)
	5000	125 123 (124)	14 12 (13)	30 29 (30)	21 21 (21)	8 6 (7)
S9 mix (+)	Solvent control	155 152 (154)	15 15 (15)	49 43 (46)	30 36 (33)	14 18 (16)
	5	140 140 (140)	14 13 (14)	45 48 (47)	33 31 (32)	15 14 (15)
	10	153 142 (148)	16 15 (16)	44 40 (42)	31 32 (32)	15 13 (14)
	50	144 152 (148)	18 15 (17)	41 38 (40)	40 34 (37)	10 13 (12)
	100	150 153 (152)	15 16 (16)	39 40 (40)	40 36 (38)	14 11 (13)
	500	153 138 (146)	12 14 (13)	41 45 (43)	39 39 (39)	13 10 (12)
	1000	140 150 (145)	14 15 (15)	44 46 (45)	28 40 (34)	11 12 (12)
	5000	145 147 (146)	16 13 (15)	31 40 (36)	37 38 (38)	9 10 (10)
Positive control not requiring S9 mix	Name	AF-2	SA	AF-2	AF-2	9-AA
	Concentration (μ g/plate)	0.01	0.5	0.01	0.1	80
	Number of colonies/plate	531 520 (526)	552 519 (536)	230 241 (236)	583 567 (575)	629 636 (633)
Positive control requiring S9 mix	Name	2-AA	2-AA	2-AA	2-AA	2-AA
	Concentration (μ g/plate)	1	2	10	0.5	2
	Number of colonies/plate	1010 997 (1004)	237 218 (228)	940 985 (963)	599 592 (596)	211 195 (203)

^{a)} : The average number of colonies in each concentration.

Solvent : Distilled water for injection

PROJECT No. H-97642

Appendix 2. Reverse mutation test of Monacolin Powder 8000F in *S. typhimurium* and *E. coli* (Mutagenicity test)

With (+) or Without (-) S9mix	Test substance concentration (μ g/plate)	Number of revertants (number of colonies/plate) ^{a)}				
		Base-pair substitution type			Frameshift type	
		TA 100	TA 1535	WP 2 uvrA	TA 98	TA 1537
S9 mix (-)	Solvent control	149	15	32	24	9
		146 (146)	16 (15)	37 (35)	28 (24)	8 (
		143	14	37	21	7
	312.5	142	15	35	22	6
		140 (141)	10 (13)	43 (39)	26 (24)	9 (
		150	15	34	28	9
625	149 (150)	13 (14)	32 (33)	22 (25)	5 (
	156	16	39	25	10	
	1250	149 (153)	14 (15)	42 (41)	29 (27)	7 (
2500	140	11	41	22	6	
	152 (146)	18 (15)	40 (41)	27 (25)	7 (
	5000	145	19	32	21	8
S9 mix (+)	Solvent control	148	12	39	38	12
		153 (151)	16 (14)	47 (43)	38 (37)	12 (
		151	15	44	36	15
	312.5	152	14	43	46	11
		158 (155)	17 (16)	45 (44)	42 (44)	13 (
		159	13	38	45	12
625	144 (152)	9 (11)	54 (46)	47 (46)	11 (
	152	11	42	39	11	
	1250	148 (150)	15 (13)	46 (44)	44 (42)	10 (
2500	147	13	46	38	11	
	152 (150)	18 (16)	49 (48)	37 (38)	10 (
	5000	158	18	44	42	10
Positive control not requiring S9 mix	Name	AF-2	SA	AF-2	AF-2	9-AA
	Concentration (μ g/plate)	0.01	0.5	0.01	0.1	80
	Number of colonies/plate	491	524	208	573	628
Positive control requiring S9 mix	Name	2-AA	2-AA	2-AA	2-AA	2-AA
	Concentration (μ g/plate)	1	2	10	0.5	2
	Number of colonies/plate	1051	250	1062	609	260
		1017 (1034)	294 (272)	1050 (1056)	600 (605)	264 (26

^{a)} : The average number of colonies in each concentration.

Solvent : Distilled water for injection

PROJECT No. H-97

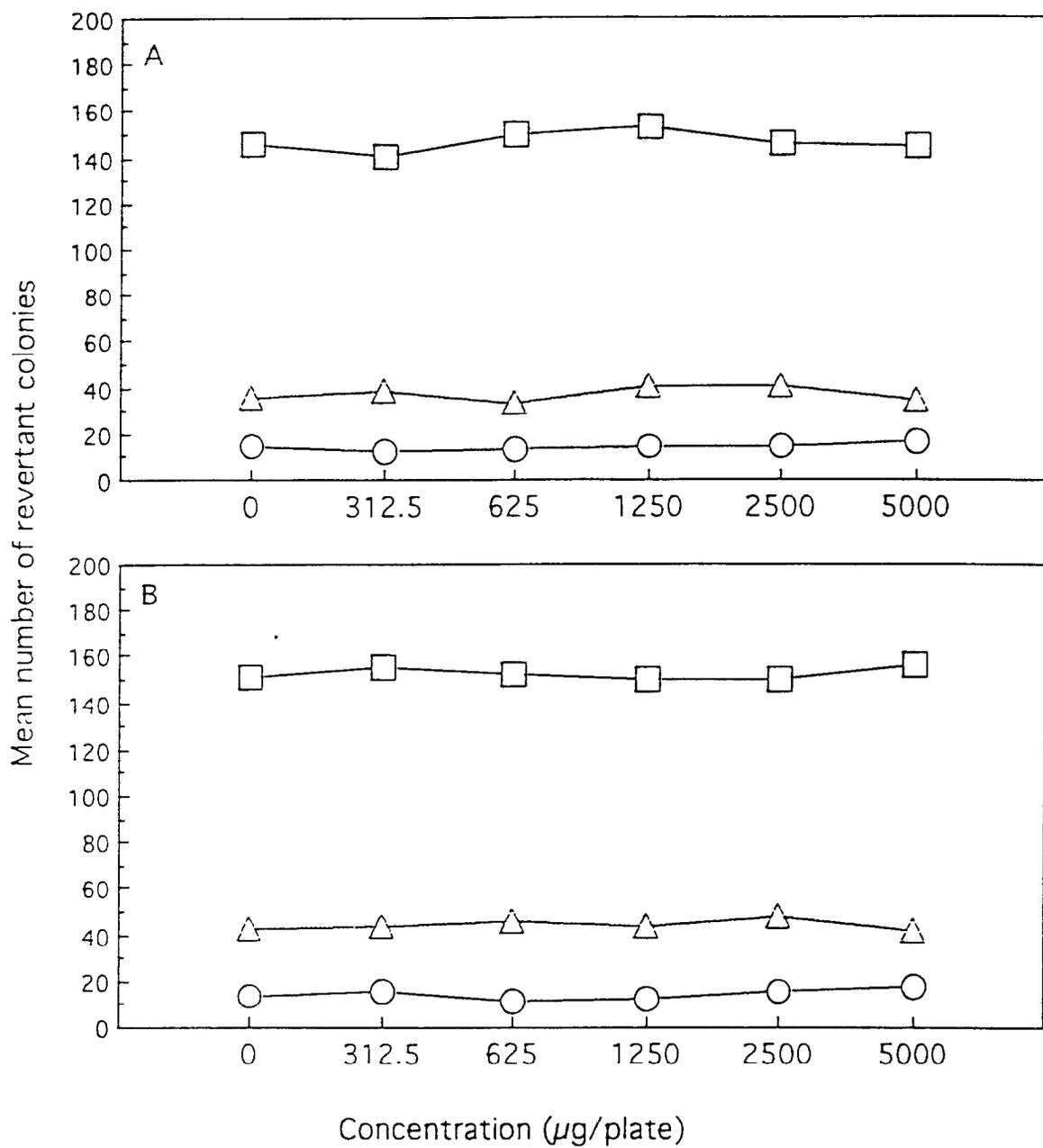


Fig. 1. Dose depending curve of Monacolin Powder 8000F (Mutagenicity test)
 A : Without metabolic activation system (-S9)
 B : With metabolic activation system (+S9)
 □ : TA100 ; ○ : TA1535; △ : WP2uvrA

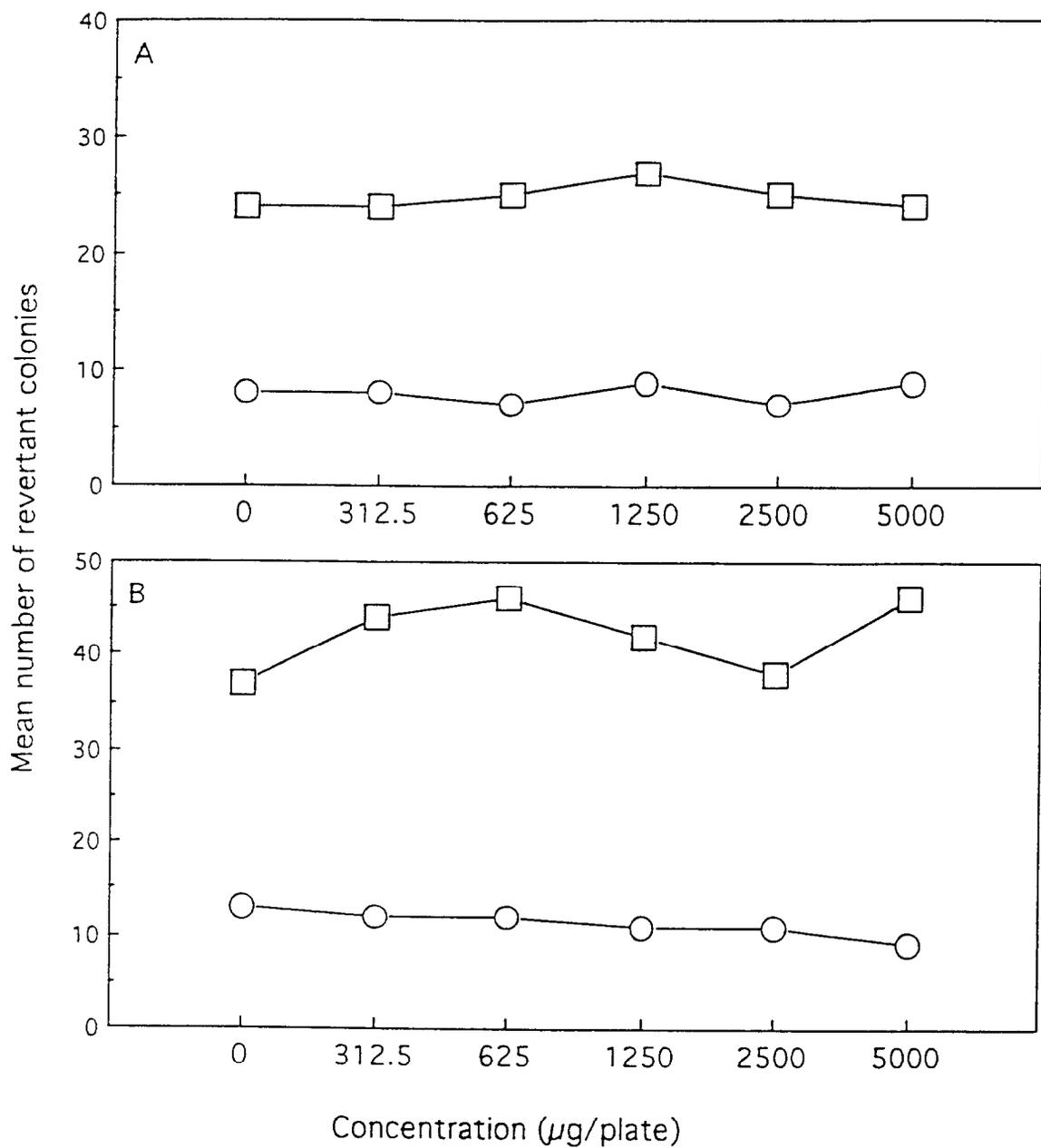


Fig. 2. Dose depending curve of Monacolin Powder 8000F (Mutagenicity test)

A : Without metabolic activation system (-S9)

B : With metabolic activation system (+S9)

□ : TA98 ; ○ : TA1537

PROJECT No. H-97642

**Hepatic Toxicity of
Monacolin Powder 8000F in Rats**

PROJECT No. H-98454

Final Report

NEMRI

(Nippon Experimental Medical Research Institute, Co., Ltd.)

