



Memorandum

DOCKETS TRANSMITTAL MEMO

Date: *OCT 27, 2003* 8 5 6 4 '03 NOV 20 P 2 :05
From: Interdisciplinary Scientist, Division of Dietary Supplement Programs, Office of
Nutritional Products, Labeling and Dietary Supplements, HFS-821
Subject: 75-Day Premarket Notification of New Dietary Ingredients
To: Dockets Management Branch, HFA-305

Subject of the Notification: Sesamin

Firm: Ullman, Shapiro & Ullman (Representative for Suntory Ltd)

Date Received by FDA: 12/27/02

90-Day Date: 3/27/03

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 substance should be placed on public display in docket number 95S-0316 as soon possible since it is past the 90-day date. Thank you for your assistance.

Gloria Chang
Gloria Chang, R.Ph./Interdisciplinary Scientist

Attachments

95S-0316

RPT/63



MAR 12 2003

Marc S. Ullman
Ullman, Shapiro & Ullman, LLP
Counselors At Law
299 Broadway, Suite 1700
New York, New York 10007

Dear Mr. Ullman:

This letter acknowledges receipt of your new dietary ingredient notification, dated November 21, 2002, that was originally filed with the Food and Drug Administration (FDA) on November 22, 2002. Subsequently, we requested additional information in accordance with Title 21 of the Code of Federal Regulations (21 CFR) Part 190.6. We received the addendum with this information from you on December 27, 2002, which is the new effective filing date. Your notification is for the substance Sesamin, which you assert to be a new dietary ingredient. You state in your notification that the recommended use is one gelcap containing 10 mg of Sesamin along with natural vitamin E, wheat germ oil, and other ingredients.

In accordance with 21 C.F.R. 190.6 (c), FDA is acknowledging its receipt of a notification for a new dietary ingredient. For 75 days after the filing date, you must not introduce or deliver for introduction into interstate commerce any dietary supplement that contains Sesamin.

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.

Your notification states that the intended use of your client's product is as "As a dietary supplement, for use in supporting healthy liver function including improved detoxification of active metabolites, alcohol, toxic compounds, and environmental carcinogens and for its vitamin E sparing effect in the liver." Under 21 U.S.C. 321(g)(1)(B), if a product is implicitly or expressly represented as being intended for use in the diagnosis, cure, mitigation, treatment, or prevention of a disease, it may be subject to regulation under the drug provisions of the Act. Claims that a product is intended to protect a consumer from environmental carcinogens may subject a product to regulation as a drug under the Act and not as a dietary supplement. Your client can find information on claims that may be made in the labeling of dietary supplements pursuant to 21 U.S.C. 343(r)(6) in the final rule on structure/function claims published in the January 6, 2000 Federal Register (65 FR 1000).

Page 2 - Marc S. Ullman

Your notification will be kept confidential for 90 days after the filing date of December 27, 2002. After March 27, 2003, the notification will be placed on public display at FDA's Docket Management Branch in docket number 95S-0316. Prior to March 27, 2003, you may wish to identify in writing specifically what information in your notifications you believe is proprietary, trade secret or otherwise confidential information, which should not be disclosed to the public.

If you have any questions concerning this matter, please contact us at (301) 436-2375.

Sincerely yours,



Susan Walker, M.D.
Acting Director,
Division of Dietary Supplement Programs
Office of Nutritional Products, Labeling
and Dietary Supplements
Center for Food Safety
and Applied Nutrition

NDI 185
CTS 82668

NDI185 OML Sesamin

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December 23, 2002

Via Fed Ex

Division of Standards and Labeling Regulations
Office of Nutritional Products, Labeling, and Dietary Supplements (HFS-820)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: Addendum to Notification for Sesamin - Project No. 82668

Dear Ms. Chang:

Per your request, Suntory Ltd. is hereby amending its Notification of intent to market the New Dietary Ingredient, Sesamin. Enclosed is a revised copy of the expert panel report as provided by Dr. Myoda along with the additional articles you requested. References 25, 26, 27, and 28 have been inserted in section 4.4b (Anti-oxidative Effects of Sesamin) and section 5 (Safety Data):

24. K. Akimoto *et al.*, *J. Brewing Soc. Japan*, 89, 787 (1994) (in Japanese) (previously listed but not attached).
25. Y. Toyota, *Japan Food Sciences*, 39, 41 (2000) (in Japanese) (citing Reference 26 and 27).
26. T. Ikeda *et al.*, Protective Effects of Sesamin for Serum Lipid Peroxidation during Heard Exercise, *25th General Assembly of the Japan Medical Congress 1999, Tokyo* (in Japanese with English translation).
27. Y. Nishijima *et al.*, Mode of Protective Action fo Sesamin for Serum Lipid Peroxidation, *25th General Assembly of the Japan Medical Congress 1999, Tokyo* (in Japanese with English translation).

Rec.
12/27/02
M. Chang

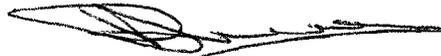
ULLMAN, SHAPIRO & ULLMAN, LLP

To: Division of Standards and Labeling Regulations
From: Marc S. Ullman; Vanessa Riviere
Re: Addendum to Notification for Sesamin - Project No. 82668
Date: December 23, 2002
Page 2

28. R. V. Cooney *et al.*, *Nutrition and Cancer* 39, 66-71 (2001) (previously cited but not attached).

Very truly yours,

ULLMAN, SHAPIRO & ULLMAN, LLP



Marc S. Ullman,
Vanessa Riviere,
Awaiting admission in New York & New
Jersey

Encls.

1. Introduction:

What are Sesamin and SesaminE?

“SesaminE” is the trademark of Sesamin (CAS Registry No. 607-80-7) containing gels manufactured by Suntory, Ltd. of Japan. Source of sesamin is sesame seeds. Sesame seed has been considered and consumed as a very healthy food for over 6,000 years throughout the world. The main component of sesame seed is fatty acids occupying over 50% of its weight, with protein as second major, 20%. Other components include several vitamins, carbohydrates, minerals, dietary fibers, and distinctive sesame lignans. Over 50% of sesame lignans is a compound named “Sesamin” comprising about 0.5 to 1.0% of total sesame seed weight. Recently, scientific research has shown that very small components of sesame lignans perform very important physiological functions in the body.

How SesaminE is produced?

Sesamin is obtained from whole sesame seeds as described in Attachment 2. Sesame seeds are pressed and sesame oil separated by filtration. After oil is separated, residues are subjected to molecular distillation, then crystallized to give Sesamin with >97% purity. Sesamin thus obtained has a geometrical isomer, Episesamin (CAS Registry No. 607-80-7) at the ratio of one to one (Fig. 1). Episesamin is formed during the refining process of non-roasted sesame seed oil. Efficacy of isolated episesamin is equal to that of Sesamin.

Natural vitamin E from soybeans and wheat germ oil are added to the purified sesamin and capsulized to give SesaminE gels. Other ingredients, namely glycerin and gelatin, to make gels are all natural products.

Minimum daily-recommended dose of one gel (600mg) contains:
Sesamin, 10mg; protein, 0.20g; carbohydrate, 0.04g; lipid, 0.61g; sodium, 0.66 mg; vitamin E (as alpha-tocopherol), 60mg; calories, 6.42kcal.
Total weight: 870mg, Fill weight: 600mg, Shell weight:270mg

2. Chemistry Considerations Concerning Sesamin

2.1 Chemical Name: Sesamin

2.2 Chemical Abstract Service (CAS) Registry Number: [607-80-7]

2.3 Chemical Synonyms:

5,5'-(tetrahydro-1*H*,3*H*-furo[3,4-*c*]furan-1,4-diyl)bis-1,3-benzodioxol;
tetrahydro-1,4-bis[3,4-(methylenedioxy)phenyl]-1*H*,3*H*-furo[3,4-*c*]furan;
2,6-bis(3,4-methylenedioxyphenyl)-3,7-dioxabicyclo[3.3.0]octane;
3,4:3',4'-bis(methylenedioxy)-7,9':7',9-diepoxyignan; Psudocubebin

2.4 Chemical Structure: See Fig. 1

2.5 Molecular Formula: C₂₀H₁₈O₆

2.6 Molecular Weight: 354.359

2.7 Chemical Properties:

d-form. Needles from ethanol, melting point 122-123C, $[\alpha]_D^{20} +64.5^\circ$
(*c*=1.75 in chloroform)

2.8 Chemical Name: (+) Episesamin

2.9 Chemical Abstract Service (CAS) Registry Number: [133-03-9]

2.10 Chemical Synonyms:

[1*R*-(1*α*,3*α*,4*β*,6*αβ*)]-5,5'-(tetrahydro-1*H*,3*H*-furo[3,4-*c*]furan-1,4-diyl) bis-1,3-
benzo-dioxole; Asarinin;

2.11 Chemical Structure: See Fig. 1

2.12 Molecular Formula: C₂₀H₁₈O₆

2.13 Molecular Weight: 354.359

2.14 Physical and Chemical Properties

Crystals from ethanol, melting point, 121-122, $[\alpha]_D^{25} -124^\circ$ (chloroform). Poorly
Soluble in water.

3. Proposed and Intended Uses

3.1 Proposed Use:

As a dietary supplement under the DSHEA regulations in the U.S. and applicable health regulations in Canada. We may in the future want to use SesaminE in special foods for dietary support in the U.S. as a bulk ingredient in complex nutritional foods for special

dietary needs.

3.2 Intended Use:

As a dietary supplement, for use in supporting healthy liver function including improved detoxification of active metabolites, alcohol, toxic compounds, and environmental carcinogens and for its vitamin E sparing effect in the liver.

4. Biological Functions of Sesamin

Biological functions of Sesamin have been investigated aggressively for 20 years, including inhibitory effect of delta-5 desaturase (1), reduction of serum cholesterol level (2-6), reduction of hypertension (7-9), anti-oxidant activity (10), protection of hepatic functions from disorder (11), protection from generation of breast cancer (12), and immunoactivating functions (13,14). In addition to functions of sesamin itself, synergistic effects with alfa-tocopherol have also been reported (5). Serum alcohol level is lower with alcohol administration with sesamin in rat experiments. Similar results in human experiments were obtained (24).

Listing of participants and titles at Sesamin Forum held in Kyoto, March 6, 2001 indicates the many interesting aspects of biological activities by sesamin.

Prof. S. Shimizu, Kyoto University, "Encounter with Sesamin"

Prof. Y. Matsumura, Osaka University of Pharmaceutical Sciences, "Anti-High Blood Pressure"

Prof. R. Sawada, Ochanomizu University, "Lipid Metabolism; Stimulation of Degradation of Fatty Acids and Control of n-6/n-3 Balance"

Dr. M. Nagai, Suntory Ltd., "Sesamin anti-Oxidation Mechanism"

Prof. K. Nagai, Osaka University, "Effects of Sesamin and Astaxanthin on Rat Behavior"

Prof. K. Yamashita, Sugiyama Jogakuen University, "Anti-aging Effects"

Prof. T. Moritani, Kyoto University, "Sesamin and Exercise"

Prof. Emeritus T. Ishikawa, Tokyo University, "Protective Action for Liver Cancer"

Prof. Emeritus H. Wada, Osaka University, "Concluding Remarks"

4.1 Metabolism of Sesamin

Sesamin has been investigated for multiple physiological functions such as anti-oxidant activity *in vivo*; however, its metabolism has not been clarified to date.

The metabolites of a reaction of Sesamin with rat liverS9 mixture with presence of co-enzyme were analyzed. Results indicated that Sesamin molecule was modified with one of its methylenedioxyphenyl moiety into catechol moiety with two hydroxyl groups by the liver enzymes (Fig. 2). Although Sesamin itself does not show any functions *in vitro*, the catechol type modified Sesamin acts as a scavenger for active oxygen and also shows protective effect of generation of fatty acid peroxide. The results of *in vivo* metabolism of Sesamin indicated that

orally ingested Sesamin is absorbed mainly through the portal vein, transferred into liver efficiently, and then modified to catechol type by liver enzymes. The catechol type sesamin is conjugated with glucuronic acid and excreted into bile as it is or further modified methoxy form. Results also indicate that the transferring ratio of Sesamin and its metabolite into serum is much smaller than that in the liver.

4.2 Reduce serum cholesterol

a) Animal studies

The reducing effects of serum cholesterol level in rats or hamsters have been clarified in recent studies (2-5), and the effects found to be enhanced under coexistence of alfa-tocopherol (5). The influence of Sesamin and alfa-tocopherol to rat hypercholesterolemia induced by cholesterol overload was investigated. After free ingestion of 0.05 and 0.2% of Sesamin contained diet for two weeks, serum cholesterol level of rats reduced by dose depended mode compared with control. Furthermore, 1.0% addition of alfa-tocopherol, which does not show any influence by itself, reduced serum cholesterol level synergistically with Sesamin (Table I-I). Alfa-tocopherol also shows dose-dependence for serum cholesterol level with presence of Sesamin (Table I-II). These effects indicate that the anti-oxidative effect of alfa-tocopherol considerably retaining Sesamin's effects.

b) Human studies

Reduction of serum cholesterol levels by Sesamin was also confirmed in humans. Hirata *et al.* reported positive effect of Sesamin on twelve hypercholesterolemia patients by single blind experiment (6).