STATEMENT OF COMPLIANCE

Title: Hepatic toxicity of Monacolin Powder 8000F in rats
Project No.: H-98454

The undersigned Managing Director of NEMRI affirms that this study reported in this manuscript was carried out under Non-GLP standards with reference to the NEMRI SOP.

Signature: Masamine Aiuchi Date: Oct. - 14 - 1998
Masamine Aiuchi, D.V.M., Ph.D.
Managing Director
NEMRI

STUDY IDENTIFICATION

Title. Hepatic toxicity of Monacolin Powder 8000F in rats
Project No.: H-98454

Test Materials Monacolin Powder 8000F

Objectives

Monacolin Powder 8000F is extracted from *Monascus Pilosus*, including an active substance Monacolin J. The present study was done to assess whether Monacolin Powder 8000F, 0.8% of Monacolin J formulation, has the hepatic toxicity in rats.

Sponsor and Study Monitor

Name. Maruzen Pharmaceutical Co., Ltd.
Address: 14703-10, Mukaihigashi, Onomichi, Hiroshima, 722-0062 Japan
TEL 0848-44-2200, FAX 0848-20-6006
Monitor Toshimitsu Kambara

Contractor and Study Director

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Address: 3303-58, Ohdo, Agatsuma, Agatsuma, Gunma, 377-0931 Japan
TEL 0279-69-2216, FAX 0279-69-2851
Study director Tomohiko Hasegawa

Study Location

Name: Haruna Laboratory of NEMRI
Address: 3303-58, Ohdo, Agatsuma, Agatsuma, Gunma, 377-0931 Japan

Archiving of Records and Documents

The relevant documents and raw data obtained from the present study will be retained at the archives of Haruna Laboratories of NEMRI for 5 years after the termination of the study. The subsequent retention will be described under approval of the sponsor.

Study Timetable

Protocol approval:	August 24, 1998
Animal receipt:	August 8, 1998
Experimental period:	September 4, 1998 – September 21, 1998
Submission of draft report:	October 7, 1998
Submission of final report:	October 12, 1998

PERSONNEL

Title: Hepatic toxicity of Monacolin Powder 8000F in rats
Project No.: H-98454

It is certified that the present manuscript and data were obtained under Non-GLP standards with reference to the NEMRI SOP.

Signature: T. Hasegawa Date: Oct. 14, 1998
Tomohiko Hasegawa, M.S.
Study director

Signature: K. Kawai Date: Oct. 14, 1998
Keiko Kawai
Pharmacological unit

Signature: M. Kuniyama Date: Oct 14 '98
Mineo Kuniyama, Ph.D.
Pharmacological unit

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APPENDIX 1-3	Day 7: Effects of Monacolin Powder 8000F on plasma GOT, GPT, glucose and hepatic weight in rats
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APPENDIX 2	Effect of Monacolin Powder 8000F on body weight in rats
APPENDIX 3	Effect of Monacolin Powder 8000F on food intake in rats

I. Summary

This study was undertaken to assess whether Monacolin Powder 8000F (0.8% Monacolin J formulation) has the hepatic toxicity in rats. The test substance was dissolved with distilled water to prepare 2 and 10 g/kg solutions. By using 3 animals per group, the single oral administration of the vehicle or the test substance solutions was employed. Blood was removed under ether anesthetization on 1, 3, 7 and 14 days after the administration. Plasma GOT, GPT, glucose and wet hepatic weight were measured. Monacolin Powder 8000F at both doses had no significant changes in plasma GOT, GPT, glucose, wet hepatic weight and body weight. However, food intake was significantly decreased only on 4 days after the 2 g/kg administration, and 1 days after the 10 g/kg administration. These results suggest that Monacolin Powder 8000F at oral doses of 10 g/kg and less has no hepatic toxicity in rats.

II. Materials and Methods

1. Test substance

(1) Name and property

Monacolin Powder 8000F is a light red powder with an odor of *koji* mold, containing 0.8 % Monacolin J, an active estimated substance. In this study, the substance with lot No. 00404028 was supplied by the sponser. Upon receipt on August 21, 1998, it was stored at room temperature under light protective conditions.

(2) Preparation

The test substance was dissolved with distilled water (WASSER, Fuso Pharmaceutical Industrial Co., Ltd.) to prepare 2 and 10 g/kg solution..

(3) Administration route and justification

The oral administration was selected with reference to the estimated clinical administration route.

(4) Administration dosage and justification

The preliminary study shows that Monacolin Powder 8000F at 10 g/kg has no side-effects such as diarrhea and death. Thus, 2 and 10 g/kg was selected as the administration doses in the present study. As the test substance solution was high viscosity, 20 ml/kg was selected as the maximal dosing volume.

2. Animals

(1) Species and strain

Forty five male Sprague-Dawley rats of 5 weeks old were purchased from Charles River Japan (795 Shimofurusawa, Atsugi, Kanagawa, Japan). On procurement day, the animals were weighed and numbered, followed by discrimination by painting their

backs with saturated picric acid solution. The cages were also identified by colored labels on which the animal and project number were defined. During more than 7 days quarantine/acclimation period, body weight and gross behavior were measured once a day. On the termination day of this period, they were divided into 13 groups including a surplus group of 9 animals to avoid bias of body weight among the groups.

The animals were housed by two in a rat stainless bracket cage of the room No. 3 of animal care unit for pharmacology. The room was maintained at a temperature of $22 \pm 3^{\circ}\text{C}$ and relative humidity of $55 \pm 15\%$. The rate of ventilation was 12 times per hour with all-fresh system. A 12-hour light (06:00-18:00)-dark cycle was conditioned with an illumination of 150-300 lux.

A standard laboratory solid food CE-2 was purchased from CREA Japan (2-20-14, Aobadai, Meguro, Tokyo, Japan). Analysis data of each lot of the food used was supplied by CREA Japan. Drinking water was given to the animals *ad-libitum*. The quality of the drinking water was analyzed by the Incorporated Pharmaceutist Association of Gunma Environmental Health Center (5-18-36, Nishi-Katagai, Maebashi, Gunma, Japan) and the Gunma Prefectural Agatsuma Public Health Center (183-1, Nishi-Nakanojo, Nakanojo, Agatsuma, Gunma, Japan). No contamination in the diet and in water which could affect the results of the present study, was observed.

(2) Justification

Rats were selected based on lots of background data and widely-use in toxicological tests.

3. Methods

(1) Administration, blood sampling and measurement of hepatic weight

Thirty six animals of 6 weeks old, were divide into 3 groups, vehicle, 2 and 10 g/kg of Monacolin Powder 8000F group. Each group consisted of 12 animals, which were also separated by three into 4 groups on the basis of blood sampling points. The vehicle or the test substance was orally given to animals. Under ether anesthetization, blood was removed on 1, 3, 7 and 14 days after the administration, and heparinized. The liver

was also removed and weighed after the blood sampling. The blood was centrifuged at 4°C, 3000 rpm for 10 min to obtain the plasma. The plasma was stocked at -80 °C until measurement of GOT, GPT and glucose

(2) **Measurement of GOT, GPT and glucose**

By using an autoanalyzer (7070, Hitachi), GOT and GPT were measured according to the standard method of Japan Clinical Chemical Association. Glucose was measured by hexokinase-G6PDH method.

(3) **Measurement of body weight and food intake**

Body weight and food intake were measured once a day for 14 days from the proceeding day of the administration to the day of the last blood sampling.

(4) **Statistical analysis**

The data are shown as the mean \pm SEM. The statistical analysis was performed by Dunnett's multiple comparison test.

4. **Unpredicted accidents and deviation from protocol**

Neither unpredicted accidents affecting the reliability of the results nor the protocol deviation, occurred in the present study. The dose of Monacolin Powder 8000F was not defined in the protocol: however, 2 and 10 g/kg were selected based on the results of the preliminary study.

III. Results

1. Effects on plasma GOT, GPT, glucose and hepatic weight (FIG. 1-4, TABLE 1, APPENDIX 1-1 - 1-4)

Plasma GOT, GPT and glucose levels of the vehicle group were approximately 100 IU/dl, 40 IU/dl and 180 mg/dl, respectively, and there were no significant daily changes for 14 days. Compared with the vehicle, Monacolin Powder 8000F at both 2 and 10 g/kg showed no significant changes in plasma GOT, GPT and glucose levels for 14 days.

Wet hepatic weight of the vehicle group was slightly decreased : 5.25 and 4.32 g/100 g body weight on 1 and 14 days, respectively after the administration, but there was no significant changes. Monacolin Powder 8000F also showed no significant changes compared with the vehicle on each blood sampling day.

2. Effect on body weight (FIG. 5, TABLE 2, APPENDIX 2)

Body weight of the vehicle group was gradually increased from 176 g to 305 g. Monacolin Powder 8000F increased body weight similarly to the vehicle group, and showed no significant changes on each day.

3. Effect on food intake (FIG. 6, TABLE 3, APPENDIX 3)

Food intake of the vehicle was kept about 25-29 g for 14 days. Monacolin Powder 8000F at 2 g/kg showed a slight but significant decrease in food intake on 4 days after the administration ($P<0.05$). Ten g/kg group also decreased it only on 1 day after the administration ($P<0.01$).

IV. Discussion

The present study revealed that although high doses of Monacolin Powder 8000F (2 and 10 g/kg) were orally administered to rats, plasma GOT, GPT glucose, hepatic weight and body weight were not significantly changes. These results suggest that Monacolin Powder 8000F has no hepatic toxicity.

Food intake was significantly decreased by Monacolin Powder 8000F in the present study. This decrease may result from dextrin, which is contained 90% in Monacolin Powder 8000F and a polymer of glucose. That is, blood glucose will be transiently elevated after the oral administration of Monacolin Powder 8000F, and it can be estimated that the high calory intake may led animals to suppress food intake.

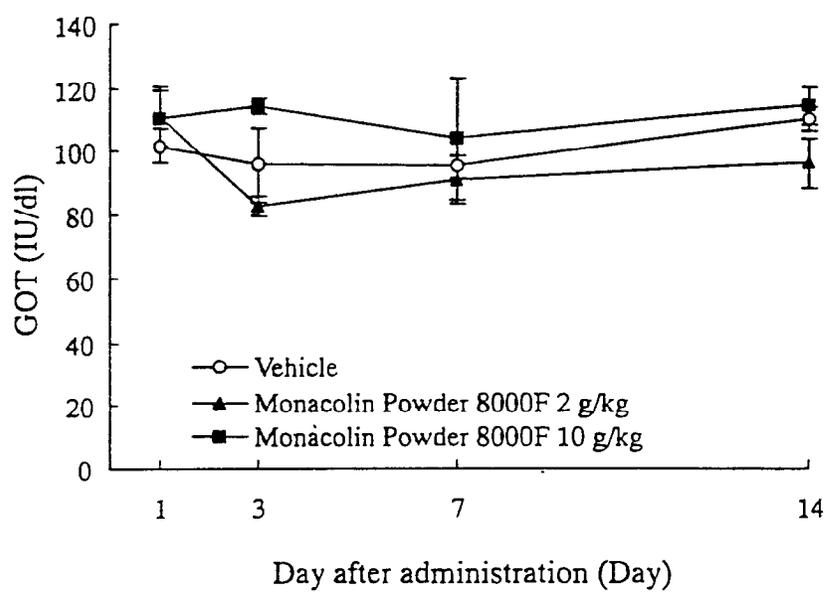


FIG. 1 Effect of Monacolin Powder 8000F on plasma GOT in rats

Vehicle: Distilled water (20 ml/kg)

Data show the mean \pm SEM from 3 animals.

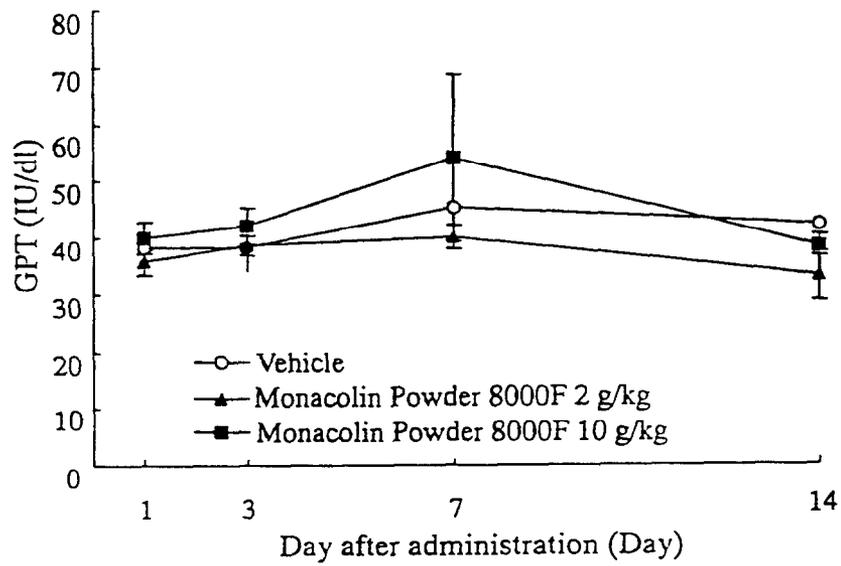


FIG. 2 Effect of Monacolin Powder 8000F on plasma GPT in rats

Vehicle: Distilled water (20 ml/kg)

Data show the mean \pm SEM from 3 animals.

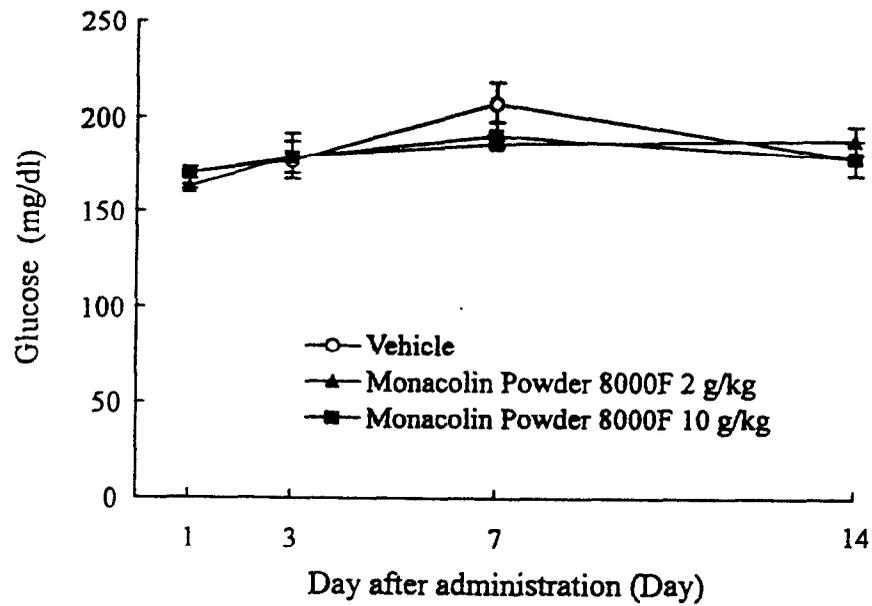


FIG. 3 Effect of Monacolin Powder 8000F on plasma glucose in rats

Vehicle: Distilled water (20 ml/kg)

Data show the mean \pm SEM from 3 animals.

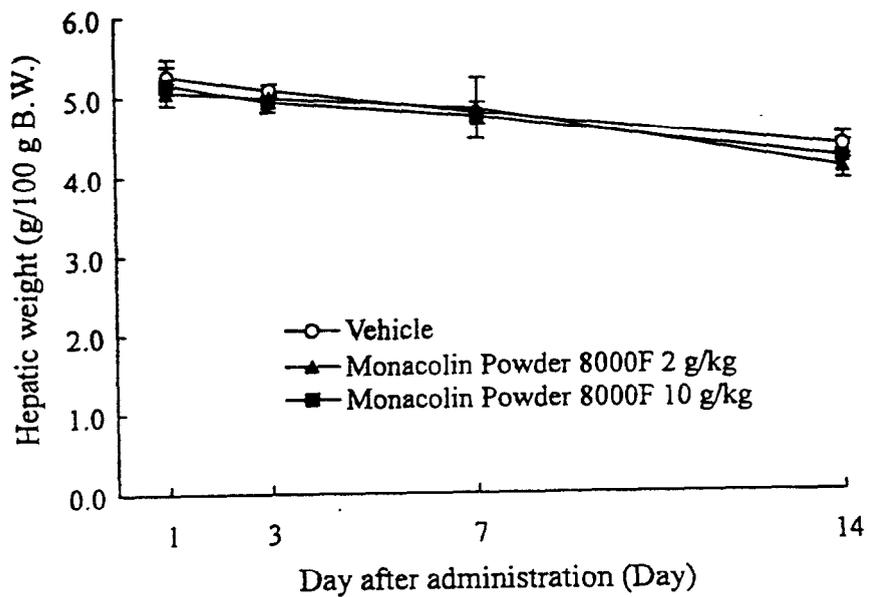


FIG. 4 Effect of Monacolin Powder 8000F on hepatic weight in rats

Vehicle: Distilled water 20 ml/kg

Data show the mean \pm SEM from 3 animals.

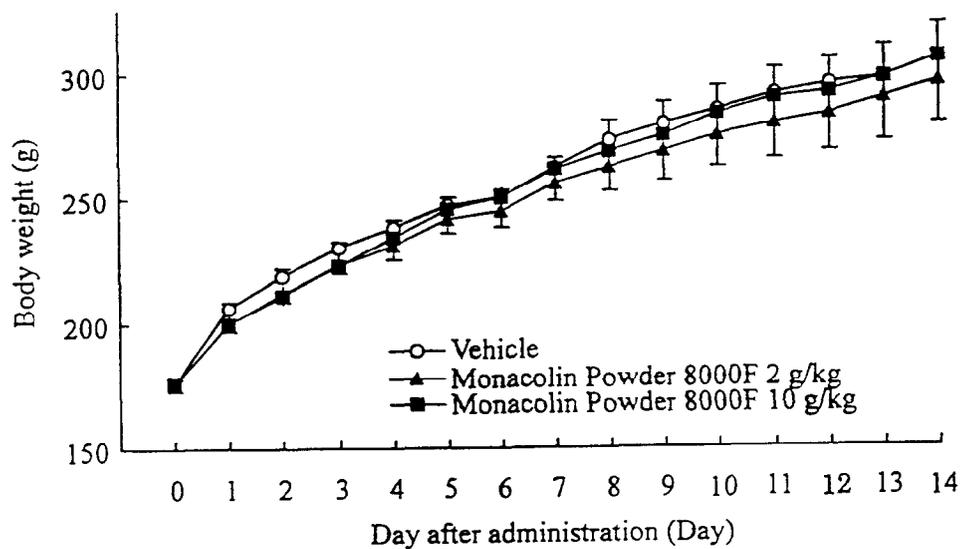


FIG. 5 Effect of Monacolin Powder 8000F on body weight in rats

Vehicle: Distilled water 20 ml/kg

Data show the mean \pm SEM from 3-12 animals.

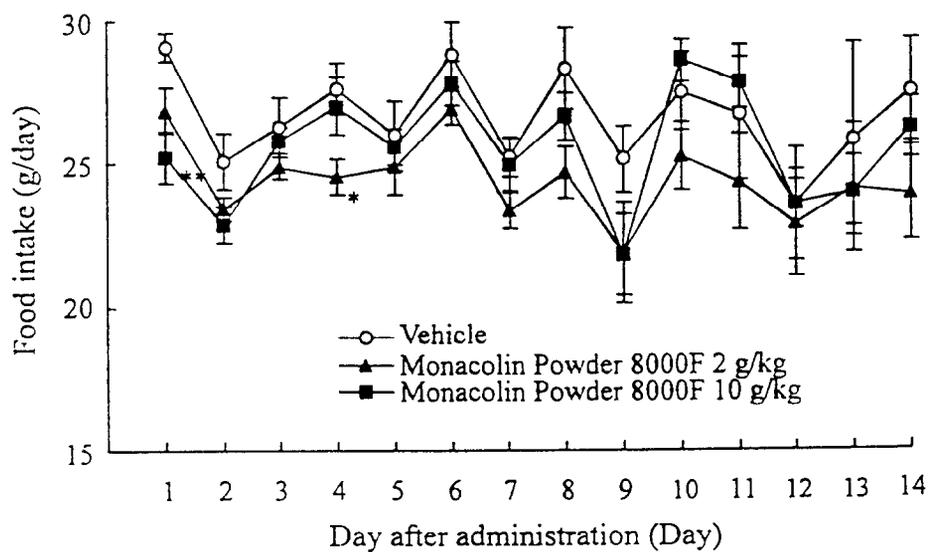


FIG. 6 Effect of Monacolin Powder 8000F on food intake in rats

Vehicle: Distilled water 20 ml/kg

Data show the mean \pm SEM from 3-12 animals.

* $P < 0.05$, ** $p < 0.01$ Significantly different from vehicle (Dunnett's multiple comparison test).

TABLE 1 Effects of Monacolin Powder 8000F on plasma GOT, GPT, glucose and hepatic weight in rats

Item	Treatment	Dose (g/kg, p.o.)	After administration			
			Day1	Day3	Day7	Day14
GOT (IU/dl)	Vehicle	-	102 ± 5.4	96 ± 11.6	95 ± 3.6	110 ± 4.1
	Monacolin Powder 8000F	2	111 ± 9.5	82 ± 3.2	91 ± 7.4	96 ± 7.8
	Monacolin Powder 8000F	10	110 ± 9.0	114 ± 2.4	104 ± 19.3	115 ± 5.9
GPT (IU/dl)	Vehicle	-	38 ± 2.2	38 ± 4.4	45 ± 5.0	42 ± 4.9
	Monacolin Powder 8000F	2	36 ± 2.3	39 ± 1.7	40 ± 2.1	33 ± 2.6
	Monacolin Powder 8000F	10	40 ± 2.6	42 ± 3.1	54 ± 14.6	38 ± 1.3
Glucose (mg/dl)	Vehicle	-	169.7 ± 2.18	176.8 ± 4.86	206.7 ± 11.80	178.6 ± 7.09
	Monacolin Powder 8000F	2	163.1 ± 0.97	179.0 ± 11.65	186.3 ± 1.33	187.9 ± 7.65
	Monacolin Powder 8000F	10	170.0 ± 2.80	178.9 ± 8.51	190.6 ± 7.08	178.9 ± 9.31
Hepatic weight (g/100 g B.W.)	Vehicle	-	5.25 ± 0.209	5.06 ± 0.069	4.78 ± 0.154	4.32 ± 0.163
	Monacolin Powder 8000F	2	5.06 ± 0.128	4.98 ± 0.172	4.81 ± 0.381	4.05 ± 0.138
	Monacolin Powder 8000F	10	5.16 ± 0.209	4.91 ± 0.109	4.73 ± 0.154	4.18 ± 0.187

Vehicle: Distilled water (20 ml/kg)

TABLE 2 Effect of Monacolin Powder 8000F on body weight in rats

Treatment	Dose (g/kg, p.o.)	Body weight (g)							
		Day0 (n=12)	Day1 (n=12)	Day2 (n=9)	Day3 (n=9)	Day4 (n=6)	Day5 (n=6)	Day6 (n=6)	Day7 (n=6)
Vehicle	-	176± 1.6	206± 2.3	219± 2.7	230± 2.5	238± 3.2	247± 2.9	250± 2.8	262± 3.3
Monacolin Powder 8000F	2	175± 2.2	200± 3.0	212± 3.0	223± 3.2	231± 5.4	241± 5.4	244± 6.2	255± 6.6
Monacolin Powder 8000F	10	176± 2.0	200± 2.8	211± 3.1	223± 3.6	234± 4.1	245± 4.2	250± 4.1	261± 4.0

Treatment	Dose (g/kg, p.o.)	Body weight (g)						
		Day8 (n=3)	Day9 (n=3)	Day10 (n=3)	Day11 (n=3)	Day12 (n=3)	Day13 (n=3)	Day14 (n=3)
Vehicle	-	273± 7.6	279± 8.5	285± 9.5	291± 10.6	295± 10.3	297± 12.7	305± 14.0
Monacolin Powder 8000F	2	262± 9.7	268± 12.2	274± 12.5	279± 13.9	283± 15.2	289± 17.4	296± 16.7
Monacolin Powder 8000F	10	268± 7.4	274± 7.9	283± 8.7	289± 9.3	292± 8.0	297± 8.7	305± 9.2

Vehicle: Distilled water (20 ml/kg)