Twelve hypercholesterolemia patients were divided into two groups. Intake of one group was nine capsules of Sesamin daily for the first four weeks and eighteen capsules daily the next four weeks, respectively. Each capsule contained 3.6 mg of Sesamin with 18 mg of alfa-tocopherol in 180 mg of wheat germ oil. Intake of other group consisted of placebo capsules containing only wheat germ oil. After eight weeks of intake, total serum cholesterol levels (T-CHO) and LDL cholesterol (LDL-C) levels of Sesamin-administered group reduced significantly (Fig.3). LDL cholesterol is considered a risk factor of arteriosclerosis; hence, Sesamin is expected to be a preventative agent of arteriosclerosis by reducing LDL cholesterol. The mechanism of cholesterol reducing effect is explained as inhibition of cholesterol uptake from intestine, promotion of cholesterol excretion into bile, inhibition of HMG-CoA reductase activity (3),

inhibition of cholesterol acetyltransferase activity (15), and activation of 7- α -hydroxylase (3).

4.3 Reduction of hypertension

Anti-hypertension effect of Sesamin is reported for DoCA-salt overload induced hypertension model and kidney clipping induced renal hypertension model (7,8). Also Sesamin showed protective effect against development and maintenance of hypertension in stroke-prone spontaneously hypertensive rats (SHRSP) (9).

Fourteen animals (SHRSP, male 6 weeks of age) were divided into two groups: normal diet group and Sesamin-diet group, both maintained on 1% NaCl drinking water. Systolic blood pressure of all animals was monitored once each week (Fig. 4). At the end of the feeding periods, cardiovascular hypertrophy and renal damage were evaluated. This showed that the Sesamin feeding group significantly suppressed development of hypertension. The left ventricle plus septum weight-to-body weight ratio was slightly, but significantly, lowered by Sesamin feeding. When the degree of vascular hypertrophy of the aorta and superior mesenteric artery was histochemically evaluated, wall thickness and wall area of those vessels were significantly decreased by the Sesamin feeding.

The mechanism of suppression of hypertension by Sesamin is not clear at this point; however, since Sesamin suppresses some kind of calcium-dependent vasoconstriction, calcium antagonism of Sesamin is considered to be part of the mechanism. Correlation between hypertension and oxygen free radicals, especially superoxide, has recently been indicated (16,17). For example, it is reported that the intravenous administration of superoxide desmutase (SOD) suppresses hypertension of SHR rats (18). This suggests that the anti-oxidative effect of Sesamin is related to suppression of hypertension.

4.4 Anti-oxidative effect

a) Animal studies

Recently, a method of radical measurement *in vivo* by means of L-band electron spin resonance spectroscopy (ESR) has achieved progress. Ogata *et al.* measured and monitored the decay of nitroxyl radical *in vivo* by using local surface-coil-type resonator (19,20). Subsequently the effect of Sesamin on decaying of nitroxyl radical in rat liver by local surface coil ESR method was studied.

Animals (Wistar, male, 10 weeks of age) ingested 250 mg/kg (body weight) of Sesamin suspended in olive oil. Three hours after ingestion, 0.2 M of 4-hydroxy-2,2,6,6-tetramethyl-piperidine-1-oxy (TEMPOL) was injected intravenously 5 times every 5 minutes, and the decay of TEMPOL from liver, kidney, and vein by surface coil then monitored.

Results show that the decay rate of TEMPOL in the liver is obviously accelerated in Sesamin administered group (Fig.5) with no effect of Sesamin observed in kidney or vein. Therefore, Sesamin was considered to show anti-oxidative function effectively in the liver. Results were consistent with those in the metabolism study.

b) Human studies

The anti-oxidative effects of Sesamin toward lipid peroxidation in humans was investigated (26). Six healthy male adults (average age 25.3 years old) participated in this experiment, and crossover trials with Sesamin (36mg) capsule and placebo capsule were carried out. The participant abstained from food for 10 hours before exercise, then ingested test capsules and carbohydrate (300kal) 2 hours before exercise. Two hours after ingestion of the test capsules, participants exercised vigorously via bicycle ergometer (HR max 80%) for 30 minutes. Blood samples were collected every 5 minutes and serum lipid peroxide (LPO) concentration measured. Results showed that the placebo group significantly increased their serum LPO 10 and 20 minutes after starting exercise. On the other hand, Sesamin group completely suppressed increase of serum LPO (Fig.6). Vigorous exercise requires huge amount of oxygen, and induced oxygen free radicals that cause generation of serum LPO. Therefore, Sesamin seems to trap oxygen free radicals generated during vigorous exercise. Additionally(27), it was confirmed that the total level of glutathione-peroxidase (total-GPX) and glutathione-S-transferase (GSH) level increased in the livers of Sesamin treated mice; therefore, Sesamin seems to be effective in inducing LPO metabolism system in the body.

4.5 Protection of liver functions

Protective effect of Sesamin from experimentally induced alcoholic hepatic disorders in mice using ethanol chamber (11) was also investigated.

Mice were divided into 2 groups of normal diet and Sesamin diet (15). Normal diet group increased serum GOP, GPT, TG and T-BIL levels significantly, and also induced fatty liver caused by continuous ethanol inhalation; however, Sesamin group suppressed such influences

(Table II). On the other hand, single administration of Sesamin (100mg/kg body weight) for hepatic disorder induced by carbontetrachloride does not suppress serum transaminase level, but reduced total cholesterol and TG levels significantly. This is also a protective effect of Sesamin from hepatic disorder (11).

It is known that excessive ethanol consumption inhibits fatty acid metabolism and accumulates fat in the liver. Sesamin is considered to suppress ethanol-induced disorders by promoting ethanol metabolism (16) and beta-oxidation of fatty acids (19).

4.6 Controlling fatty acid metabolism

Significance of the balance of n-6 and n-3 polyunsaturated fatty acid (LCPUFA) in the body has been a focus of constant attention. Ingestion of some specific fatty acids as eicosapentaenoic acid (EPA) or docosaheptaenoic acid (DHA) induces extremely unbalanced n-6/n-3 LCPUF ratio, but administration of Sesamin reduced the ratio to normal (22,23). Also influence of Sesamin for change of total fatty acid level in the body induced by gradually increasing of plant oil administration was investigated.

By increasing the administration of plant oil, total fatty acid in the liver increased; however, Sesamin intake suppressed the total fatty acid level elevation. Sesamin induces beta-oxidation of fatty acids in the liver; therefore, there is a possibility that the effect suppresses the total level increase (21).

4.7 Suntory's Seminar Slides and Texts

In collaboration with several universities Suntory has published more than 40 original articles concerning biological functions of Sesamin. On the basis of their studies slides and texts (Attachment 6) were prepared by Suntory and used for seminars. Some awkwardness might exist in English translation but findings are well summarized and were useful for the Panel's evaluation.

5. Safety Data

Report of "Two-week Repeated Oral Administration Toxicity Study of Sesamin in Mice" is attached (Attachment 3). Also "Mutagenicity Study of Sesamin" (Ames Test) was attached as

Attachment 4. There is no abnormality. LD50 for mice could not be obtained since 2.14 g/day/kg (corresponding to approximately 128 g/day/kg in human) did not kill mice.

There is the published human study by Cooney, R.V., Custer, L.J., Okinaka, L. and Adrian Franke on Effects of dietary Sesame Seeds on Plasma Tocopherol Levels, Nutrition and Cancer 39, 1: 66-71, 2001 (28). This study employed 22 grams of sesame seeds/day in humans, so the intake of Sesamin from that source was about 48 mg per day for a full week. There is no limit on the amount of sesame seeds allowed in the human diet in the US, and there are not data indicating that consumption of sesame seeds has been a problem or may be toxic in any way. In fact, there is a significant benefit in that sesame seeds are known source of tocopherols, and sesamin and other lignans in sesame seeds are known to protect tocopherols and raise plasma levels in human and animals. So, the Net effect would appear to be that sesamin is in fact beneficial rather than just non-toxic, and as far as I know there have never been any studies that indicate Sesamin may have any adverse effect.

SesaminE has been also approved as food supplement and marketed in Japan since mid 1993. Since then, sales have been increasing steadily every year. There is no accurate figures available on how many people have been using it but from sales, at least a few hundred thousand people have been using it without any adverse effects. Incidentally, a member of the Panel, Prof. Myoda, has been taking 30-50mg every day for nine years. His cholesterol level is high-normal but due to high value of HDL, high 80's to low 90's. Health check ups indicate no liver problems in spite of alcohol consumption. Not only by clinical trials described in the previous chapter, Biological Functions of Sesamin, but also these data by public consumption surely indicate that SesaminE is very safe.

As shown in Attachment 7, there are no detectable 34 kinds of pesticides and heavy metals.

6. Stability/Shelf Life of SesaminE

SesaminE is very stable. No significant change was found after two years storage at 25°C. Even accelerated storage test at 40°C, under 70% humidity for one year did not alter quality. After two years under these conditions, capsule decay was observed. However, Sesamin content was not changed and the peroxide value was still within a reasonable range (Attachment 5).

7. Conclusion

The liver plays important roles in accumulation of energy, synthesis and/or degradation of physiologically important molecules, and detoxification. SesaminE suppresses liver cell damage by its anti-oxidative effect and most importantly control functions of metabolic enzymes. Physiological functions of plant polyphenol such as catechin have been widely studied; however, it is difficult to be transferred into the liver because it has some hydrophilic moieties. The significant feature of Sesamin is that it can be transferred very smoothly into the liver, and then converted to polyphenols. This converted Sesamin is

able to trap oxygen free radicals in the liver cells.

As the conclusion, the above data indicated that SesaminE is not only safe for human consumption but also useful for keeping good health.

References (Revised)

1. S. Shimizu *et al.*, *Lipids*, **26**, 512 (1991)
2. M. Sugano *et al.*, *Agric. Biol. Chem.*, **54**, 2660 (1990)
3. N. Hirose *et al.*, *J. Lipid Res.*, **32**, 629 (1991)
4. T. Ogawa *et al.*, *Carcinogenesis*, **15**, 1663 (1994)
5. A. Nakabayashi *et al.*, *Internal. J. Nutr. Res.*, **65**, 162 (1995)
6. F. Hirata *et al.*, *Atherosclerosis*, **122**, 135 (1996)
7. Y. Matsumura *et al.*, *Biol. Pharm. Bull.*, **18**, 1016 (1995)
8. S. Kita *et al.*, *Ibid.*, **18**, 1283 (1995)
9. Y. Matsumura *et al.*, *Ibid.*, **21**, 469 (1998)
10. K. Yamashita *et al.*, *Lipids*, **30**, 1019 (1995)
11. K. Akimoto *et al.*, *Ann. Nutr. Metab.*, **37**, 218 (1993)
12. N. Hirose *et al.*, *Anticancer Research*, **12**, 1259 (1992)
13. JY. Gu *et al.*, *Biosci. Biotech. Biochem.*, **58**, 1885 (1994)
14. JY. Gu *et al.*, *Ibid.*, **59**, 2198 (1995)
15. R. U. Sawada *et al.*, *Biosci. Biotech. Biochem.*, **58**, 2144 (1994)
16. X. Zhang *et al.*, *Am. J. Physiol.*, **259**, 497 (1990)
17. M. Torii *et al.*, *Jpn. Heart J.*, **30**, 586 (1989)
18. K. Nakazone *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, **88**, 10045 (1991)
19. H. Hirata *et al.*, *Rev. Sci. Instrum.*, **66**, 4529 (1995)
20. Y. Lin *et al.*, *MAGMA*, **5**, 99 (1997)
21. L. Ashakumary *et al.*, *Metabolism*, **48**, 1303 (1999)
22. R. U. Sawada *et al.*, *Biosci. Biotech. Biochem.*, **59**, 2268 (1995)
23. R. U. Sawada *et al.*, *Lipids*, **33**, 567 (1998)
24. K. Akimoto *et al.*, *J. Brewing Soc. Japan*, **89**, 787 (1994) (in Japanese)
25. Y. Toyota, *Japan Food Sciences*, **39**, 41 (2000)(review in Japanese)
26. T. Ikeda *et al.*, Abstract, 25th General Assembly of the Japan medical Congress
*The Commemorative symposium of the Japanese Society of Physical
Fitness and Sports Medicine*, 1999, Tokyo
27. Y. Nishijima *et al.*, *Ibid*, Abstract
28. R. V. Cooney *et al.*, *Nutrition and Cancer*, **39**, 66-71 (2001)

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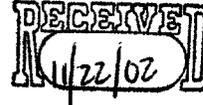
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Via Fed Ex

November 21, 2002

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Food and Drug Administration
5100 Paint Branch Parkway
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Re: New Dietary Ingredient Notification for Sesamin

Dear Sir/Madame,

Pursuant to 21 CFR §190.6, please be advised that Suntory, Ltd. of Osaka, Japan,¹ is hereby providing you with notification of its intent to market a New Dietary Ingredient, namely Sesamin. Suntory will commence marketing of its Sesamin product 75 days after acknowledgment of your receipt of this notification, unless otherwise instructed by your agency. Enclosed with this original document are two additional copies of Suntory submission and the attachments thereto.

Based upon the following, Suntory respectfully submits that there are no safety issues relating to its intended marketing of Sesamin:

¹ The company's facilities are located at Dojimahama 2-1-40, Kita-ku, Osaka, Osaka 530-8203, Japan.