TABLE 3 Effect of Monacolin Powder 8000F on food intake in rats

Treatment	Dose (g/kg, p.o.)	Food intake (g/day)						
		Day1 (n=12)	Day2 (n=9)	Day3 (n=9)	Day4 (n=6)	Day5 (n=6)	Day6 (n=6)	Day7 (n=6)
Vehicle	-	29.1 ± 0.47	25.1 ± 0.98	26.3 ± 1.03	27.6 ± 0.90	26.0 ± 1.21	28.8 ± 1.13	25.2 ± 0.70
Monacolin Powder 8000F	2	26.9 ± 0.81	23.4 ± 0.43	24.9 ± 0.46	24.5 ± 0.62 *	24.9 ± 0.97	27.0 ± 0.56	23.3 ± 0.61
Monacolin Powder 8000F	10	25.2 ± 0.91**	22.8 ± 0.65	25.8 ± 0.54	27.0 ± 1.01	25.6 ± 0.64	27.9 ± 0.76	25.0 ± 0.90

Treatment	Dose (g/kg, p.o.)	Food intake (g/day)						
		Day8 (n=3)	Day9 (n=3)	Day10 (n=3)	Day11 (n=3)	Day12 (n=3)	Day13 (n=3)	Day14 (n=3)
Vehicle	-	28.3 ± 1.38	25.1 ± 1.17	27.5 ± 1.36	26.7 ± 2.33	23.5 ± 1.97	25.8 ± 3.39	27.5 ± 1.80
Monacolin Powder 8000F	2	24.7 ± 0.92	21.9 ± 1.77	25.2 ± 1.20	24.3 ± 1.68	22.9 ± 1.86	24.1 ± 2.25	23.9 ± 1.68
Monacolin Powder 8000F	10	26.6 ± 0.84	21.8 ± 1.42	28.6 ± 0.75	27.8 ± 0.86	23.5 ± 0.86	24.0 ± 1.22	26.2 ± 1.06

Vehicle: Distilled water (20 ml/kg)

* P<0.05, ** P<0.01 Significantly different from vehicle (Dunnett's multiple comparison test)



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APPENDIX 1-1 Day1: Effects of Monacolin Powder 8000F on plasma GOT, GPT, glucose and hepatic weight in rats

Treatment	Animal #	Body weight (g)	GOT (IU/dl)	GPT (IU/dl)	Glucose (mg/dl)	Hepatic weight	
						Wet weight (g)	Relative weight (g/100 g B.W.)
Vehicle 20 ml/kg	M980828- 32	197	94	40	174.1	11.086	5.63
	M980828- 10	205	99	34	167.6	10.057	4.91
	M980828- 12	199	112	41	167.5	10.393	5.22
	n	3	3	3	3	3	3
	mean	200	102	38	169.7	10.512	5.25
	SEM	2.4	5.4	2.2	2.18	0.3029	0.209
Monacolin Powder 8000F 2 g/kg	M980828- 18	177	129	32	162.2	8.521	4.81
	M980828- 45	203	97	40	165.0	10.658	5.25
	M980828- 23	203	107	35	162.0	10.359	5.10
	n	3	3	3	3	3	3
	mean	194	111	36	163.1	9.846	5.06
	SEM	8.7	9.5	2.3	0.97	0.6683	0.128
Monacolin Powder 8000F 10 g/kg	M980828- 33	186	128	45	166.7	9.330	5.02
	M980828- 24	207	104	36	175.6	11.529	5.57
	M980828- 27	200	99	39	167.8	9.774	4.89
	n	3	3	3	3	3	3
	mean	198	110	40	170.0	10.211	5.16
	SEM	6.2	9.0	2.6	2.80	0.6713	0.209

Vehicle: Distilled water (20 ml/kg)



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APPENDIX 1-2 Day3: Effects of Monacolin Powder 8000F on plasma GOT, GPT, glucose and hepatic weight in rats

Treatment	Animal #	Body weight (g)	GOT (IU/dl)	GPT (IU/dl)	Glucose (mg/dl)	Hepatic weight	
						Wet weight (g)	Relative weight (g/100 g B.W.)
Vehicle 20 ml/kg	M980828- 41	241	77	30	174.9	11.875	4.93
	M980828- 39	225	117	45	169.5	11.602	5.16
	M980828- 38	228	93	40	186.0	11.639	5.10
	n	3	3	3	3	3	3
	mean	231	96	38	176.8	11.705	5.06
	SEM	4.9	11.6	4.4	4.86	0.0854	0.069
Monacolin Powder 8000F 2 g/kg	M980828- 25	215	76	37	177.8	11.042	5.14
	M980828- 07	221	85	37	159.5	10.255	4.64
	M980828- 11	227	86	42	199.8	11.742	5.17
	n	3	3	3	3	3	3
	mean	221	82	39	179.0	11.013	4.98
	SEM	3.5	3.2	1.7	11.65	0.4294	0.172
Monacolin Powder 8000F 10 g/kg	M980828- 31	203	111	38	180.2	10.276	5.06
	M980828- 13	223	113	40	163.6	10.483	4.70
	M980828- 42	224	119	48	193.0	11.144	4.97
	n	3	3	3	3	3	3
	mean	217	114	42	178.9	10.634	4.91
	SEM	6.8	2.4	3.1	8.51	0.2617	0.109

Vehicle: Distilled water (20 ml/kg)



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APPENDIX 1-3 Day7: Effects of Monacolin Powder 8000F on plasma GOT, GPT, glucose and hepatic weight in rats

Treatment	Animal #	Body weight (g)	GOT (IU/dl)	GPT (IU/dl)	Glucose (mg/dl)	Hepatic weight	
						Wet weight (g)	Relative weight (g/100 g B.W.)
Vehicle 20 ml/kg	M980828- 30	257	88	38	187.8	12.229	4.76
	M980828- 35	262	100	55	204.0	11.862	4.53
	M980828- 40	268	97	43	228.4	13.557	5.06
	n	3	3	3	3	3	3
	mean	262	95	45	206.7	12.549	4.78
	SEM	3.2	3.6	5.0	11.80	0.5148	0.154
Monacolin Powder 8000F 2 g/kg	M980828- 15	246	80	36	188.5	11.745	4.77
	M980828- 34	244	87	43	183.9	10.182	4.17
	M980828- 21	276	105	41	186.6	15.151	5.49
	n	3	3	3	3	3	3
	mean	255	91	40	186.3	12.359	4.81
	SEM	10.3	7.4	2.1	1.33	1.4670	0.381
Monacolin Powder 8000F 10 g/kg	M980828- 26	250	140	83	203.9	12.471	4.99
	M980828- 08	263	74	42	179.7	12.491	4.75
	M980828- 17	272	98	37	188.3	12.116	4.45
	n	3	3	3	3	3	3
	mean	262	104	54	190.6	12.359	4.73
	SEM	6.4	19.3	14.6	7.08	0.1219	0.154

Vehicle: Distilled water (20 ml/kg)



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APPENDIX 1-4 Day14: Effects of Monacolin Powder 8000F on plasma GOT, GPT, glucose and hepatic weight in rats

Treatment	Animal #	Body weight (g)	GOT (IU/dl)	GPT (IU/dl)	Glucose (mg/dl)	Hepatic weight	
						Wet weight (g)	Relative weight (g/100 g B.W.)
Vehicle 20 ml/kg	M980828- 19	317	103	34	191.9	14.675	4.63
	M980828- 16	277	111	41	167.7	11.745	4.24
	M980828- 14	321	117	51	176.2	13.101	4.08
	n	3	3	3	3	3	3
	mean	305	110	42	178.6	13.174	4.32
	SEM	14.0	4.1	4.9	7.09	0.8465	0.163
Monacolin Powder 8000F 2 g/kg	M980828- 44	263	81	29	182.3	10.480	3.98
	M980828- 22	315	106	38	203.0	13.605	4.32
	M980828- 43	311	102	32	178.3	11.990	3.86
	n	3	3	3	3	3	3
	mean	296	96	33	187.9	12.025	4.05
	SEM	16.7	7.8	2.6	7.65	0.9021	0.138
Monacolin Powder 8000F 10 g/kg	M980828- 29	293	122	41	166.8	11.184	3.82
	M980828- 06	299	119	37	172.7	12.802	4.28
	M980828- 36	323	103	37	197.2	14.342	4.44
	n	3	3	3	3	3	3
	mean	305	115	38	178.9	12.776	4.18
	SEM	9.2	5.9	1.3	9.31	0.9118	0.187

Vehicle: Distilled water (20 ml/kg)

APPENDIX 2 Effect of Monacolin Powder 8000F on body weight in rats

Treatment	Animal #	Body weight (g)														
		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Vehicle 20 ml/kg	M980828- 32	167	197	-	-	-	-	-	-	-	-	-	-	-	-	-
	M980828- 10	175	205	-	-	-	-	-	-	-	-	-	-	-	-	-
	M980828- 12	176	199	-	-	-	-	-	-	-	-	-	-	-	-	-
	M980828- 41	174	222	232	241	-	-	-	-	-	-	-	-	-	-	-
	M980828- 39	177	206	214	225	-	-	-	-	-	-	-	-	-	-	-
	M980828- 38	178	207	219	228	-	-	-	-	-	-	-	-	-	-	-
	M980828- 30	169	196	209	221	230	239	243	257	-	-	-	-	-	-	-
	M980828- 35	180	207	220	229	240	247	253	268	-	-	-	-	-	-	-
	M980828- 40	184	212	227	237	244	250	254	262	-	-	-	-	-	-	-
	M980828- 19	176	207	219	234	237	251	251	263	278	282	289	295	299	309	317
	M980828- 16	172	199	207	220	228	238	241	249	258	263	267	271	275	272	277
	M980828- 14	187	216	225	238	248	256	259	272	283	292	299	307	310	311	321
	n	12	12	9	9	6	6	6	6	3	3	3	3	3	3	3
	mean	176	206	219	230	238	247	250	262	273	279	285	291	295	297	305
SEM	1.6	2.3	2.7	2.5	3.2	2.9	2.8	3.3	7.6	8.5	9.5	10.6	10.3	12.7	14.0	
Monacolin Powder 8000F 2 g/kg	M980828- 18	163	177	-	-	-	-	-	-	-	-	-	-	-	-	
	M980828- 45	179	203	-	-	-	-	-	-	-	-	-	-	-	-	
	M980828- 23	181	203	-	-	-	-	-	-	-	-	-	-	-	-	
	M980828- 25	166	195	202	215	-	-	-	-	-	-	-	-	-	-	
	M980828- 07	177	201	211	221	-	-	-	-	-	-	-	-	-	-	
	M980828- 11	181	206	217	227	-	-	-	-	-	-	-	-	-	-	
	M980828- 15	166	194	205	215	221	231	233	246	-	-	-	-	-	-	
	M980828- 34	174	194	203	215	222	231	237	244	-	-	-	-	-	-	
	M980828- 21	185	215	225	238	248	260	267	276	-	-	-	-	-	-	
	M980828- 44	169	192	203	210	216	227	226	234	243	244	249	251	253	254	263
	M980828- 22	177	205	219	229	238	244	248	258	268	279	282	291	301	308	315
	M980828- 43	184	214	222	233	243	251	255	269	275	282	290	294	296	304	311
	n	12	12	9	9	6	6	6	6	3	3	3	3	3	3	3
	mean	175	200	212	223	231	241	244	255	262	268	274	279	283	289	296
SEM	2.2	3.0	3.0	3.2	5.4	5.4	6.2	6.6	9.7	12.2	12.5	13.9	15.2	17.4	16.7	
Monacolin Powder 8000F 10 g/kg	M980828- 33	161	186	-	-	-	-	-	-	-	-	-	-	-	-	
	M980828- 24	179	207	-	-	-	-	-	-	-	-	-	-	-	-	
	M980828- 27	177	200	-	-	-	-	-	-	-	-	-	-	-	-	
	M980828- 31	167	181	194	203	-	-	-	-	-	-	-	-	-	-	
	M980828- 13	178	200	212	223	-	-	-	-	-	-	-	-	-	-	
	M980828- 42	180	196	214	224	-	-	-	-	-	-	-	-	-	-	
	M980828- 26	171	195	206	215	223	235	238	250	-	-	-	-	-	-	
	M980828- 08	179	204	213	227	236	244	253	263	-	-	-	-	-	-	
	M980828- 17	183	212	221	235	245	255	258	272	-	-	-	-	-	-	
	M980828- 29	173	197	208	217	225	236	241	251	259	265	272	280	282	287	293
	M980828- 06	174	201	207	222	229	240	244	259	263	268	276	280	287	289	299
	M980828- 36	187	216	226	239	246	260	263	272	283	290	300	308	308	314	323
	n	12	12	9	9	6	6	6	6	3	3	3	3	3	3	3
	mean	176	200	211	223	234	245	250	261	268	274	283	289	292	297	305
SEM	2.0	2.8	3.1	3.6	4.1	4.2	4.1	4.0	7.4	7.9	8.7	9.3	8.0	8.7	9.2	

Vehicle: Distilled water
 - Body weight was not determination because after dissection

APPENDIX 3 Effect of Monacolin Powder 8000F on food intake in rats

Treatment	Animal #	Food intake (g)													
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Vehicle 20 ml/kg	M980828- 32	31.7	-	-	-	-	-	-	-	-	-	-	-	-	-
	M980828- 10	27.4	-	-	-	-	-	-	-	-	-	-	-	-	-
	M980828- 12	26.9	-	-	-	-	-	-	-	-	-	-	-	-	-
	M980828- 41	28.3	22.6	24.6	-	-	-	-	-	-	-	-	-	-	-
	M980828- 39	30.5	27.4	27.3	-	-	-	-	-	-	-	-	-	-	-
	M980828- 38	29.5	23.8	23.9	-	-	-	-	-	-	-	-	-	-	-
	M980828- 30	27.7	24.5	22.9	26.6	23.5	24.8	24.3	-	-	-	-	-	-	-
	M980828- 35	30.2	21.4	23.6	25.4	23.9	28.9	24.4	-	-	-	-	-	-	-
	M980828- 40	30.0	27.2	26.0	27.7	25.2	31.9	24.5	-	-	-	-	-	-	-
	M980828- 19	30.3	31.0	32.4	31.0	31.3	31.4	28.6	31.1	26.1	29.1	27.4	25.5	31.9	29.5
	M980828- 16	26.7	23.5	26.4	25.6	24.5	26.4	24.1	26.9	22.8	24.8	22.4	19.6	20.2	23.9
	M980828- 14	29.9	24.4	29.6	29.3	27.5	29.5	25.3	27.0	26.5	28.6	30.4	25.5	25.3	29.1
	n	12	9	9	6	6	6	6	3	3	3	3	3	3	3
	mean	29.1	25.1	26.3	27.6	26.0	28.8	25.2	28.3	25.1	27.5	26.7	23.5	25.8	27.5
SEM	0.47	0.98	1.03	0.90	1.21	1.13	0.70	1.38	1.17	1.36	2.33	1.97	3.39	1.80	
Monacolin Powder 8000F 2 g/kg	M980828- 18	21.0	-	-	-	-	-	-	-	-	-	-	-	-	-
	M980828- 45	28.1	-	-	-	-	-	-	-	-	-	-	-	-	-
	M980828- 23	28.0	-	-	-	-	-	-	-	-	-	-	-	-	-
	M980828- 25	26.9	21.4	25.8	-	-	-	-	-	-	-	-	-	-	-
	M980828- 07	25.3	23.6	25.0	-	-	-	-	-	-	-	-	-	-	-
	M980828- 11	30.1	24.5	24.0	-	-	-	-	-	-	-	-	-	-	-
	M980828- 15	25.7	22.8	22.8	25.5	23.3	25.0	23.5	-	-	-	-	-	-	-
	M980828- 34	24.2	21.7	24.5	23.9	27.2	28.1	22.7	-	-	-	-	-	-	-
	M980828- 21	27.0	24.7	27.5	23.6	28.1	28.8	23.7	-	-	-	-	-	-	-
	M980828- 44	26.4	23.1	24.3	24.0	24.9	26.0	22.3	23.1	18.6	23.7	21.3	19.5	19.9	21.1
	M980828- 22	27.8	25.1	24.2	23.0	21.9	27.1	21.8	26.3	22.3	24.4	27.1	25.9	27.6	26.9
	M980828- 43	32.0	23.9	26.1	27.1	23.7	26.7	26.0	24.6	24.7	27.6	24.5	23.2	24.8	23.8
	n	12	9	9	6	6	6	6	3	3	3	3	3	3	3
	mean	26.9	23.4	24.9	24.5	24.9	27.0	23.3	24.7	21.9	25.2	24.3	22.9	24.1	23.9
SEM	0.81	0.43	0.46	0.62	0.97	0.56	0.61	0.92	1.77	1.20	1.68	1.86	2.25	1.68	
Monacolin Powder 8000F 10 g/kg	M980828- 33	21.9	-	-	-	-	-	-	-	-	-	-	-	-	-
	M980828- 24	25.6	-	-	-	-	-	-	-	-	-	-	-	-	-
	M980828- 27	23.1	-	-	-	-	-	-	-	-	-	-	-	-	-
	M980828- 31	20.6	21.3	24.9	-	-	-	-	-	-	-	-	-	-	-
	M980828- 13	24.7	20.3	25.1	-	-	-	-	-	-	-	-	-	-	-
	M980828- 42	22.0	25.5	25.1	-	-	-	-	-	-	-	-	-	-	-
	M980828- 26	27.3	23.8	23.8	26.2	26.8	26.0	26.7	-	-	-	-	-	-	-
	M980828- 08	25.6	21.2	26.5	26.1	23.8	28.9	21.8	-	-	-	-	-	-	-
	M980828- 17	29.8	23.9	27.0	30.1	24.2	29.1	27.7	-	-	-	-	-	-	-
	M980828- 29	23.3	20.8	23.7	23.3	24.8	26.7	24.1	25.9	19.4	27.3	28.4	21.9	24.2	24.6
	M980828- 06	29.5	23.8	27.6	26.9	26.0	26.0	23.5	25.7	21.7	28.5	26.1	24.8	21.8	25.8
	M980828- 36	29.1	25.0	28.2	29.4	27.8	30.4	25.9	28.3	24.3	29.9	28.9	23.9	26.0	28.2
	n	12	9	9	6	6	6	6	3	3	3	3	3	3	3
	mean	25.2	22.8	25.8	27.0	25.6	27.9	25.0	26.6	21.8	28.6	27.8	23.5	24.0	26.2
SEM	0.91	0.65	0.54	1.01	0.64	0.76	0.90	0.84	1.42	0.75	0.86	0.86	1.22	1.06	

Vehicle: Distilled water

-: Food intake was not determination because after dissection

(3) Clinical Research Study

Title : Effects of active constituent of MONACOLIN
POWDER 8000F (Monacolin J) on blood lipids and
hepatic toxicity in Watanabe heritable hyperlipidemic
(WHHL) rabbits

Sep. 18, 1998

T. Hasegawa

Tomohiko Hasegawa
Pharmacological unit, Nippon Experimental
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Summary

The present study was undertaken to assess whether active constituent of MONACOLIN POWDER 8000F reduced blood cholesterol and induce hepatic toxicity in WHHL rabbits. The control (30.4 % ethanol solution), test substance at the dose of 100 or 300 mg/kg was given orally once a day for 14 days. Blood was removed before and 1, 3, 7 and 14 days after the administration, and plasma total cholesterol, HDL-cholesterol, triglyceride, GOP and GPT were measured. The liver was also removed on the next day after the last administration, and histopathological evaluation was done. WHHL rabbits showed about 800 mg/dl of plasma total cholesterol before the administration. The control had no changes in total cholesterol, but increased both GOT and GPT levels (TABLE 1). The test substance at 100 and 300 mg/kg decreased the lipid levels approximately by 20% and 40 %, respectively 14 days after the administration, while both doses had no changes in GOT and GPT levels (TABLE 1). Histopathological changes were not observed in the liver treated with the substances. Thus, the present results suggest the possibility that active constituent of MONACOLIN POWDER 8000F has hypolipidemic effect but no hepatic toxicity in inheritable hyperlipidemic model of WHHL rabbits.

TABLE 1

Effects of active constituent of MONACOLIN POWDER 8000F (Monacolin J)
on blood GOT, GPT, total-cholesterol(T-CHO), triglyceride
and HDL-cholesterol (HDL-C) levels in WHHL rabbits
PROJECT No.: H-98401

Item	Treatment	Animal #	Day0	Day3	Day7	Day10	Day14
GOT (IU/l)	Distilled water	M980706-02	38	18	33	30	39
		M980706-04	33	39	33	30	53
	30.4 % Ethanol	M980706-02	20	37	27	53	33
		M980706-04	23	91	53	114	110
	100mg/kg	M980706-01	24	42	65	44	54
		M980706-03	25	18	21	27	33
	300mg/kg	M980706-06	55	106	54	47	15
		M980706-05	38	64	33	31	21
GPT (IU/l)	Distilled water	M980706-02	45	36	48	28	44
		M980706-04	48	45	46	36	54
	30.4 % Ethanol	M980706-02	35	42	41	55	48
		M980706-04	43	49	54	70	84
	100mg/kg	M980706-01	46	63	88	60	89
		M980706-03	46	42	50	34	55
	300mg/kg	M980706-06	46	62	61	54	53
		M980706-05	53	59	50	50	45
Total-cholesterol (mg/dl)	Distilled water	M980706-02	689.1	645.3	686.8	625.6	729.8
		M980706-04	1078.0	1032.2	972.4	898.4	874.2
	30.4 % Ethanol	M980706-02	803.3	723.4	798.5	791.6	799.7
		M980706-04	927.8	849.1	938.8	894.6	935.8
	100mg/kg	M980706-01	807.9	806.2	764.3	699.9	720.0
		M980706-03	828.8	698.9	684.0	599.1	636.6
	300mg/kg	M980706-06	877.5	673.2	601.0	577.6	560.0
		M980706-05	848.8	777.9	488.7	478.0	503.0
Triglyceride (mg/dl)	Distilled water	M980706-02	260.0	229.7	237.1	205.9	234.3
		M980706-04	181.1	235.4	191.5	164.7	167.5
	30.4 % Ethanol	M980706-02	243.0	349.5	381.0	283.9	279.0
		M980706-04	209.7	228.6	155.1	147.9	159.5
	100mg/kg	M980706-01	217.1	222.2	316.9	249.5	262.6
		M980706-03	224.3	304.9	263.8	252.2	248.2
	300mg/kg	M980706-06	174.9	231.0	351.1	374.6	337.8
		M980706-05	229.7	344.6	347.9	601.4	480.0
HDL-C (mg/dl)	Distilled water	M980706-02	5.0	6.2	6.4	5.0	5.7
		M980706-04	3.7	5.5	3.8	3.7	3.6
	30.4 % Ethanol	M980706-02	8.3	9.1	6.9	7.5	6.7
		M980706-04	5.1	6.7	3.3	4.9	3.3
	100mg/kg	M980706-01	4.9	5.5	5.7	4.2	4.9
		M980706-03	4.4	3.1	4.1	2.8	3.5
	300mg/kg	M980706-06	3.3	5.8	5.3	4.3	3.6
		M980706-05	4.2	4.8	6.6	7.2	5.2

Physiological Studies of MONACOLIN POWDER

Test Item	Result
Change in serum total cholesterol	450 mg of MONACOLIN POWDER 8000 F applied daily to 7 human subjects for 3 weeks. Average 8 % of serum total cholesterol reduced.
Change in atherogenesis index	450 mg of MONACOLIN POWDER 8000 F applied daily to 7 human subjects for 3 weeks. Reduction of atherogenesis index seen with all the subject.

Please refer to attached test report

**Cholesterol Reducing Effect of
MONACOLIN POWDER 8000 F**

July, 1998

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14703-10 Mukaihigashi Onomichi City

Hiroshima 722-0062 Japan

Tel : 81-848-44-2200 Fax : 81-848-44-6851

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1. Preface

Monascus bacterium, such as *Monascus pilosus*, products are used as natural pigments by the food industry, and are believed to be good health food materials because they contain a cholesterol reducing agent, monacolin. This study was intended to confirm the cholesterol reducing effect of MONACOLIN POWDER 8000F in in-house subjects.

2. Subjects and Methods

Blood tests were conducted on 22 in-house adult normal males (25 to 55 years old) to select seven subjects with high serum total cholesterol values. The subjects received three MONACOLIN POWDER 8000F tablets a day (each tablet containing 150 mg of MONACOLIN POWDER 8000 F; total daily dose of 450 mg) for three weeks. Blood samples were collected before, and 1, 2, 3, and 4 weeks (1 week after discontinuing administration) after the start of administration to determine serum cholesterol, HDL cholesterol, LDL cholesterol, neutral fat, and lipoprotein values. Diets were not restricted during the study period. Blood collection and screening were entrusted to a local medical institution, and blood samples were collected from 4:00pm to 4:30pm every Monday.

(Before starting this study, all the subjects were fully apprised of the significance and contents of the study, and submitted signed consent forms. At the same time, to respect subject safety and personal rights, blood pressure and liver functions were checked, and the results were provided to them together with physicians' directions.)