ULLMAN, SHAPIRO & ULLMAN, LLP

To: Division of Standards and Labeling Regulations
From: Marc S. Ullman; Vanessa Riviere, Law Clerk
Re: New Dietary Ingredient Notification for Sesamin
Date: November 21, 2002

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1. Suntory Ltd., was founded in 1899 as a food and liquor business. The company has since successfully expanded into the pharmaceutical, flower and health food industries. In 1992, building on its expertise in biotechnology, Suntory started the Consumer Health Products Division to develop new health food products. The company currently maintains restaurants, offices and production plants throughout the world. All Suntory production plants engage in TPM (Total Productive Maintenance) for quality assurance. The company is also introducing the internationally recognized sanitation control system HACCP to achieve even higher quality assurance.
2. Sesamin is obtained from pressing whole sesame seeds and separating the sesame oil by filtration. Sesame seeds have been consumed as food for over 6,000 years throughout the world and are a known source of tocopherol. Sesamin and other lignans in sesame seeds are also known to protect tocopherals and raise plasma levels in humans and animals.
3. The name of the new dietary ingredient is Sesamin, which will initially be marketed under the trade name SesaminE.TM SesaminE is intended for use as a dietary supplement in supporting healthy liver function. It has been approved and marketed as a food supplement in Japan since 1993. Records indicate that at least a few hundred thousand people have been safely using the product. There are no known studies indicating adverse effects associated with the consumption of Sesamin.
4. SesaminE will first be marketed in capsule form. Natural vitamin E from soybeans and wheat germ oil are added to the purified Sesamin and capsulized to produce SesaminE gels. The minimum daily recommended dose of one gel (600mg) contains sesamin (10mg), protein (0.20g), carbohydrate (0.04g), lipid (0.51g), sodium (0.66mg), and vitamin E (60mg) (as alpha-tocopherol).
5. The biological functions of Sesamin have been investigated aggressively for 20 years, including the inhibitory effect of delta-5 desaturase, the reduction of serum cholesterol level, the reduction of hypertension, the anti-oxidant activity, the protection of hepatic functions from disorder, the protection from generation of breast cancer, and immunoactivating functions.

ULLMAN, SHAPIRO & ULLMAN, LLP

To: Division of Standards and Labeling Regulations
From: Marc S. Ullman, Vanessa Riviere, Law Clerk
Re: New Dietary Ingredient Notification for Sesamin
Date: November 21, 2002

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6. As noted in the Expert Panel Report,² the results of *in vivo* metabolism of Sesamin indicate that orally ingested Sesamin is absorbed mainly through the portal vein, transferred into the liver efficiently, and then modified to catechol type by liver enzymes. Sesamin is also considered to show an anti-oxidative function in the liver.
7. Animal studies as well as human studies confirm the reduction of serum cholesterol levels by Sesamin. A single blind study of twelve hypercholesterolemia patients showed that after eight weeks of intake, total serum cholesterol levels and LDL cholesterol levels of the Sesamin administered group were significantly reduced.
8. The Expert Panel Report further indicates that Sesamin has been shown to have a protective effect against the development and maintenance of hypertension in stroke-prone spontaneously hypertensive rats.
9. The attached Toxicity Study of Sesamin concludes that there are no abnormal symptoms associated with the administration of Sesamin [Attachment 3]. The attached Mutagenic Study concludes that Sesamin has no mutagenic activity on the Ames/Salmonella test. After reviewing the toxicology reports and technical information, Dr. Kim C. Krumhar, Ph.D. (curriculum vitae attached), concludes that Sesamin presents “no more safety concern than the consumption of reasonable quantities of dietary sesame seeds, wheat germ oil and gelatin.” [Attachment 7].
10. A Sesamin Forum was held in Tokyo in March 2001. The Panel, consisting of eight speakers including seven professors, unanimously concluded that Sesamin is not only safe but is considered to be Generally Recognized as Safe (GRAS) based on scientific procedures. Seminar slides and texts are included as Attachment 6.
11. The Expert Panel concludes that “SesaminE is not only safe for human consumption but also useful for keeping good health.”

Based on the foregoing, we believe that FDA should accept this filing on behalf of Suntory, Inc. as providing sufficient evidence that SesaminE, Sesamin extract, can reasonably be expected to be safe for human consumption.

² The Expert Panel consists of T. Timothy Myoda, Ph.D., Sakayu Shimizu, Ph.D., and Toshio Moritani, Ph.D. Their respective curriculum vitae are attached as Attachment 1.

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If you require any additional information, please direct call all correspondence to the undersigned.

Very truly yours,

ULLMAN, SHAPIRO & ULLMAN, LLP



Marc S. Ullman,
Vanessa Riviere,
Awaiting admission in New Jersey
Not admitted in New York

Encls.

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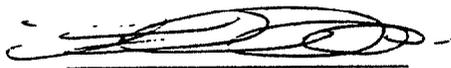
Expert Panel Statements
Use of SesaminE in Dietary Supplements

The undersigned, an independent panel of recognized experts (the Expert Panel), qualified by their scientific and/or medical training, and relevant national and international experience in evaluation of foods and food supplements, was requested by Suntory Ltd. to determine the safety and efficacy of SesaminE for use as a new dietary ingredient in dietary supplements. The qualifications of the Expert Panel members are evidenced in their curriculum vitae, provided in Appendix 1.

A comprehensive search of the available literature concerning nutritional, safety and toxicity through December 2001 was conducted by Prof. T. Timothy Myoda, Ph.D. and made available to the other members of the Panel, Prof. Sakayu Shimizu, Ph.D. and Prof. Toshio Moritani, Ph.D. The Panel independently evaluated pertinent articles, material provided by Suntory and all other data and information deemed appropriate or necessary. The Panel considered specifically data and information relating to biological activities and physical and chemical properties of SesaminE, as well as daily intake of SesaminE as in conventional foods and dietary supplements.

Following evaluations of all the data and information, the Panel conferred and also met several times with representatives from Suntory. The Panel also participated in a Sesamin Forum held in Kyoto, March 6, 2001 featuring eight speakers including seven professors covering different aspects of SesaminE activity. The Panel unanimously concluded that under intended use, appropriate food grade specifications and current Japanese Good Manufacturing practices for foods and dietary supplements SesaminE is not only safe and useful but is believed to be qualified as Generally Recognized as Safe (GRAS) based on scientific procedures. The attached are summarized reports and information considered by the Panel and which establish the bases for the Panel's conclusions.

By:


T. Timothy Myoda, Ph.D. Sept. 16, '02
Date


Sakayu Shimizu, Ph.D. 11/09/02
Date


Toshio Moritani, Ph.D. 11/09/02
Date

1. Introduction:

What are Sesamin and SesaminE?

“SesaminE” is the trademark of Sesamin (CAS Registry No. 607-80-7) containing gels manufactured by Suntory, Ltd. of Japan. Source of sesamin is sesame seeds. Sesame seed has been considered and consumed as a very healthy food for over 6,000 years throughout the world. The main component of sesame seed is fatty acids occupying over 50% of its weight, with protein as second major, 20%. Other components include several vitamins, carbohydrates, minerals, dietary fibers, and distinctive sesame lignans. Over 50% of sesame lignans is a compound named “Sesamin” comprising about 0.5 to 1.0% of total sesame seed weight. Recently, scientific research has shown that very small components of sesame lignans perform very important physiological functions in the body.

How SesaminE is produced?

Sesamin is obtained from whole sesame seeds as described in Attachment 2. Sesame seeds are pressed and sesame oil separated by filtration. After oil is separated, residues are subjected to molecular distillation, then crystallized to give Sesamin with >97% purity. Sesamin thus obtained has a geometrical isomer, Episesamin (CAS Registry No. 607-80-7) at the ratio of one to one (Fig. 1). Episesamin is formed during the refining process of non-roasted sesame seed oil. Efficacy of isolated episesamin is equal to that of Sesamin.

Natural vitamin E from soybeans and wheat germ oil are added to the purified sesamin and capsulized to give SesaminE gels. Other ingredients, namely glycerin and gelatin, to make gels are all natural products.

Minimum daily-recommended dose of one gel (600mg) contains:
Sesamin, 10mg; protein, 0.20g; carbohydrate, 0.04g; lipid, 0.61g; sodium, 0.66 mg; vitamin E (as alpha-tocopherol), 60mg; calories, 6.42kcal.
Total weight: 870mg, Fill weight: 600mg, Shell weight:270mg

2. Chemistry Considerations Concerning Sesamin

2.1 Chemical Name: Sesamin

2.2 Chemical Abstract Service (CAS) Registry Number: [607-80-7]

2.3 Chemical Synonyms:

5,5'-(tetrahydro-1*H*,3*H*-furo [3,4-*c*] furan-1,4-diyl)bis-1,3-benzodioxol;
tetrahydro-1,4-bis [3,4-(methylenedioxy)phenyl] -1*H*,3*H*-furo [3,4-*c*] furan;
2,6-bis(3,4-methylenedioxyphenyl)-3,7-dioxabicyclo [3.3.0] octane;
3,4:3',4'-bis(methylenedioxy)-7,9':7',9-diepoxy lignan; Pseudocubebin

2.4 Chemical Structure: See Fig. 1

2.5 Molecular Formula: C₂₀H₁₈O₆

2.6 Molecular Weight: 354.359

2.7 Chemical Properties:

d-form. Needles from ethanol, melting point 122-123C, $[\alpha]_D^{20} -64.5^\circ$
(*c*=1.75 in chloroform)

2.8 Chemical Name: (+) Episesamin

2.9 Chemical Abstract Service (CAS) Registry Number: [133-03-9]

2.10 Chemical Synonyms:

[1*R*-(1*α*,3*αα*,4*β*,6*αβ*)]-5,5'-(tetrahydro-1*H*,3*H*-furo [3,4-*c*] furan-1,4-diyl) bis-1,3-
benzo-dioxole; Asarinin;

2.11 Chemical Structure: See Fig. 1

2.12 Molecular Formula: C₂₀H₁₈O₆

2.13 Molecular Weight: 354.359

2.14 Physical and Chemical Properties

Crystals from ethanol, melting point, 121-122, $[\alpha]_D^{25} -124^\circ$ (chloroform). Poorly
Soluble in water.

3. Proposed and Intended Uses

3.1 Proposed Use:

As a dietary supplement under the DSHEA regulations in the U.S. and applicable health regulations in Canada. We may in the future want to use SesaminE in special foods for dietary support in the U.S. as a bulk ingredient in complex nutritional foods for special

dietary needs.

3.2 Intended Use:

As a dietary supplement, for use in supporting healthy liver function including improved detoxification of active metabolites, alcohol, toxic compounds, and environmental carcinogens and for its vitamin E sparing effect in the liver.

4. Biological Functions of Sesamin

Biological functions of Sesamin have been investigated aggressively for 20 years, including inhibitory effect of delta-5 desaturase (1), reduction of serum cholesterol level (2-6), reduction of hypertension (7-9), anti-oxidant activity (10), protection of hepatic functions from disorder (11), protection from generation of breast cancer (12), and immunoactivating functions (13,14). In addition to functions of sesamin itself, synergistic effects with alfa-tocopherol have also been reported (5). Serum alcohol level is lower with alcohol administration with sesamin in rat experiments. Similar results in human experiments were obtained (24).

Listing of participants and titles at Sesamin Forum held in Kyoto, March 6, 2001 indicates the many interesting aspects of biological activities by sesamin.

Prof. S. Shimizu, Kyoto University, "Encounter with Sesamin"

Prof. Y. Matsumura, Osaka University of Pharmaceutical Sciences, "Anti-High Blood Pressure"

Prof. R. Sawada, Ochanomizu University, "Lipid Metabolism: Stimulation of Degradation of Fatty Acids and Control of n-6/n-3 Balance"

Dr. M. Nagai, Suntory Ltd., "Sesamin anti-Oxidation Mechanism"

Prof. K. Nagai, Osaka University, "Effects of Sesamin and Astaxanthin on Rat Behavior"

Prof. K. Yamashita, Sugiyama Jogakuen University, "Anti-aging Effects"

Prof. T. Moritani, Kyoto University, "Sesamin and Exercise"

Prof. Emeritus T. Ishikawa, Tokyo University, "Protective Action for Liver Cancer"

Prof. Emeritus H. Wada, Osaka University, "Concluding Remarks"

4.1 Metabolism of Sesamin

Sesamin has been investigated for multiple physiological functions such as anti-oxidant activity *in vivo*; however, its metabolism has not been clarified to date.

The metabolites of a reaction of Sesamin with rat liverS9 mixture with presence of co-enzyme were analyzed. Results indicated that Sesamin molecule was modified with one of its methylenedioxyphenyl moiety into catechol moiety with two hydroxyl groups by the liver enzymes (Fig. 2). Although Sesamin itself does not show any functions *in vitro*, the catechol type modified Sesamin acts as a scavenger for active oxygen and also shows protective effect of generation of fatty acid peroxide. The results of *in vivo* metabolism of Sesamin indicated that

orally ingested Sesamin is absorbed mainly through the portal vein, transferred into liver efficiently, and then modified to catechol type by liver enzymes. The catechol type sesamin is conjugated with glucuronic acid and excreted into bile as it is or further modified methoxy form. Results also indicate that the transferring ratio of Sesamin and its metabolite into serum is much smaller than that in the liver.

4.2 Reduce serum cholesterol

a) Animal studies

The reducing effects of serum cholesterol level in rats or hamsters have been clarified in recent studies (2-5), and the effects found to be enhanced under coexistence of alfa-tocopherol (5). The influence of Sesamin and alfa-tocopherol to rat hypercholesterolemia induced by cholesterol overload was investigated. After free ingestion of 0.05 and 0.2% pf Sesamin contained diet for two weeks, serum cholesterol level of rats reduced by dose depended mode compared with control. Furthermore, 1.0% addition of alfa-tocopherol, which does not show any influence by itself, reduced serum cholesterol level synergistically with Sesamin (Table I-I). Alfa-tocopherol also shows dose-dependence for serum cholesterol level with presence of Sesamin (Table I-II). These effects indicate that the anti-oxidative effect of alfa-tocopherol considerably retaining Sesamin's effects.

b) Human studies

Reduction of serum cholesterol levels by Sesamin was also confirmed in humans. Hirata *et al.* reported positive effect of Sesamin on twelve hypercholesterolemia patients by single blind experiment (6).

Twelve hypercholesterolemia patients were divided into two groups. Intake of one group was nine capsules of Sesamin daily for the first four weeks and eighteen capsules daily the next four weeks, respectively. Each capsule contained 3.6 mg of Sesamin with 18 mg of alfa-tocopherol in 180 mg of wheat germ oil. Intake of other group consisted of placebo capsules containing only wheat germ oil. After eight weeks of intake, total serum cholesterol levels (T-CHO) and LDL cholesterol (LDL-C) levels of Sesamin-administered group reduced significantly (Fig.3). LDL cholesterol is considered a risk factor of arteriosclerosis; hence, Sesamin is expected to be a preventative agent of arteriosclerosis by reducing LDL cholesterol. The mechanism of cholesterol reducing effect is explained as inhibition of cholesterol uptake from intestine, promotion of cholesterol excretion into bile, inhibition of HMG-CoA reductase activity (3),

inhibition of cholesterol acetyltransferase activity (15), and activation of 7- α -hydroxylase (3).

4.3 Reduction of hypertension

Anti-hypertension effect of Sesamin is reported for DoCA-salt overload induced hypertension model and kidney clipping induced renal hypertension model (7,8). Also Sesamin showed protective effect against development and maintenance of hypertension in stroke-prone spontaneously hypertensive rats (SHRSP) (9).

Fourteen animals (SHRSP, male 6 weeks of age) were divided into two groups: normal diet group and Sesamin-diet group, both maintained on 1% NaCl drinking water. Systolic blood pressure of all animals was monitored once each week (Fig. 4). At the end of the feeding periods, cardiovascular hypertrophy and renal damage were evaluated. This showed that the Sesamin feeding group significantly suppressed development of hypertension. The left ventricle plus septum weight-to-body weight ratio was slightly, but significantly, lowered by Sesamin feeding. When the degree of vascular hypertrophy of the aorta and superior mesenteric artery was histochemically evaluated, wall thickness and wall area of those vessels were significantly decreased by the Sesamin feeding.

The mechanism of suppression of hypertension by Sesamin is not clear at this point; however, since Sesamin suppresses some kind of calcium-dependent vasoconstriction, calcium antagonism of Sesamin is considered to be part of the mechanism. Correlation between hypertension and oxygen free radicals, especially superoxide, has recently been indicated (16,17). For example, it is reported that the intravenous administration of superoxide desmutase (SOD) suppresses hypertension of SHR rats (18). This suggests that the anti-oxidative effect of Sesamin is related to suppression of hypertension.

4.4 Anti-oxidative effect

a) Animal studies

Recently, a method of radical measurement *in vivo* by means of L-band electron spin resonance spectroscopy (ESR) has achieved progress. Ogata *et al.* measured and monitored the decay of nitroxyl radical *in vivo* by using local surface-coil-type resonator (19,20). Subsequently the effect of Sesamin on decaying of nitroxyl radical in rat liver by local surface coil ESR method was studied.

Animals (Wistar, male, 10 weeks of age) ingested 250 mg/kg (body weight) of Sesamin suspended in olive oil. Three hours after ingestion, 0.2 M of 4-hydroxy-2,2,6,6-tetramethyl-piperidine-1-oxy (TEMPOL) was injected intravenously 5 times every 5 minutes, and the decay of TEMPOL from liver, kidney, and vein by surface coil then monitored.

Results show that the decay rate of TEMPOL in the liver is obviously accelerated in Sesamin administered group (Fig.5) with no effect of Sesamin observed in kidney or vein. Therefore, Sesamin was considered to show anti-oxidative function effectively in the liver. Results were consistent with those in the metabolism study.

b) Human studies

The anti-oxidative effects of Sesamin toward lipid peroxidation in humans was investigated. Six healthy male adults (average age 25.3 years old) participated in this experiment, and crossover trials with Sesamin (36mg) capsule and placebo capsule were carried out. The participant abstained from food for 10 hours before exercise, then ingested test capsules and carbohydrate (300kal) 2 hours before exercise. Two hours after ingestion of the test capsules, participants exercised vigorously via bicycle ergometer (HR max 80%) for 30 minutes. Blood samples were collected every 5 minutes and serum lipid peroxide (LPO) concentration measured. Results showed that the placebo group significantly increased their serum LPO 10 and 20 minutes after starting exercise. On the other hand, Sesamin group completely suppressed increase of serum LPO (Fig.6). Vigorous exercise requires huge amount of oxygen, and induced oxygen free radicals that cause generation of serum LPO. Therefore, Sesamin seems to trap oxygen free radicals generated during vigorous exercise. Additionally, it was confirmed that the total level of glutathione-peroxidase (total-GPX) and glutathione-S-transferase (GSH) level increased in the livers of Sesamin treated mice; therefore, Sesamin seems to be effective in inducing LPO metabolism system in the body.

4.5 Protection of liver functions

Protective effect of Sesamin from experimentally induced alcoholic hepatic disorders in mice using ethanol chamber (11) was also investigated.

Mice were divided into 2 groups of normal diet and Sesamin diet (15). Normal diet group increased serum GOP, GPT, TG and T-BIL levels significantly, and also induced fatty liver caused by continuous ethanol inhalation; however, Sesamin group suppressed such influences

(Table II). On the other hand, single administration of Sesamin (100mg/kg body weight) for hepatic disorder induced by carbontetrachloride does not suppress serum transaminase level, but reduced total cholesterol and TG levels significantly. This is also a protective effect of Sesamin from hepatic disorder (11).

It is known that excessive ethanol consumption inhibits fatty acid metabolism and accumulates fat in the liver. Sesamin is considered to suppress ethanol-induced disorders by promoting ethanol metabolism (16) and beta-oxidation of fatty acids (19).

4.6 Controlling fatty acid metabolism

Significance of the balance of n-6 and n-3 polyunsaturated fatty acid (LCPUFA) in the body has been a focus of constant attention. Ingestion of some specific fatty acids as eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) induces extremely unbalanced n-6/n-3 LCPUF ratio, but administration of Sesamin reduced the ratio to normal (22,23). Also influence of Sesamin for change of total fatty acid level in the body induced by gradually increasing of plant oil administration was investigated.

By increasing the administration of plant oil, total fatty acid in the liver increased; however, Sesamin intake suppressed the total fatty acid level elevation. Sesamin induces beta-oxidation of fatty acids in the liver; therefore, there is a possibility that the effect suppresses the total level increase (21).

4.7 Suntory's Seminar Slides and Texts

In collaboration with several universities Suntory has published more than 40 original articles concerning biological functions of Sesamin. On the basis of their studies slides and texts (Attachment 6) were prepared by Suntory and used for seminars. Some awkwardness might exist in English translation but findings are well summarized and were useful for the Panel's evaluation.

5. Safety Data

Report of "Two-week Repeated Oral Administration Toxicity Study of Sesamin in Mice" is attached (Attachment 3). Also "Mutagenicity Study of Sesamin" (Ames Test) was attached as

Attachment 4. There is no abnormality. LD50 for mice could not be obtained since 2.14 g/day/kg (corresponding to approximately 128 g/day/kg in human) did not kill mice.

There is the published human study by Cooney, R.V., Custer, L.J., Okinaka, L. and Adrian Franke on Effects of dietary Sesame Seeds on Plasma Tocopherol Levels, Nutrition and Cancer 39, 1: 66-71, 2001. This study employed 22 grams of sesame seeds/day in humans, so the intake of Sesamin from that source was about 48 mg per day for a full week. There is no limit on the amount of sesame seeds allowed in the human diet in the US, and there are not data indicating that consumption of sesame seeds has been a problem or may be toxic in any way. In fact, there is a significant benefit in that sesame seeds are known source of tocopherols, and sesamin and other lignans in sesame seeds are known to protect tocopherols and raise plasma levels in human and animals. So, the Net effect would appear to be that sesamin is in fact beneficial rather than just non-toxic, and as far as I know there have never been any studies that indicate Sesamin may have any adverse effect.

SesaminE has been also approved as food supplement and marketed in Japan since mid 1993. Since then, sales have been increasing steadily every year. There is no accurate figures available on how many people have been using it but from sales, at least a few hundred thousand people have been using it without any adverse effects. Incidentally, a member of the Panel, Prof. Myoda, has been taking 30-50mg every day for nine years. His cholesterol level is high-normal but due to high value of HDL, high 80's to low 90's. Health check ups indicate no liver problems in spite of alcohol consumption. Not only by clinical trials described in the previous chapter, Biological Functions of Sesamin, but also these data by public consumption surely indicate that SesaminE is very safe.

As shown in Attachment 7, there are no detectable 34 kinds of pesticides and heavy metals.

6. Stability/Shelf Life of SesaminE

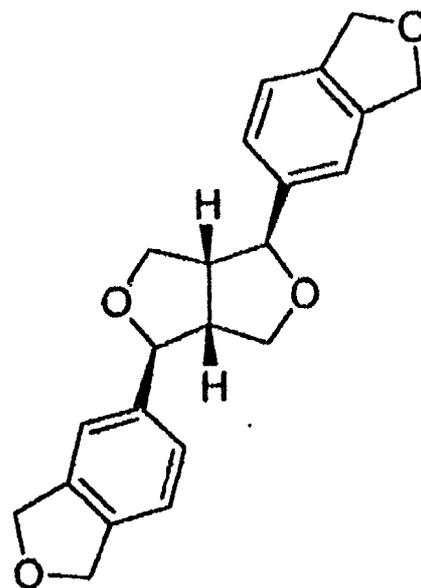
SesaminE is very stable. No significant change was found after two years storage at 25°C. Even accelerated storage test at 40°C, under 70% humidity for one year did not alter quality. After two years under these conditions, capsule decay was observed. However, Sesamin content was not changed and the peroxide value was still within a reasonable range (Attachment 5).

7. Conclusion

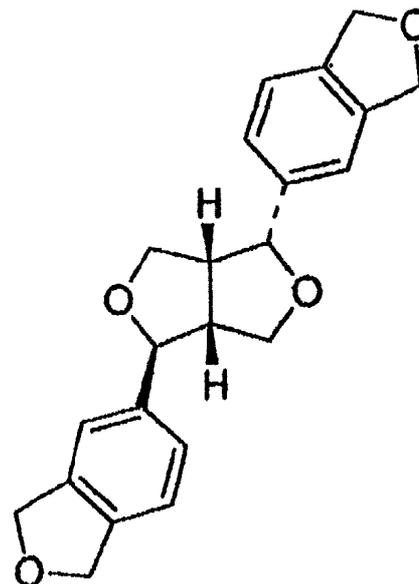
The liver plays important roles in accumulation of energy, synthesis and/or degradation of physiologically important molecules, and detoxification. SesaminE suppresses liver cell damage by its anti-oxidative effect and most importantly control functions of metabolic enzymes. Physiological functions of plant polyphenol such as catechin have been widely studied; however, it is difficult to be transferred into the liver because it has some hydrophilic moieties. The significant feature of Sesamin is that it can be transferred very smoothly into the liver, and then converted to polyphenols. This converted Sesamin is

able to trap oxygen free radicals in the liver cells.

As the conclusion, the above data indicated that SesaminE is not only safe for human consumption but also useful for keeping good health.



Sesamin



Episesamin

Fig. 1.
Chemical structure of sesamin and episesamin.