※Formula for MONACOLIN 8000F Tablet

Ingredient	Amount (%)
MONACOLIN POWDER 8000F	
Total	100.0

*300 mg / tablet

3. Results

1) Change in Serum Total Cholesterol

Table 1 shows the change rate of the serum total cholesterol values in the seven selected subjects. The results showed that MONACOLIN POWDER 8000F tablets reduced total cholesterol values in all the subjects: the total cholesterol values remained reduced by an average of 7 to 8 % during the study period. There were individual differences in the cholesterol reducing effect of MONACOLIN POWDER 8000F: the total cholesterol values remained about 10 % lower during the study period in two subjects, (52-year-old male) and (49-year-old male), while it only slightly decreased in one subject, (48-year-old male). One subject, (49-year-old male) showed a gradual drop in the total cholesterol value, which amounted to more than a 10 % reduction after three weeks. Another subject, (53-year-old male), showed an almost 15 % drop after one week, with gradual increase amounting to a 6 % drop after three weeks.

Figure 1 shows the change in the serum total cholesterol values over time. The total cholesterol values returned to the pretreatment values in four of the seven subjects one week after discontinuing MONACOLIN POWDER 8000F administration. This finding indicates the total cholesterol reducing effect of MONACOLIN POWDER 8000F.

Chart 1. Change in Serum Total Cholesterol

Monitor	Before administration (mg/dl)	After administration (%)			
		After 1 Week	After 2 Week	After 3 Week	1 Week after discontinuation
(Male, 48)	222	0	-0.5	-1.4	0
(Male, 49)	260	-1.5	-5.0	-11.5	+0.8
(Male, 39)	262	-5.3	-0.8	-10.7	-0.4
(Male, 52)	250	-12.8	-9.6	-9.2	-12.8
(Male, 53)	227	-14.5	-9.7	-6.2	+0.4
(Male, 46)	260	-9.2	-12.7	-7.3	-14.6
(Male, 49)	217	-11.0	-8.8	-9.2	-9.8
Average	243	-7.8	-6.7	-7.9	-5.2

2) Change in HDL Cholesterol

Figure 2 shows that the MONACOLIN POWDER 8000F induced drop in the serum total cholesterol values is largely attributable to the drops in LDL and free cholesterol values.

As shown in Figure 3, MONACOLIN POWDER 8000F clearly increased HDL cholesterol values, and the values returned to their pretreatment levels after MONACOLIN POWDER 8000F administration was discontinued. The subjects, (52-year-old male) and (53-year-old male), who showed a remarkable drop in the total cholesterol values after one week, also showed a drop in HDL cholesterol values after one week. However, the drop rate of the HDL cholesterol values was lower than that of the total cholesterol values, and the HDL cholesterol values subsequently (after 2-3 weeks) returned to, or exceeded, pretreatment levels.

3) Change in Arteriosclerosis (atherogenesis) Index

All the subjects showed a remarkable drop in arteriosclerosis index following MONACOLIN POWDER 8000F administration (Figure 4), confirming that MONACOLIN POWDER 8000F inhibits arteriosclerosis. The index dropped especially remarkable in three of four subjects with high total cholesterol values (250mg/dl or higher) before MONACOLIN POWDER 8000F administration, such as (49-year-old male), (52-year-old male), (46-year-old male).

$$\text{*Arteriosclerosis Index} = \frac{\text{Serum total cholesterol} - \text{HDL cholesterol}}{\text{HDL cholesterol}}$$

4) Changes in Neutral Fat and Lipoproteins

Because this study did not restrict the diets of the subjects, neutral fat values greatly varied depending on what they ate before blood samples were collected. Therefore, the neutral fat reducing effect of MONACOLIN POWDER 8000F could not be determined.

Figure 6 shows the overall decrease in lipoprotein values caused by MONACOLIN POWDER 8000F, although the values increased after one week in the subjects with high neutral fat values, such as (49-year-old male) and (52-year-old male). This is probable because of the reduced cholesterol in lipoproteins.

5) Changes in Blood Pressure, GOT, GPT, and γ -GTP

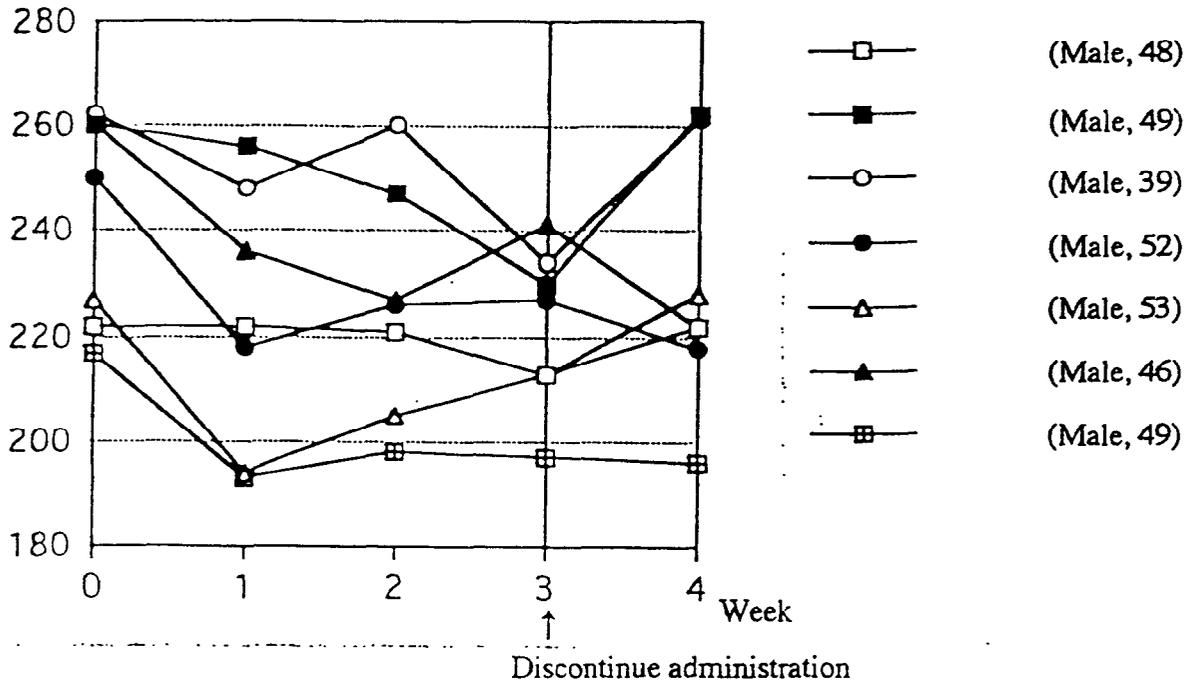
Blood pressure, GOT, GPT, and γ -GTP were also measured in the study period. The results showed that MONACOLIN POWDER 8000F, 450 mg/day, did not cause any abnormal or remarkable changes in the blood pressure, GOT, GPT, and γ -GTP values.

4. Summary

- 1) Administering 450 mg/day of MONACOLIN POWDER 8000F to seven subjects reduced their serum total cholesterol values, although individual differences were noted. The average reduction rate was 7 to 8 %.
- 2) The serum total cholesterol reduction caused by MONACOLIN POWDER 8000F resulted from reduced LDL and free cholesterol.
- 3) MONACOLIN POWDER 8000F increased HDL cholesterol.
- 4) The arteriosclerosis index calculated from the total and HDL cholesterol values evidently decreased in all the subjects, confirming that MONACOLIN POWDER 8000F inhibits arteriosclerosis.
- 5) Although neutral fat reducing effect of MONACOLIN 8000F could not be identified, MONACOLIN POWDER 8000F reduced serum lipoprotein values.
- 6) Administering 45mg/day of MONACOLIN POWDER 8000F for three weeks did not cause any abnormal or remarkable changes in the blood pressure, GOT, GPT, or γ -GTP. The cholesterol reducing effect of MONACOLIN POWDER 8000F in this study suggests the potential hypotensive effect of long-term MONACOLIN POWDER 8000F administration in hypertensive patients with hypercholesterolemia or other diseases.
- 7) Because this study did not include glucose tolerance testing, the blood sugar reducing effect of MONACOLIN POWDER 8000F could not be identified. However, previous studies with MONACOLIN POWDER 8000F tablets have demonstrated that they also remarkably reduce blood sugar.