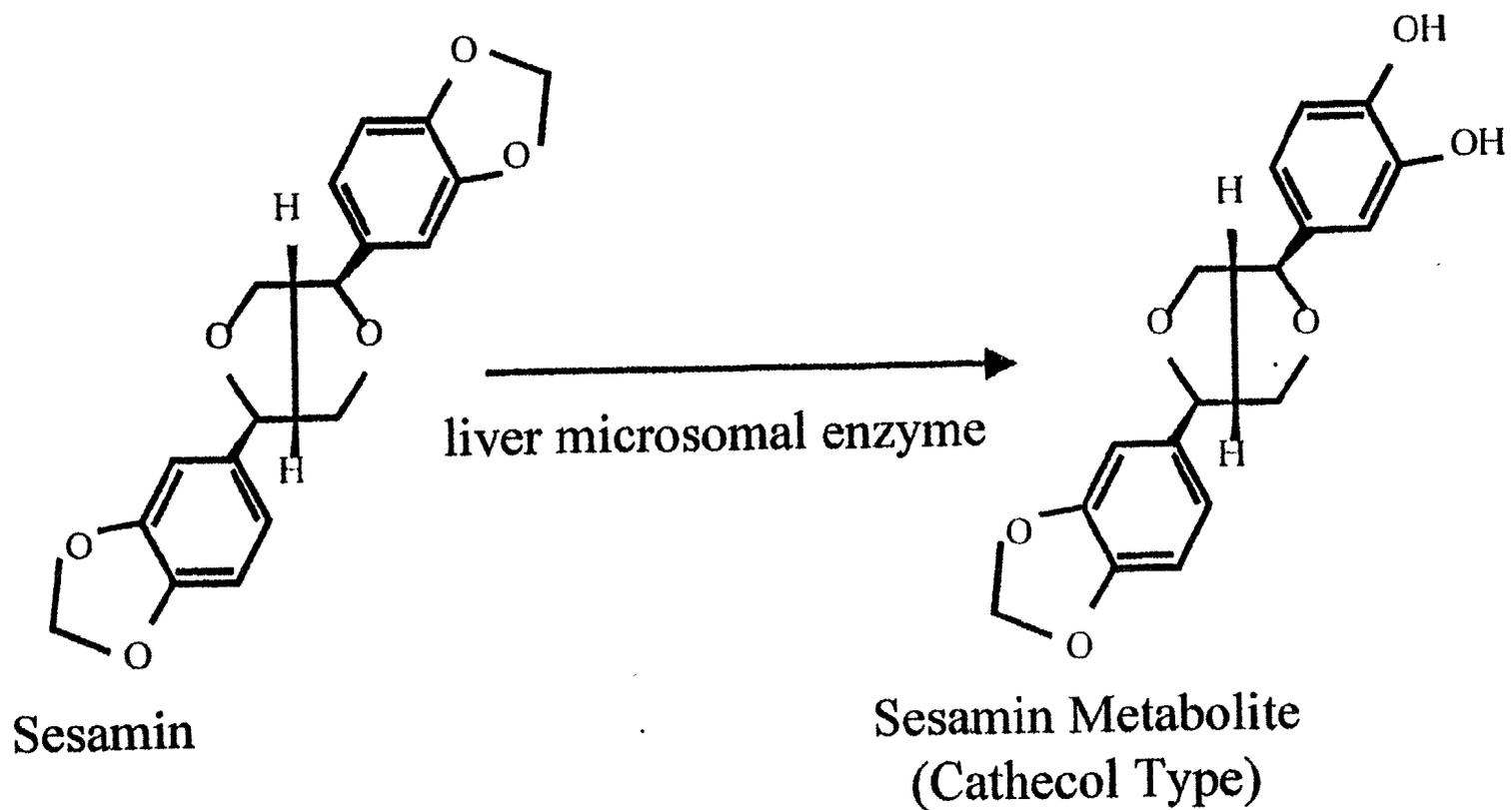
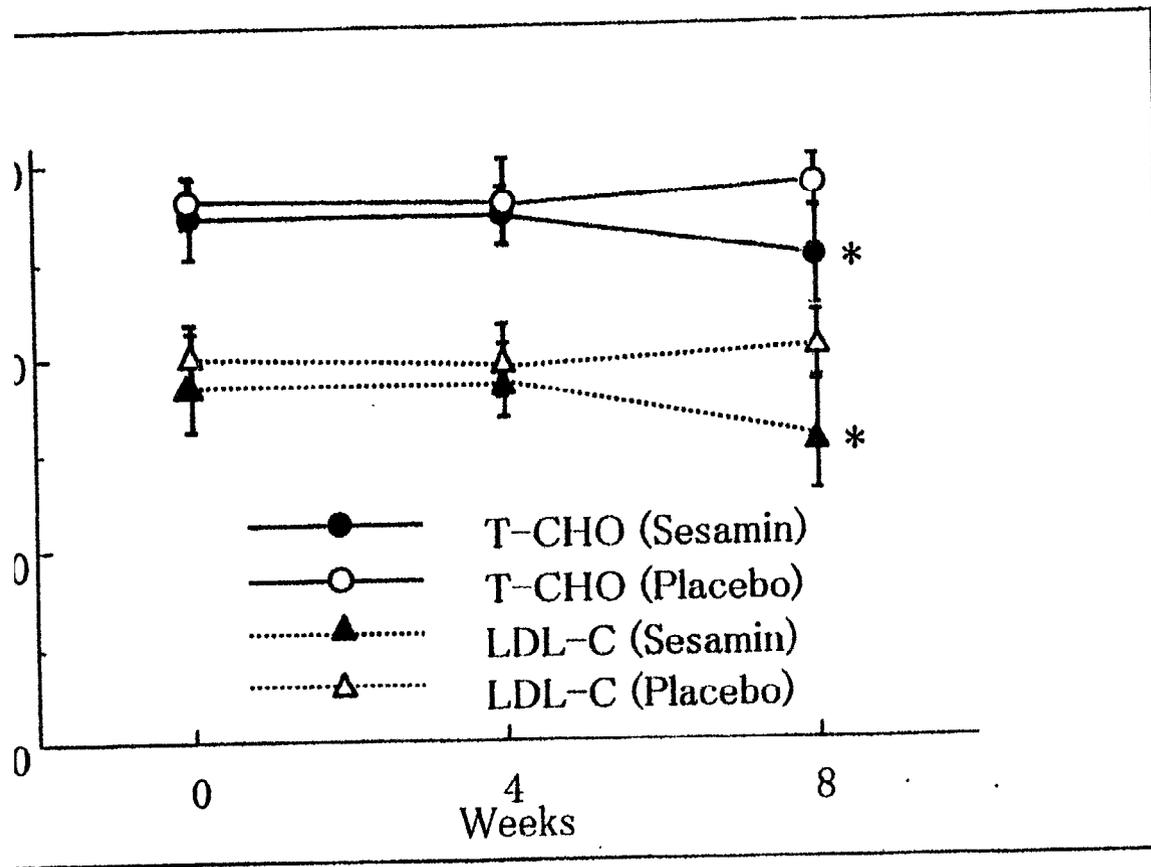
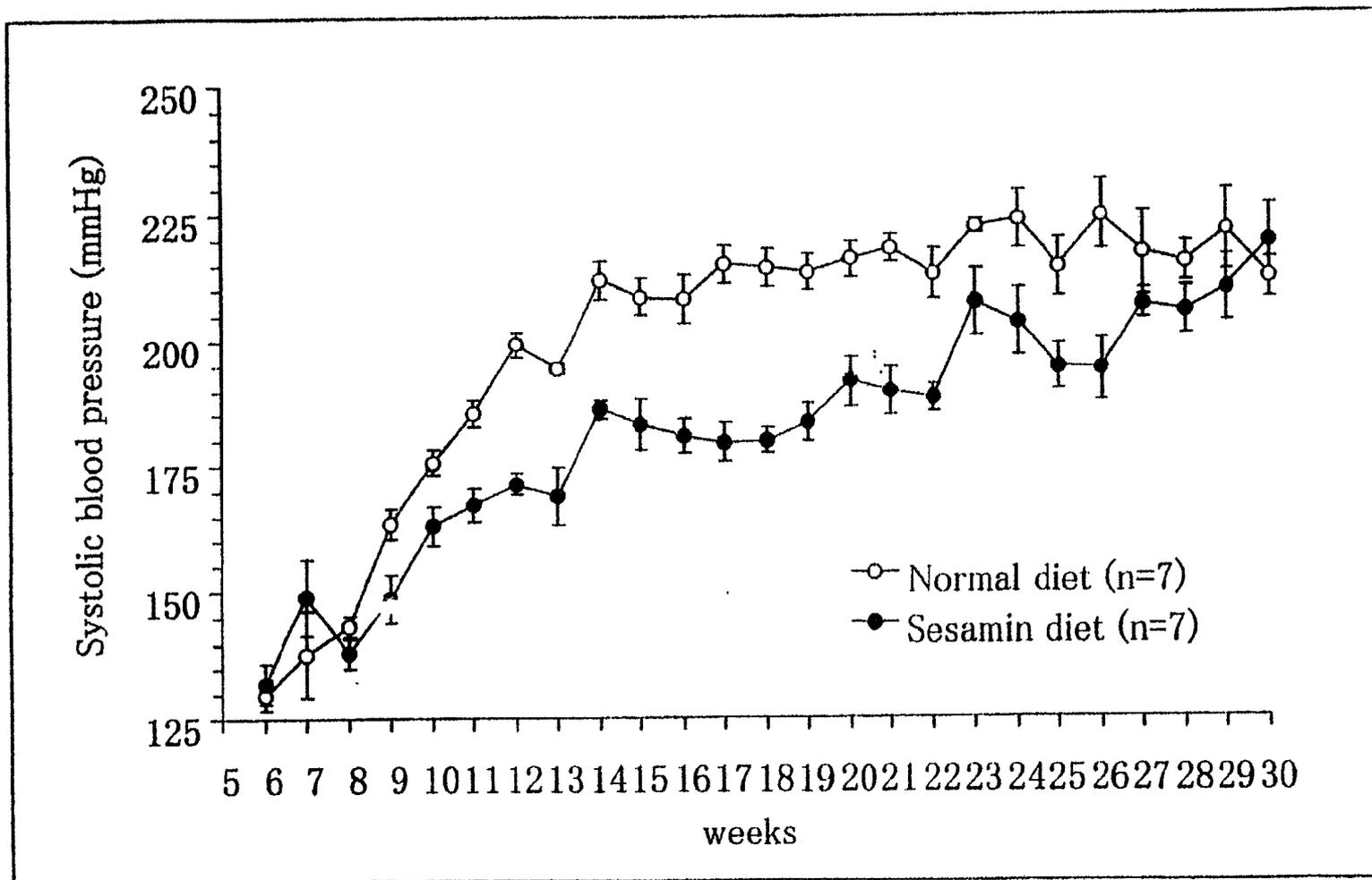


Fig. 2. Chemical Structure of Sesamin and its catechol type metabolite with liver microsomal enzyme



Effect of Sesamin on the cholesterol level of hypercholesterolemia
T-cholesterol level were measured by means of Friedewald
method \pm SD (n=6). * : p<0.05



mean \pm S.E.M. * $p < 0.001$

Fig. 4. Effects of Sesamin on DOCA-salt induced hypertension in stroke-prone spontaneously hypertensive rats (SHRSP)