Figure 1. Change in Serum Total Cholesterol

1) All monitors



2) Average

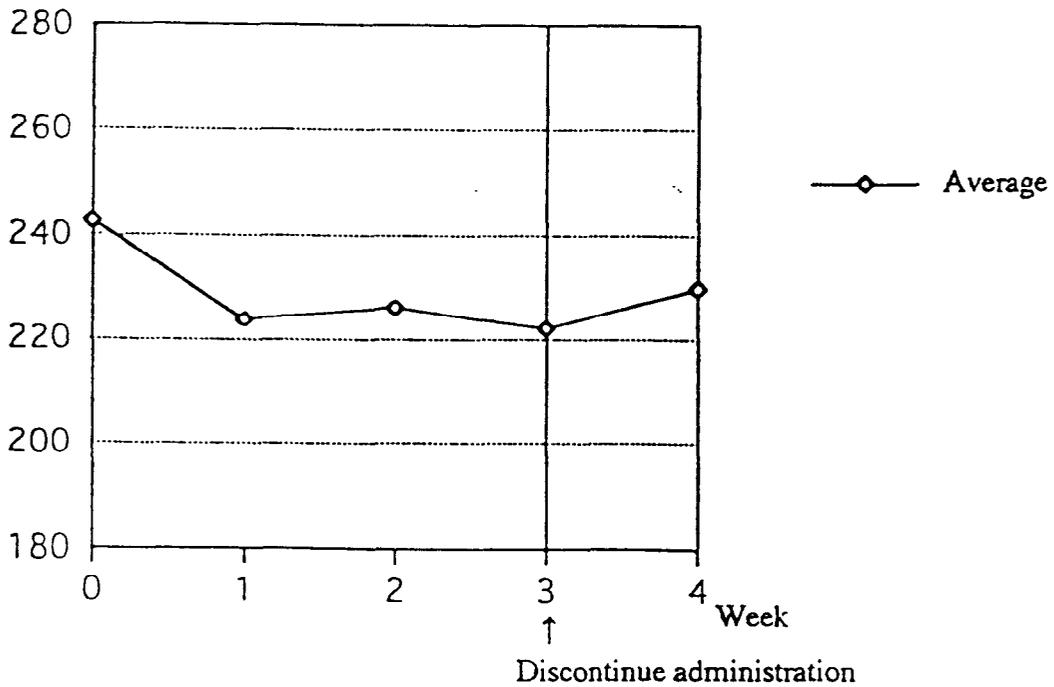


Figure 2. Change in Serum Cholesterol

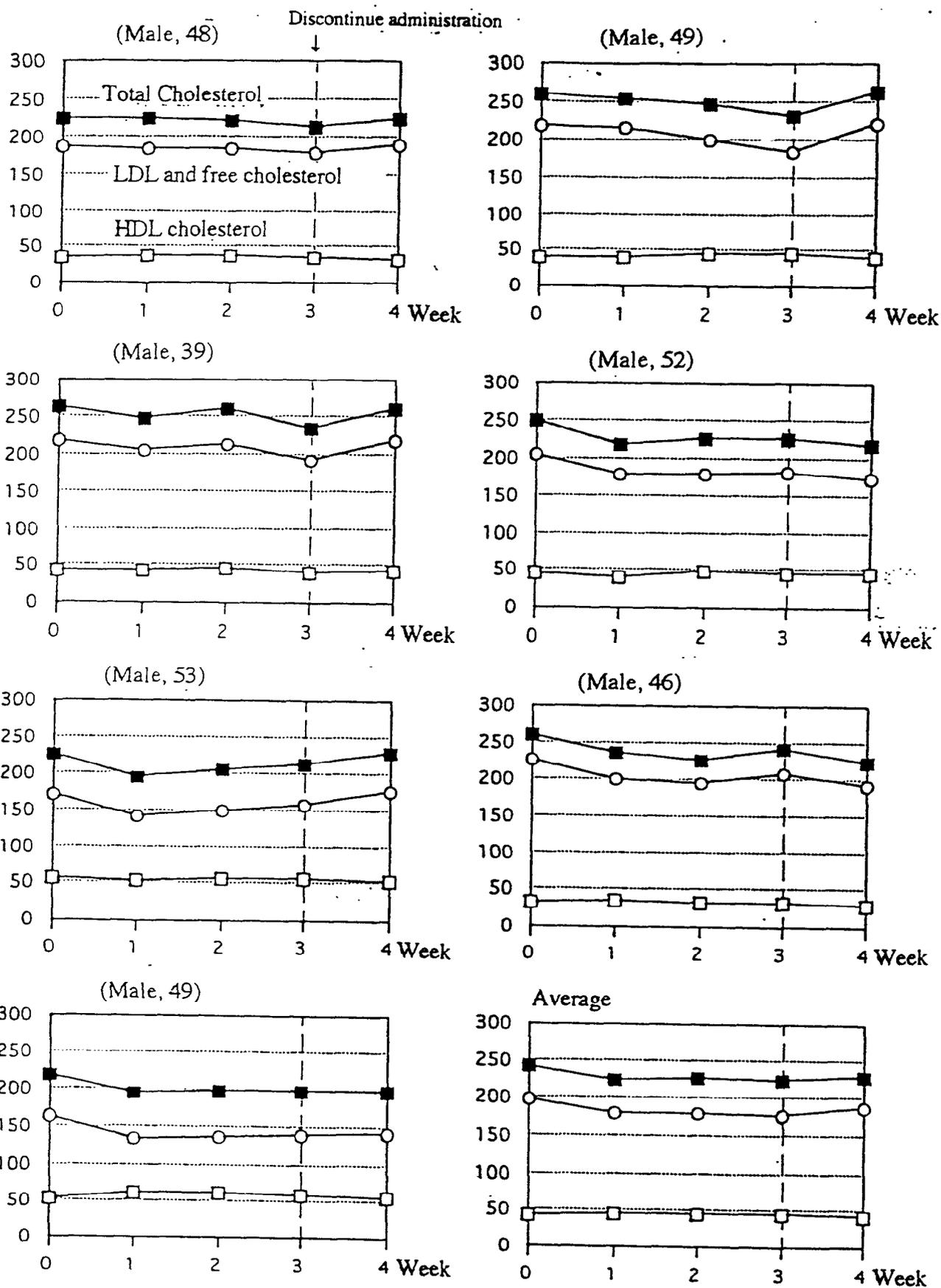
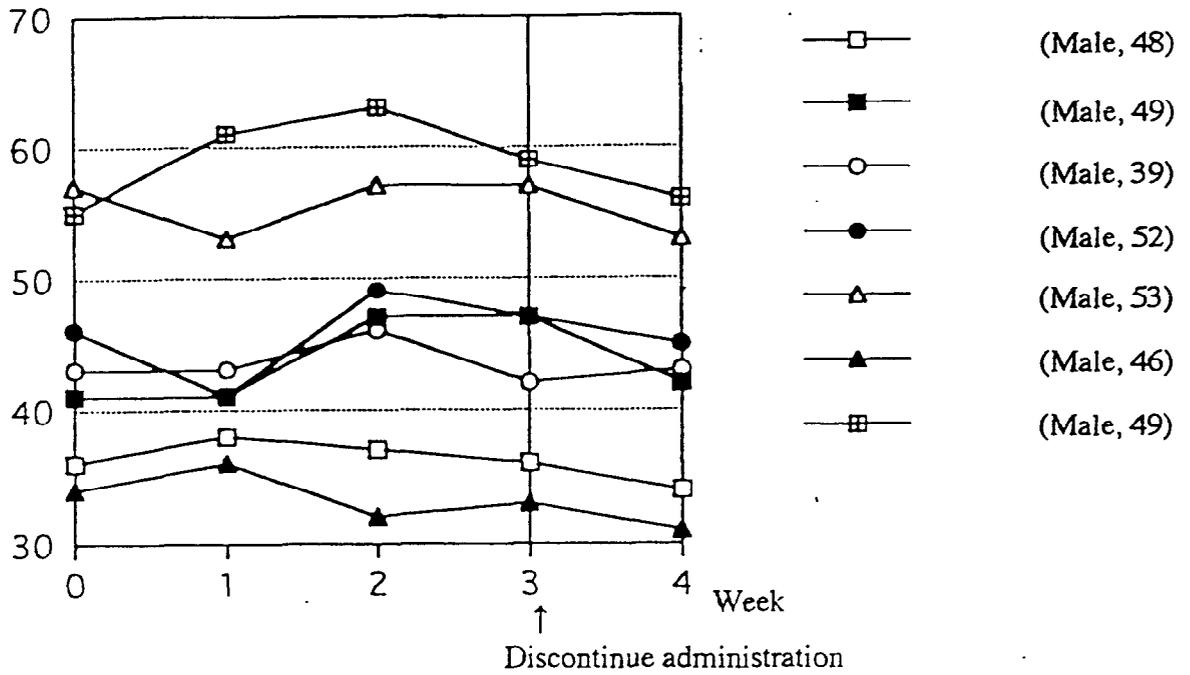


Figure 3. Change in HDL Cholesterol

1) All monitors



2) Average

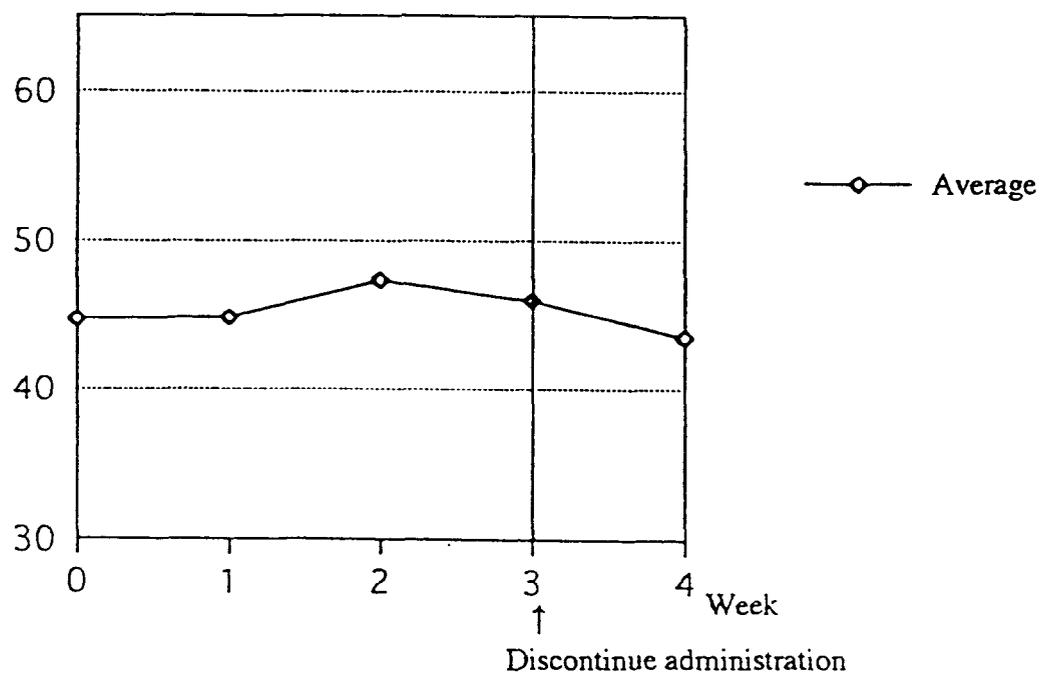
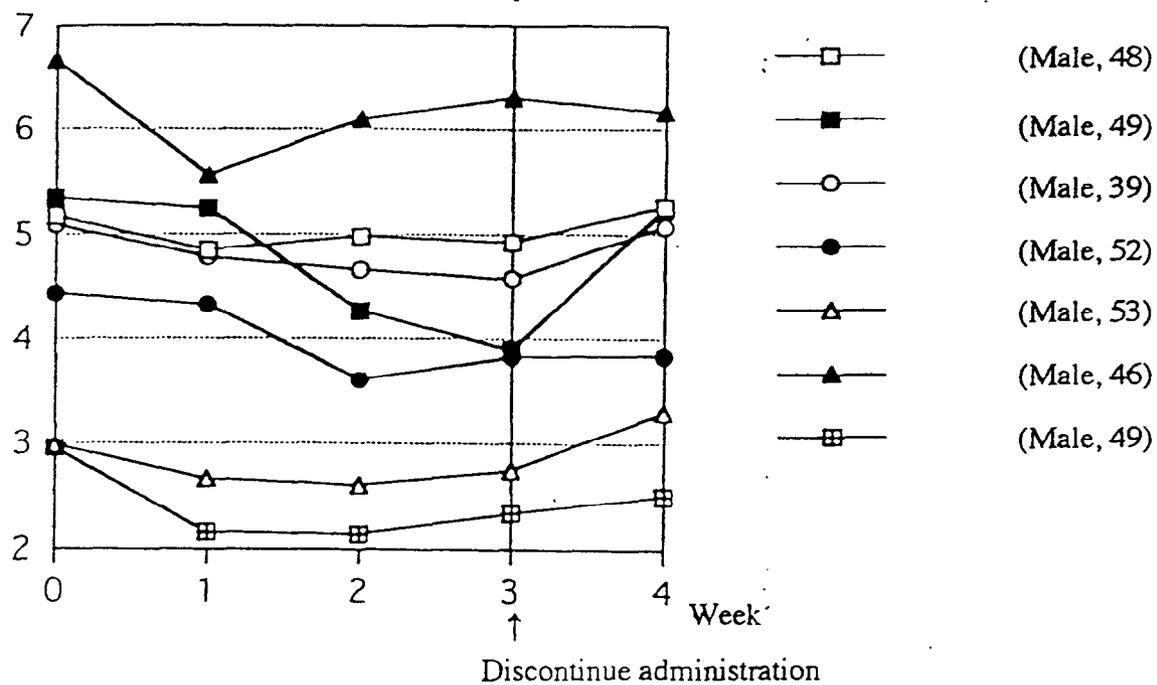


Figure 4. Change in Arteriosclerosis Index

1) All monitors



2) Average

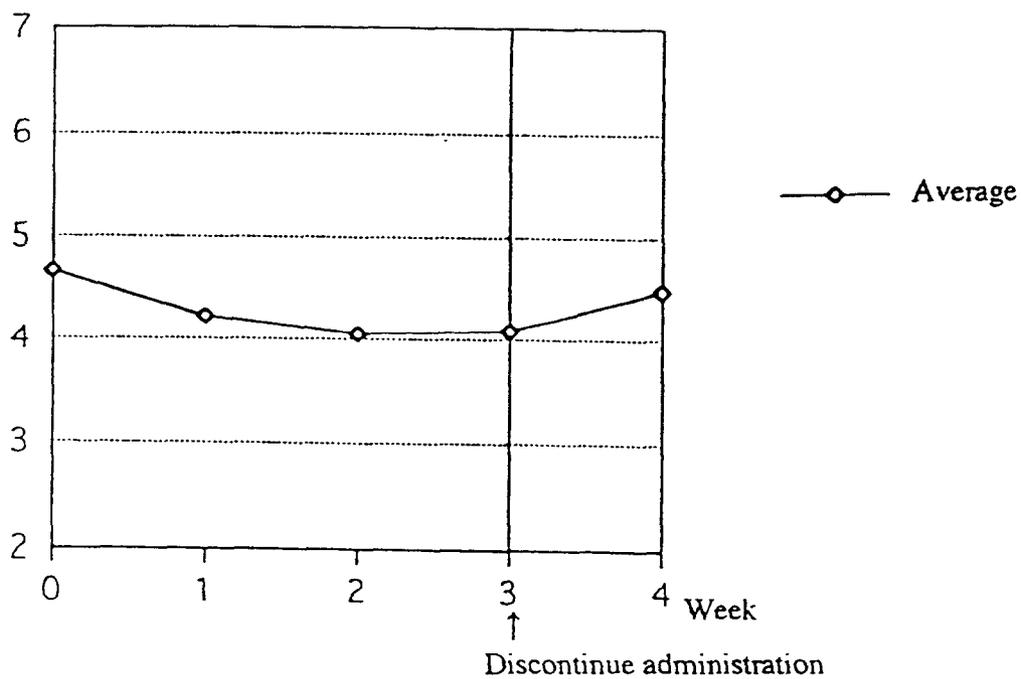
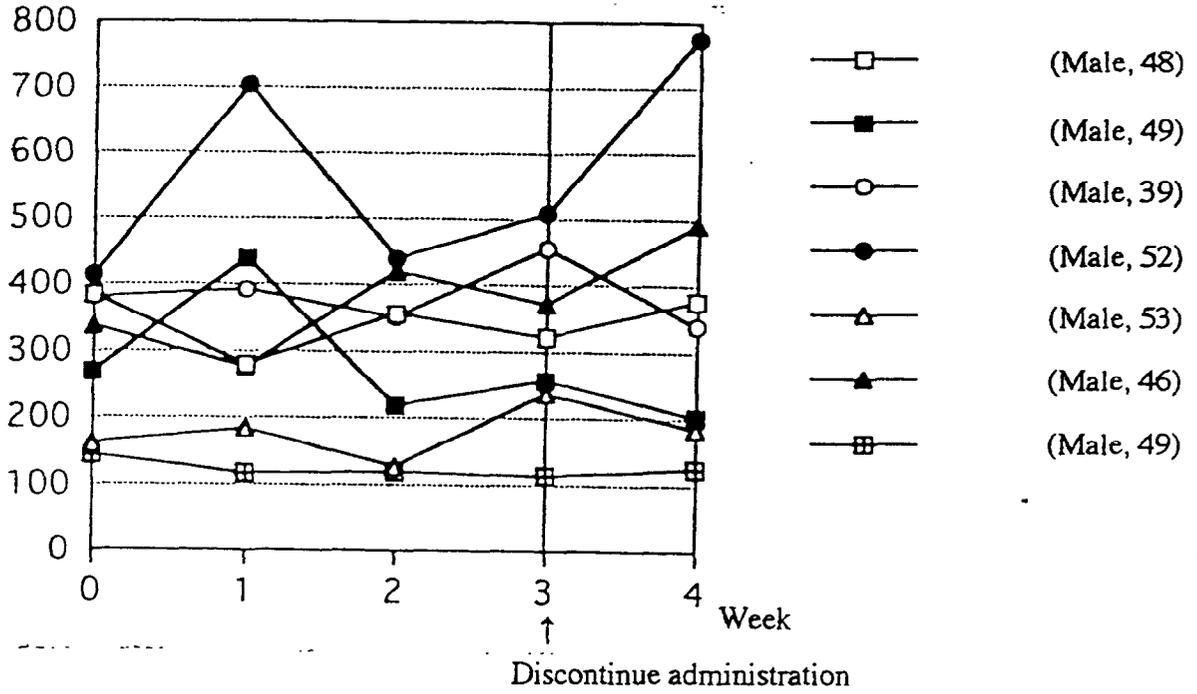


Figure 5. Change in Neutral Fat

1) All monitors



2) Average

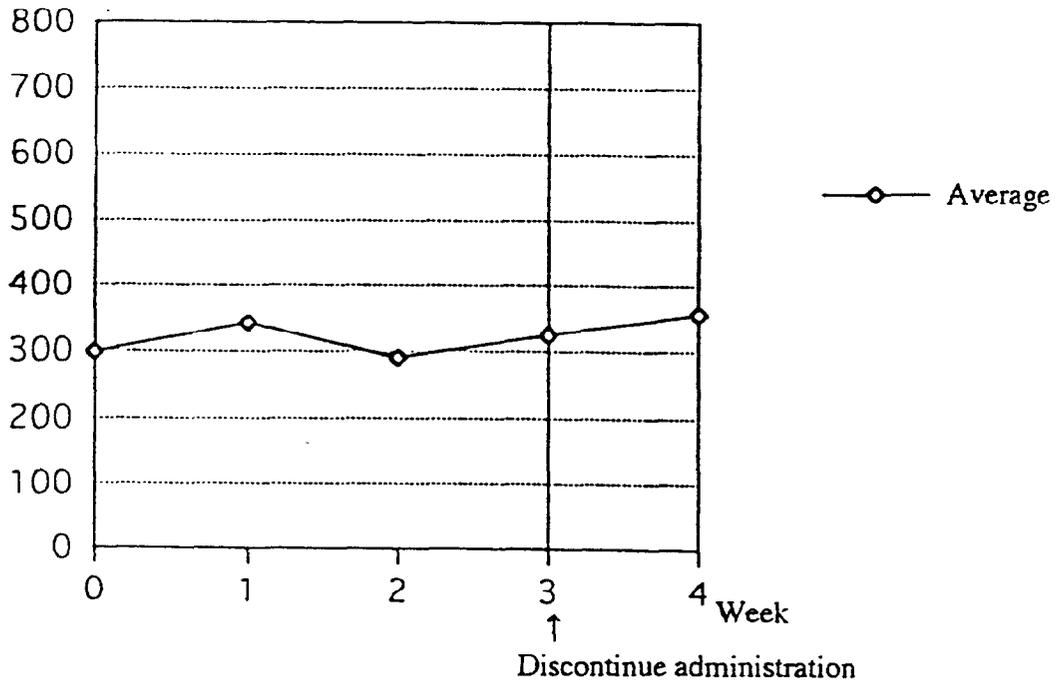
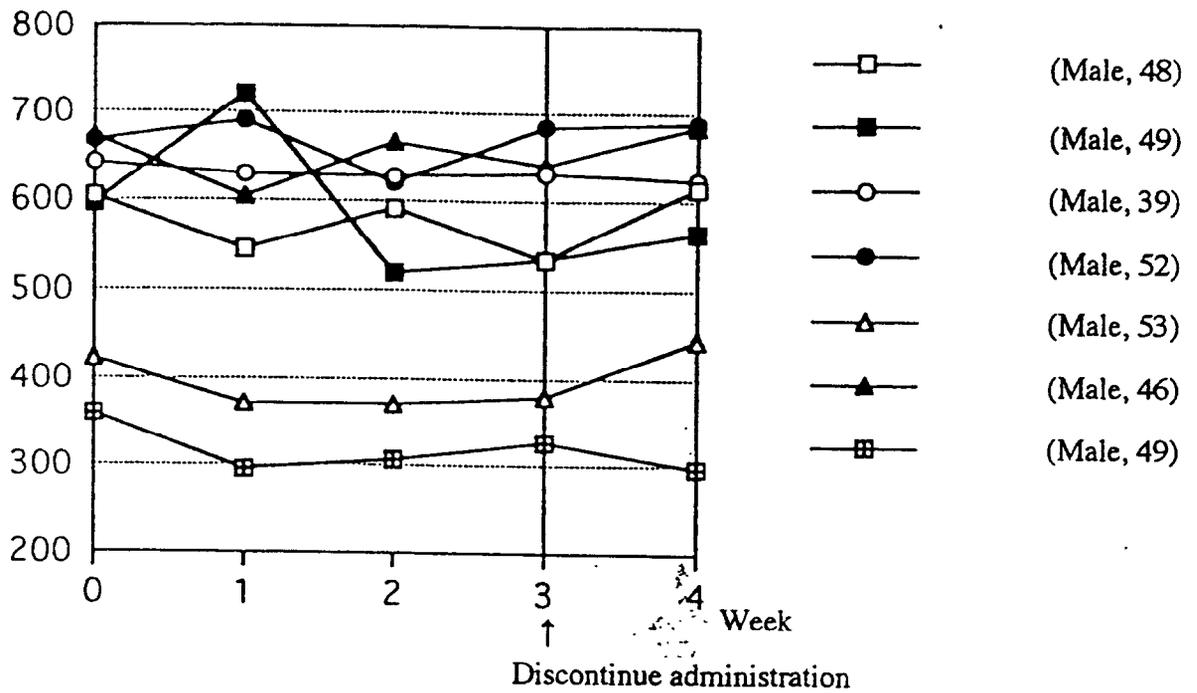
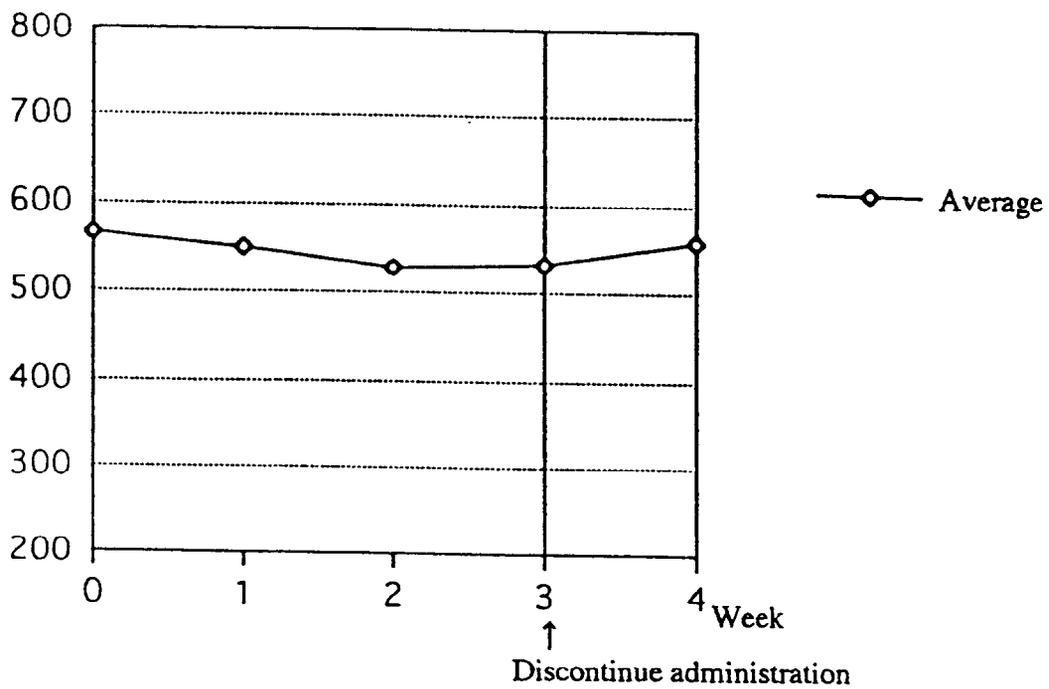


Figure 6. Change in Serum Lipoprotein

1) All monitors



2) Average



(4) Differentiation table verses other products

Comparison of Cholesterol Lowering Substances

Maruzen Pharmaceuticals Co., Ltd.

Trade Name	MONACOLIN POWDER 8000F	CHOLESTIN	MEVACOR
Manufacturer	Maruzen Pharmaceuticals Co., Ltd.	Pharmanex, Inc.	Merck & Co., Inc.
Composition	Fermented Product of Monascus(red yeast rice) 100%	Fermented Product of Monascus(red yeast rice) 100%	Lovastatin
Active Constituents	Monacolin J 0.8%	Monacolin K(Lovastatin) 0.362%* Monacolin L 0.020%* Monacolin J 0.012%*	Lovastatin 100%
Fungus	<i>Monascus plisses</i>	<i>Monascus pursuers</i>	<i>Aspergillus terreus</i>
Manufacturing Method	Purification from fermentation broth of <i>Monascus pliosus</i> .	Fermentation of proprietary strain of <i>Monascus purpureus</i> Went yeast and premium rice.	Purification from fermentation broth of <i>Aspergillus terreus</i> .
R. D. I.	450mg per day	1,200mg per day**	10 – 20 mg per day
Daily Intake of Lovastatin	0mg per day	4.34 mg per day***	10 – 20mg per day

*Analytical value at Maruzen Pharamceuticals Co., Ltd.

**Labelling information of CHOLESTIN says R. D. I. is (300mg capsule x 2) x 2 per day.

Comparison of Activity Rate:

Lovastatin : Monacolin L : Monacolin J = 100: 15 : 4 (A. Endo et al. *J. Antibiotic.* pp1148-1150, 1988)

Conclusion:

1. Lovastatin has strong cholesterol lowering effect and being classified as a medical ingredient. On the other hand it is well known that Lovastatin has a side effect such as the elevation of plasma GOT and GPT values.
2. Actually Cholestin is sold a dietary supplement and the content of Lovastatin per one capsule is small. However if the daily intake of Lovastatin is calculated according to the recommended dosage mentioned in the labeling of Cholestin which was purchased in the market in May 1998.
3. The active constituent of MONACOLIN POWDER 8000F is Monacolin J. It does not contain Lovastatin. The product has gentle effect compared to Lovastatin but it is confirmed to have no adverse effects on liver. MONACOLIN POWDER 8000F is very suitable for the use of dietary supplement.

Toxicological Studies of MONACOLIN POWDER 8000 F

Test Item	Result
Acute oral toxicity	Lethal dose $\geq 2,000$ mg / kg b.w. in mice by oral administration.
Antigenicity studies	Negative in the Ames test.

Please refer to attached test report.