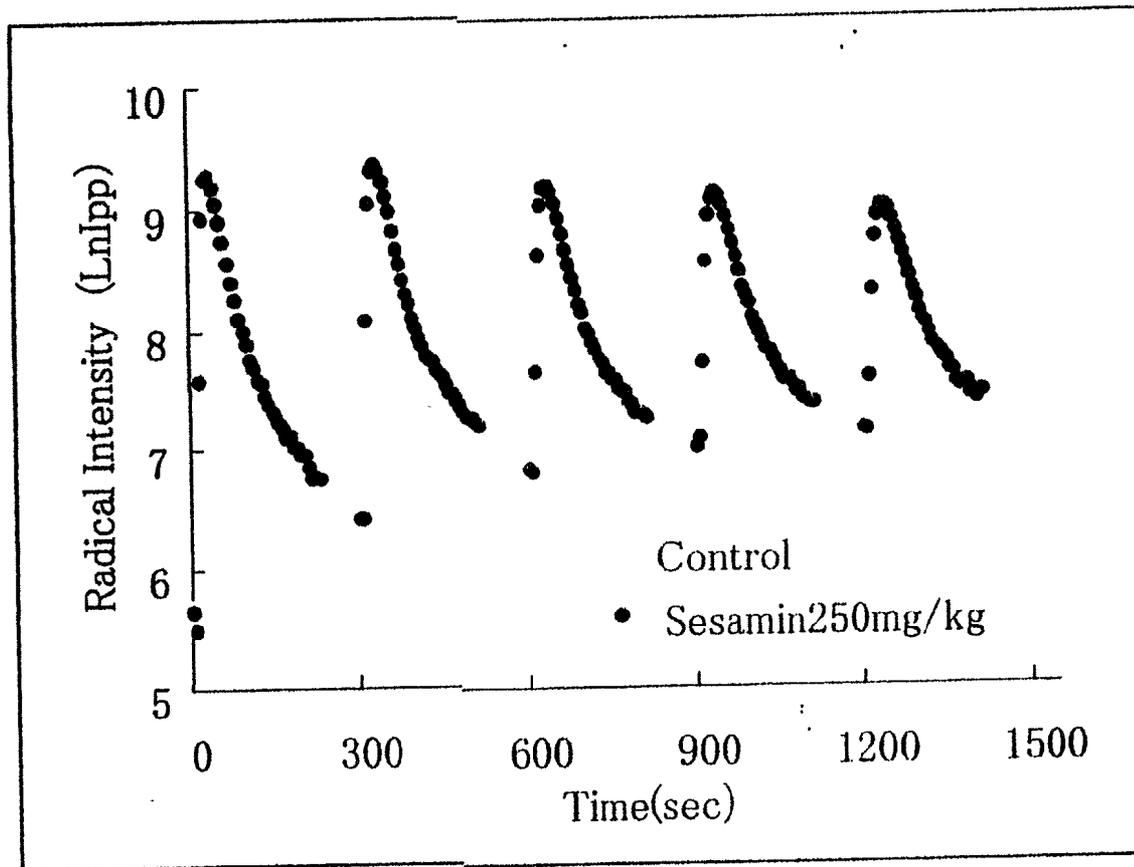


Fig. 5. Scavenging effects Sesamin on nitroxyl radical (TEMPOL) in the liver

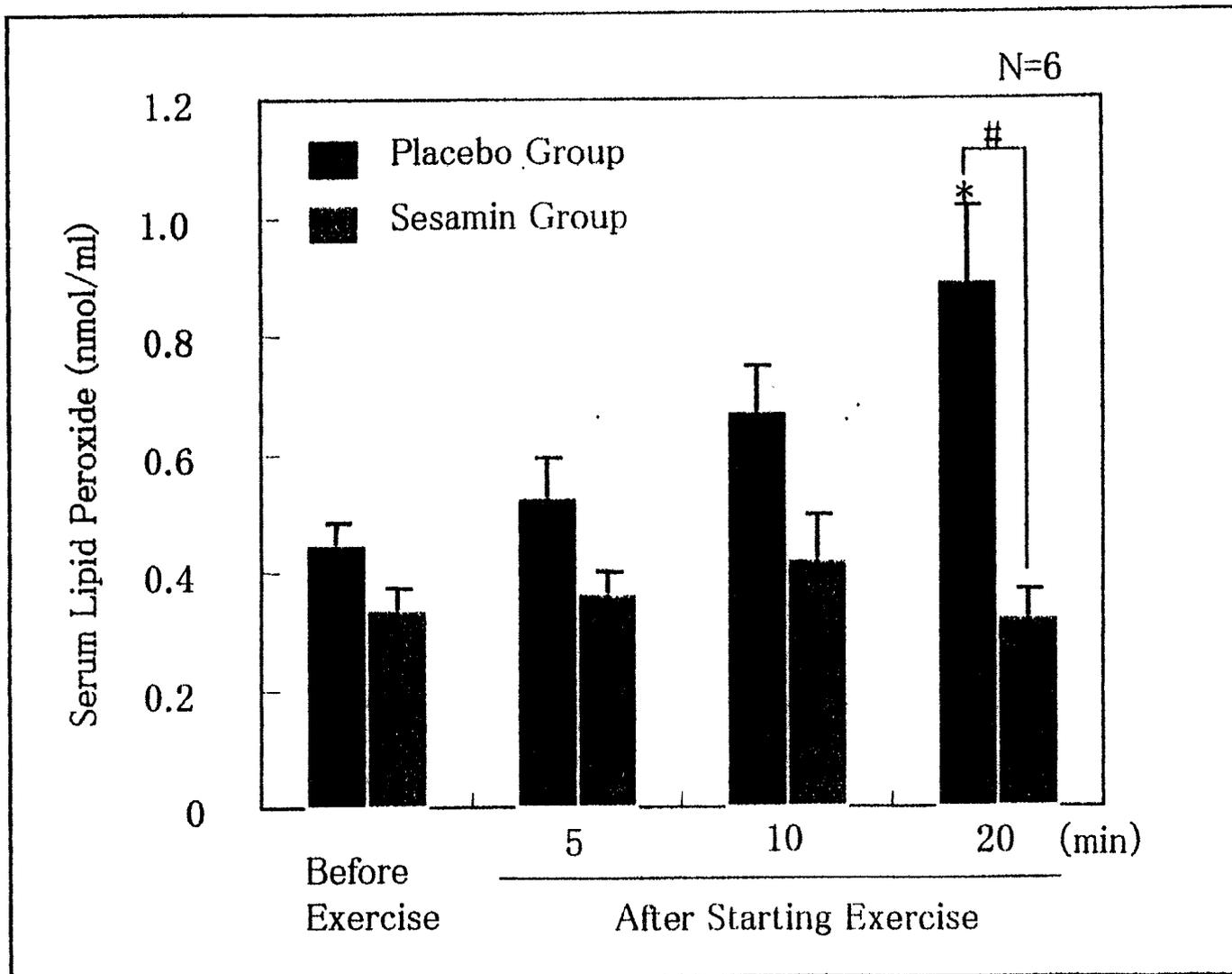


Fig. 6. Protective effect of Sesamin on serum lipid-peroxide induced by hard exercise.

Table II. Protective effects of Sesamin on hepatic disorder induced by continuous inhalation of ethanol vapor.

Groups	T-CHO mg/dl	TG mg/dl	T-Bil mg/dl	GOT IU/l	GPT IU/l
Control	91.6 ± 8.8	58.6 ± 12.1	0.47 ± 0.15	149.7 ± 76.3	26.1 ± 7.8
Ethanol	100.9 ± 10.2	237.3 ± 124.2 ^{##}	1.61 ± 1.32 [#]	312.4 ± 203.8 [#]	39.6 ± 31.9
Ethanol+sesamin	89.4 ± 8.5	83.0 ± 19.0 ^{#,*}	0.41 ± 0.04 [*]	81.6 ± 15.4 ^{#,**}	18.3 ± 1.6

[#] $p < 0.05$ and ^{##} $p < 0.01$ vs the control group

^{*} $p < 0.05$ and ^{**} $p < 0.01$ vs the ethanol group

Table I Synergistic suppressing effects of Sesamin and α -tocopherol toward the serum cholesterol level of rats

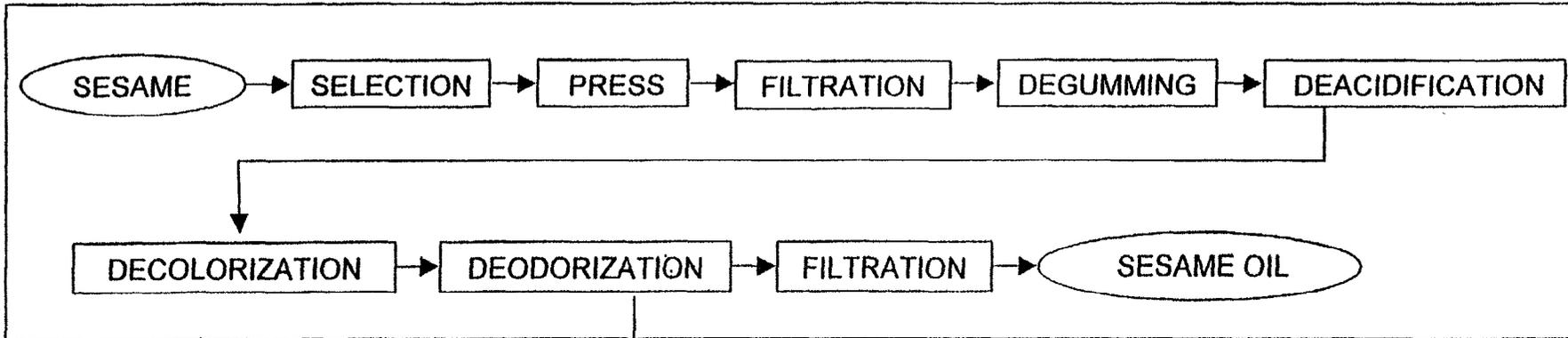
Group		No. of Animal	Serum Total Cholesterol (mg/m)
Experiment I	high-cholesterol diet	6	490 \pm 94
	+1.0% Tocopherol	6	460 \pm 76
	+0.05% Sesamin	6	437 \pm 76
	+0.05% Sesamin+1.0% Tocopherol	6	244 \pm 23 ^{*,#}
	+0.2% Sesamin	6	371 \pm 28
	+0.2% Sesamin+1.0% Tocopherol	6	149 \pm 9 ^{*,#}
Experiment II	high-cholesterol diet	9	429 \pm 24
	+0.2% Sesamin	6	374 \pm 64 [*]
	+0.2% Sesamin+0.2% Tocopherol	6	243 \pm 5 ^{*,#}
	+0.2% Sesamin+1.0% Tocopherol	6	184 \pm 13 ^{*,#}

mean \pm SE, ^{*} $p < 0.05$ vs high-cholesterol diet group

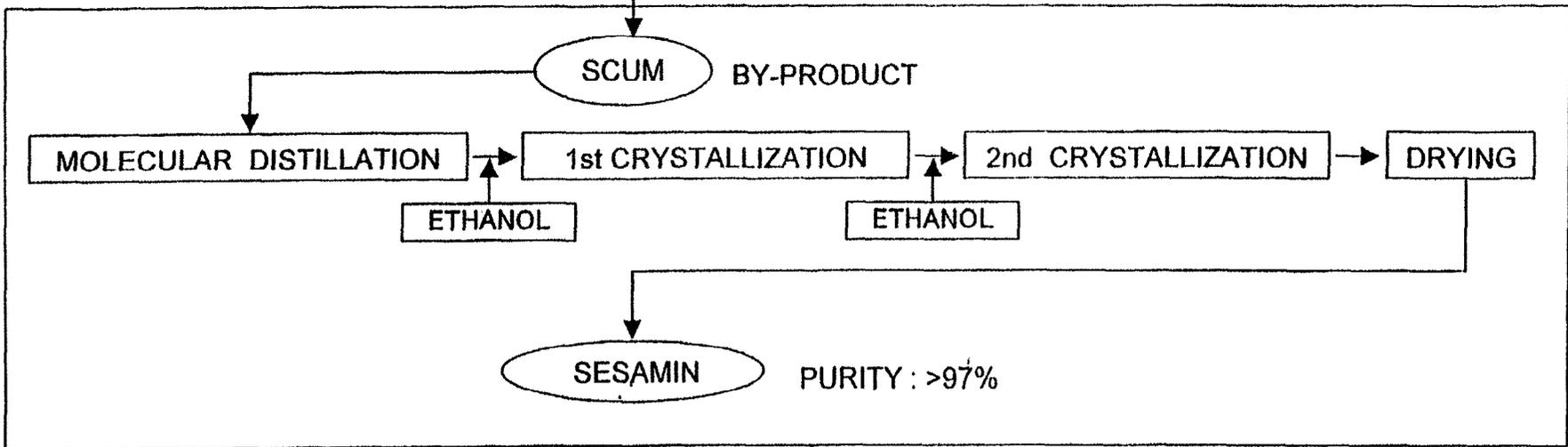
[#] $p < 0.05$ vs Sesamin diet group

SESAMIN PROCESS FLOW

SESAME OIL NORMAL PRODUCTION FLOW CHART



SESAMIN POWDER PRODUCTION FLOW CHART



Attachment 3

FINAL REPORT

Two-Week Repeated Oral Administration Toxicity Study of
Sesamin in Mice

Institute for Fundamental Research
Suntory Ltd.

SIGNATURE PAGE

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Date

INTRODUCTION

Two-week repeated oral administration toxicity study was conducted in mice, as a part of the safety study of sesamin for its use as a food material.

MATERIALS AND METHODS

1. Test material

Sesamin (Lot No. 891009) was used as test material.

2. Animals

Seven-week-old ICR male mice (SPF) were purchased from CLEA Japan Inc. and used for the test at 8 weeks of age after acclimation.

3. Testing conditions

Mice were bred in polyisopentene cages (235×325×170mm, Charles River Japan), 5 mice in a cage, in an air-conditioned room (temperature of $23.5 \pm 2.0^{\circ}\text{C}$, humidity of around $55 \pm 5\%$). Food (CE-2, CLEA Japan Inc.) and water (tap water in polycarbonate bottle) were taken freely.

4. Dose levels, group design and method of administration

Animals were divided into 2 groups of 10 mice each. Sesamin was ground in a mortar and mixed with CE-2 (radiosterilized feed) at 2% and given to mice ad libitum since it was hardly soluble in water and therefore difficult to forcibly administer orally. The control group received CE-2 supplemented with cellulose (2% cellulose feed).

5. Examination

1) Observation of general symptoms

Mice were observed for general symptoms at least once a day during the period of administration.

2) Body weight measurements

Body weight was measured right before the administration of sesamin, once a day during the first week, and every other day in the second week.

3) Food intake

Food intake was measured everyday during the first week and every other day in the second week.

4) Water intake

Water intake was measured everyday during the first week and every other day in the second week.

5) Autopsy

All mice were killed after 2 weeks of the administration, and were observed macroscopically.

6) Biochemical examination

Blood was collected before autopsy from the heart for all mice and the obtained sera were used for the following 6 examinations.

- i) Glutamate oxaloacetate transaminase
- ii) Glutamate pyruvate transaminase
- iii) Total-cholesterol
- iv) Triglyceride
- v) Alkaline phosphatase
- vi) Total-bilirubin

RESULTS

1. General symptom

No abnormal symptoms were observed in any of the groups from immediately after administration up to autopsy two weeks later (Table 1).

Table 1 LD₅₀ values

Groups	Average intake of Sesamin	Dead / Tested
Control	—	0/10
Sesamin	2.14 g/day/kg	0/10

2. Body weights

Significant loss in body weight was observed on day 1 and day 2 after administration in the group receiving the test substance, but was recovered later (Fig. 1).

3. Food and water intake

Food and water intake initially decreased rapidly in the test substance group, but recovered gradually and returned to normal levels on day 3 to 4 (Fig. 2, 3).

4. Autopsy

Red swelling of lymph nodes, enlargement of large intestine and hematoma of pancreas were observed in one case each of the test substance group. Necrotic spots (small, pin-head sized deep red spots) were observed in the liver in one case of the test substance group, but these were sporadic and are also observed sometimes in normal animals. Therefore, it cannot be concluded that these changes were caused by the administration of the test substance.

5. Biochemical examination

No significant difference was observed in any of the measurements between the test substance group and the control group (Table 2, Fig. 4).

DISCUSSION

Sesamin was mixed with the feed at 2% concentration and given to ICR mice continuously for two weeks to assess its toxicity. As the results, no abnormal symptom was observed from immediately after the administration up to the autopsy after two weeks. Decrease in food intake, which was accompanied by significant loss of body weight and decrease in water intake, was observed in the test substance group, but recovered after 3 to 4 days. These decreases were possibly due to the poor palatability of sesamin. No marked findings were observed in autopsies that could be attributed to the test substance. Also, serum analysis revealed no difference between the test substance group and the control group. The amount of sesamin intake, as calculated from the food intake, was 2.14 g/day/kg, which was a continuous intake of a fairly large amount. Based on the above results, it is concluded that the toxicity of the test substance, sesamin, in male ICR mice is very low. (The amount of sesamin intake in this experiment corresponds to approximately 128 g/day/60 kg in human).

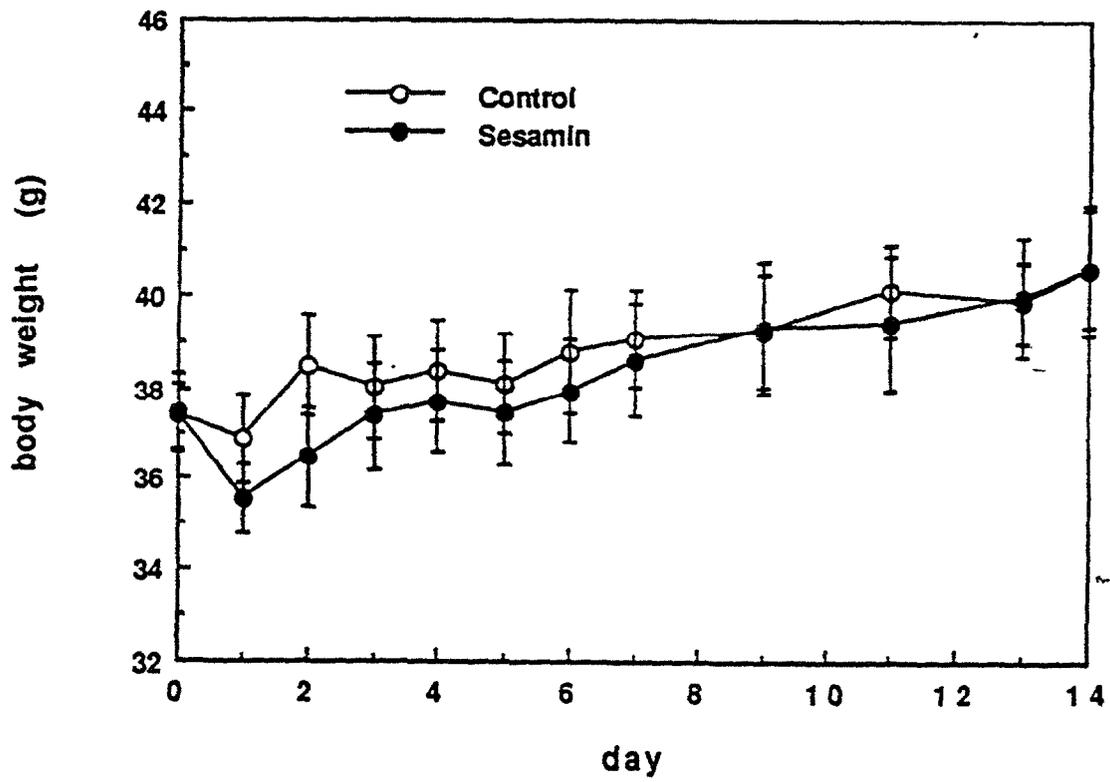


Fig.1 Body weight changes in mice treated orally with sesamin for 2 weeks

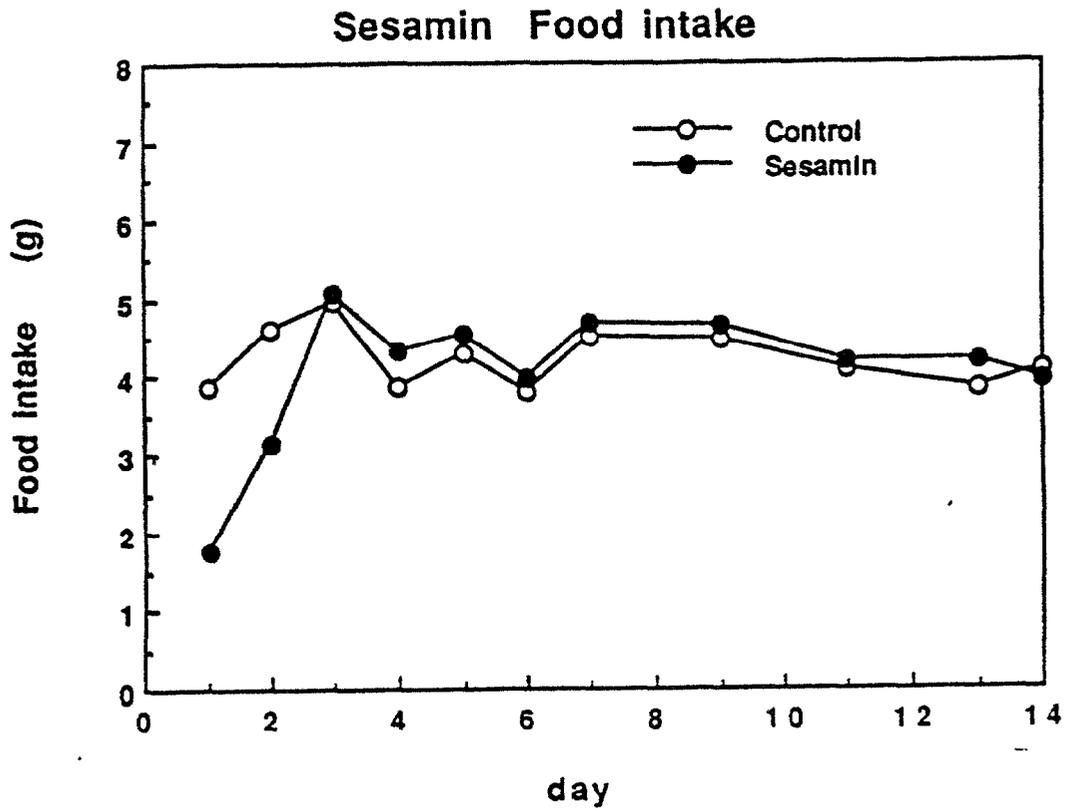


Fig.2 Food intake changes in mice treated orally with sesamin for 2 weeks

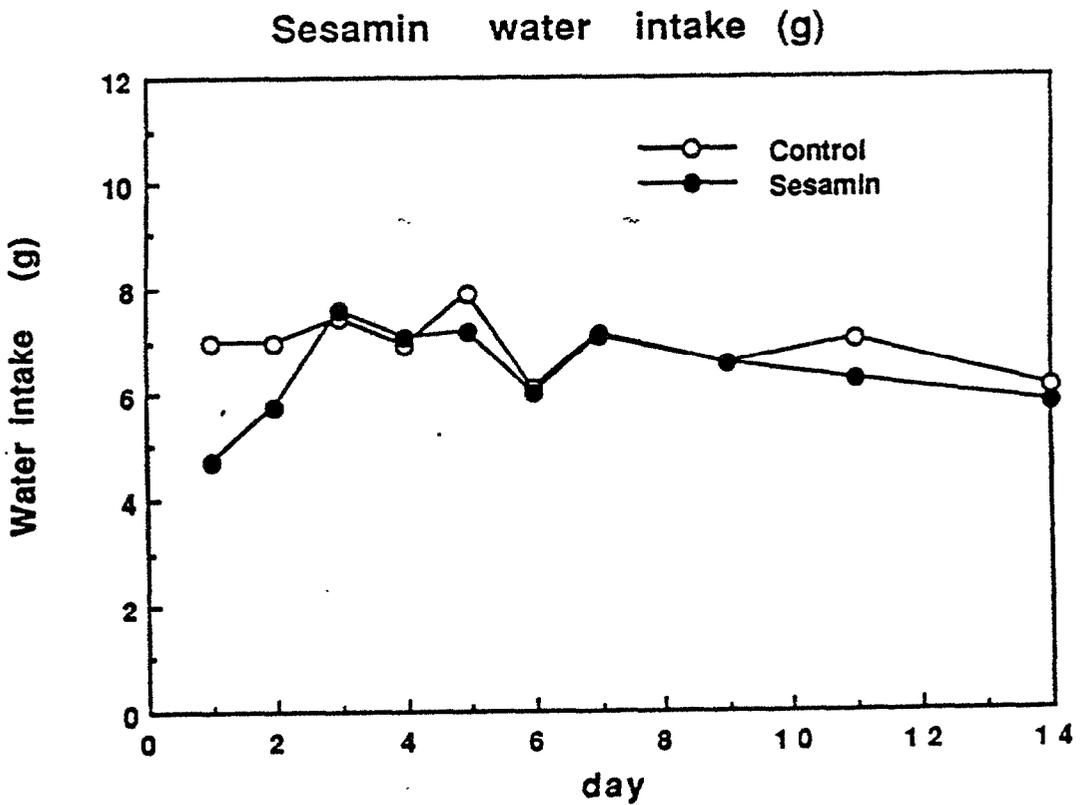


Fig.3 Water intake changes in mice treated orally with sesamin for 2 weeks

Table 2 Biochemical findings in mice treated orally with sesamin for 2 weeks

Items (unit)	Control	Sesamin
G O T (IU/l)	54.9 ± 9.78	53.3 ± 12.30
G P T (IU/l)	21.4 ± 4.97	31.8 ± 16.08
T - C H O (mg/dl)	118.6 ± 19.25	115.1 ± 20.82
T G (mg/dl)	189.4 ± 36.64	198.9 ± 85.70
A P L (IU/l)	244.3 ± 28.35	235.1 ± 35.04
T - B I L (mg/dl)	0.42 ± 0.109	0.56 ± 0.196

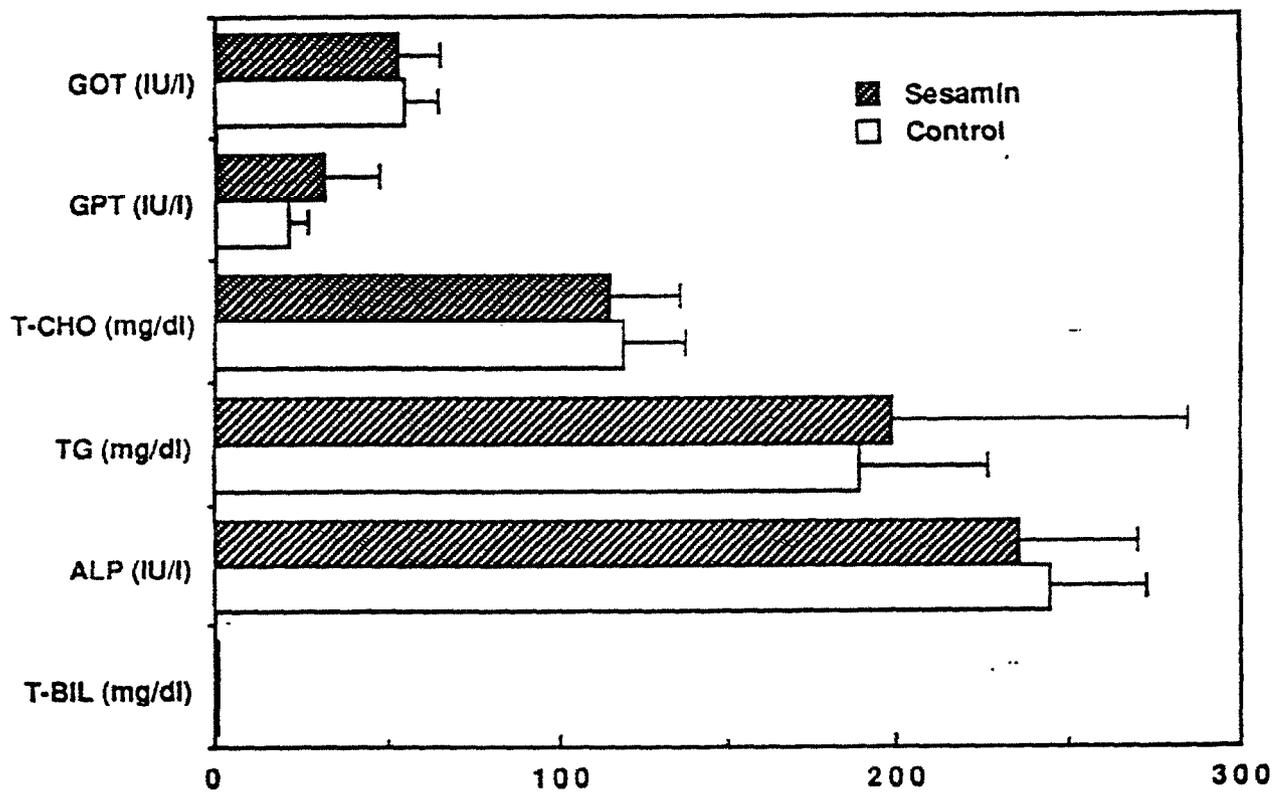


Fig.4 Biochemical findings in mice treated orally with sesamin for 2 weeks

Attachment 4

FINAL REPORT

Mutagenicity Study of Sesamin

Institute for Fundamental Research
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SIGNATURE PAGE

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SUMMARY

The mutagenicity of sesamin was evaluated on the reverse mutation test (Ames test) by *Salmonella typhimurium* strains TA97, TA98, TA100 and TA102. In all tester strains, no increase in the number of the revertant colonies was observed at dose range of 0.5-5mg/plate with or without metabolic activation. The present result showed that sesamin had no mutagenic activity on Ames/Salmonella test.

INTRODUCTION

The mutagenic activity of sesamin was evaluated with four strains of *Salmonella typhimurium*, for the safety assessment of sesamin as a food material.

MATERIALS AND METHODS

1. Test material

Sesamin (No.891009) was used as a test material.

2. Positive control substances

2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2), tert-butylhydroperoxide (t-BuOOH), 2-aminofluorene (2-AF) and benzo[a]pyrene (B[a]P) were used as positive controls.

3. Mutation test

Salmonella typhimurium strains TA97, TA98, TA100 and TA102 cultured in nutrient broth at 37°C overnight were used for mutation tests. Mutagenicity was assayed by preincubation method. 0.1ml of sample in dimethyl sulfoxide (DMSO) was mixed with 0.5ml of S9 mix or 0.1M sodium phosphate buffer (pH 7.4) and 0.1ml of bacterial culture. The mixture was incubated at 37°C for 20 min with shaking. Then 2ml of soft agar was added and the mixture was pored over minimal glucose agar plate. After incubation at 37°C for 2days, the number of revertants was counted. Test sample was assayed at 5 doses, with two plates for each dose. Negative(vehicle) and positive control experiments were performed simultaneously.

Sample giving revertants more than 200% of the control (the number of spontaneous revertants) with dose-response were regarded as positive.

RESULTS

The results of the mutagenicity assay are shown in Table 1 and Fig.1. Sesamin did not increase the number of revertants per plate of any tester strains with or without rat liver microsomal fraction (S9 mix). The vehicle control showed no increase in revertants per plate, while four positive control mutagens (AF-2, t-BuOOH, 2-AF and B[a]P) induced the expected increases in revertant colonies in each strain.

DISCUSSION

The mutagenicity of sesamin was evaluated on the reverse mutation test by *Salmonella typhimurium* strains. In all tester strains, no marked increase in the number of the revertant colonies was observed with or without metabolic activation. Based on the above results, it is concluded that sesamin has no mutagenic activity on Ames/Salmonella test.

Table 1 Reverse mutation test of sesamin in *S.typhimurium* strains

With (+) or without (-) S9 Mix	Test substance concentration ($\mu\text{g}/\text{plate}$)	Number of revertants (number of colonies/plate)			
		Base-pair substitution type		Frameshift type	
		TA100	TA102	TA97	TA98
S9 Mix (-)	Solvent control (DMSO)	105 105 140 (117)	180 164 195 (180)	159 124 135 (139)	30 20 23 (24)
	500	119 113 (116)	201 231 (216)	144 173 (159)	24 20 (22)
	1250	122 128 (125)	171 180 (176)	150 164 (157)	29 27 (28)
	2500	96 109 (103)	152 174 (163)	156 151 (154)	31 27 (29)
	3750	101 101 (101)	163 151 (157)	140 144 (142)	28 29 (29)
	5000	92 102 (97)	171 157 (164)	156 154 (155)	29 32 (31)
	S9 Mix (+)	Solvent control (DMSO)	119 134 139 (131)	294 292 332 (306)	168 196 171 (178)
500		118 145 (132)	330 312 (321)	169 192 (181)	62 62 (62)
1250		123 125 (124)	293 296 (295)	159 162 (161)	55 50 (53)
2500		122 129 (126)	269 263 (266)	182 152 (167)	65 50 (58)
3750		127 120 (124)	294 257 (276)	196 171 (184)	55 51 (53)
5000		127 89 (127)	226 202 (214)	181 203 (192)	47 53 (50)
Positive control not requiring S9 Mix		Name	AF-2	t-BuOOH	AF-2
	concentration ($\mu\text{g}/\text{plate}$)	0.02	0.5 ($\mu\text{l}/\text{plate}$)	0.1	0.1
	Number of colonies/plate	783 1022 (903)	2518 2710 (2614)	564 544 (554)	218 184 (201)
Positive control requiring S9 Mix	Name	B(a)P		2-AF	B(a)P
	concentration ($\mu\text{g}/\text{plate}$)	10		1	5
	Number of colonies/plate	602 578 (590)	()	599 596 (598)	201 236 (219)

Notes:

1. When inhibition is found against growth of the bacteria, mark the applicable value with an asterisk.
2. Fill the average number of colonies in each concentration in the ().
3. "Number of revertants"-Fill in the observed value in order beginning with low concentrations of the test substance.

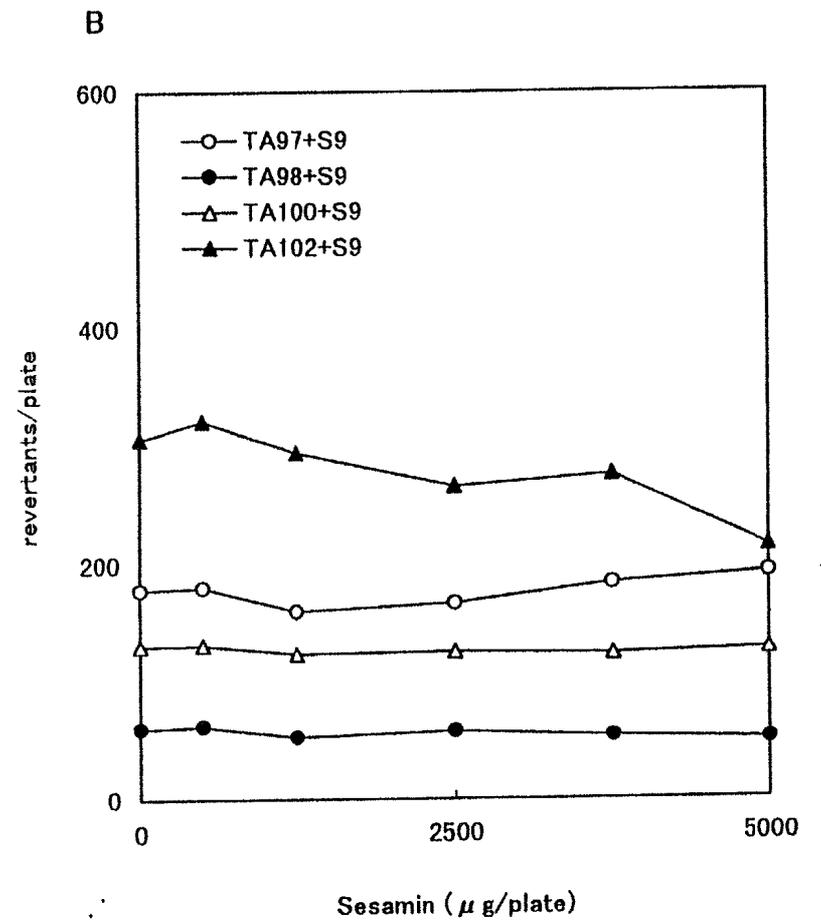
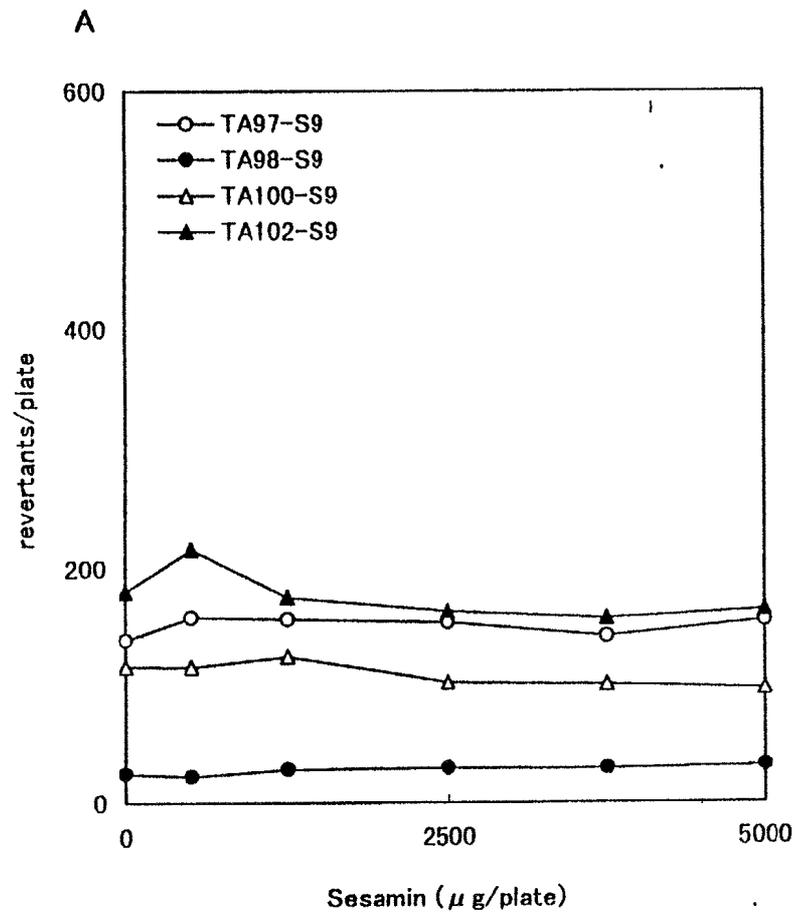


Fig.1 Dose-response curves of sesamin

A: without S9mix, B: with S9mix

Multifunctional Gift from the Nature
Sesamin

21



One of the lignans containing abundantly in sesame with antioxidant activity especially in liver.

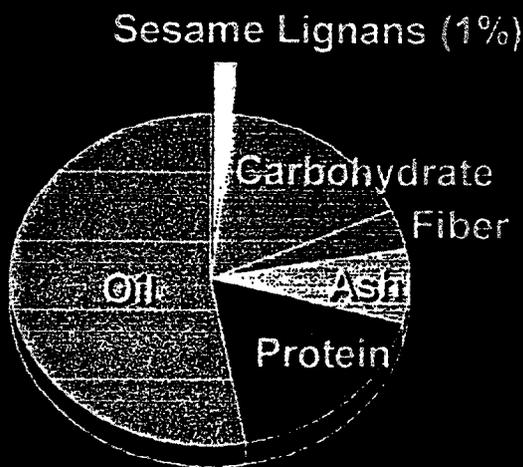
The miracle functions of sesamin are waiting for a chance of "Open Sesame".

For a long time, sesame seed has been known as a traditional health food or a medicinal plant in the East in particular, but the biochemical background of its desirable functions have not totally been appreciated.

Attachment 6

Suntory's Seminar Slides & Texts

Sesame seed contains 1% of sesame lignans

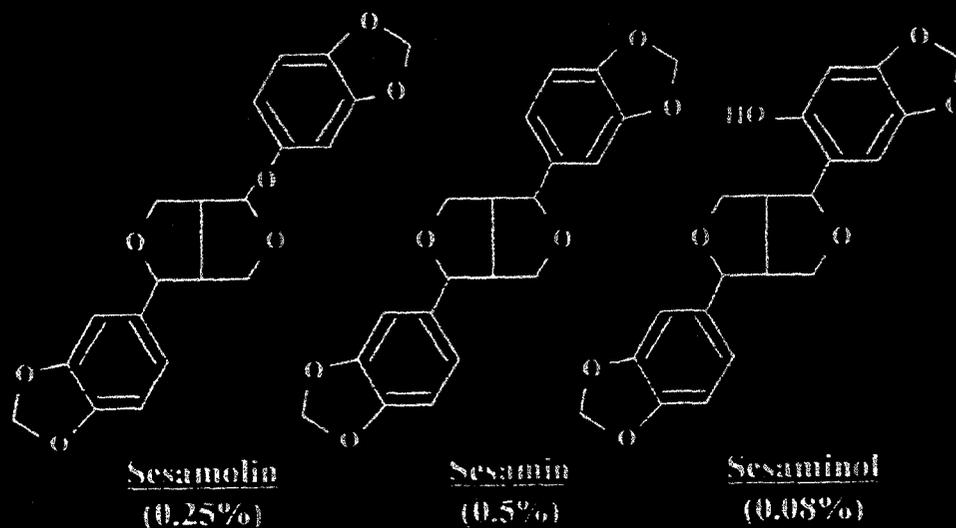


Sesame seed contains sesame oil more than 50% of its weight.

Sesame oil has been known as very stable edible oil, and this character can be attributed at least in part caused by the presence of several small amounts of lignans.

Contents of sesame lignans is about 1%.

Main Sesame Lignans



This slide shows chemical structures of main sesame lignans.

Sesaminol, the hydroxylated product of sesamol transformed from sesamol during the refining process for the production of unroasted sesame seed oil, shows antioxidant activity and play an important role for the stability of sesame oil.

Sesamin is the major lignan in sesame.

The content of sesamin in sesame seed is approximately 0.5%.

A bulk of sesamin is removed from the edible sesame oil during the refining process, but even in the edible grade products, unroasted sesame oil still contains enough amounts of sesamin.

As can be understood from the chemical structure, sesamin itself does not exhibit antioxidant activity *in vitro*, in contrast to sesaminol.

This is one of the reasons why sesamin has not been attracted nutritional and physiological interest, although studies on other lignans have suggested their diverse biological functions.

Agenda

1. A Meet with Sesamin
2. Sesamin for those who are fond of Alcohol
3. Sesamin has hypocholesterolemic activity
4. Anti-Oxidative Role of Sesamin
5. Sesamin for Athletes
6. Sesamin inhibits liver carcinogenesis
7. Enhancement of vitamin E and DHA level in blood

This is the agenda of my talk today.

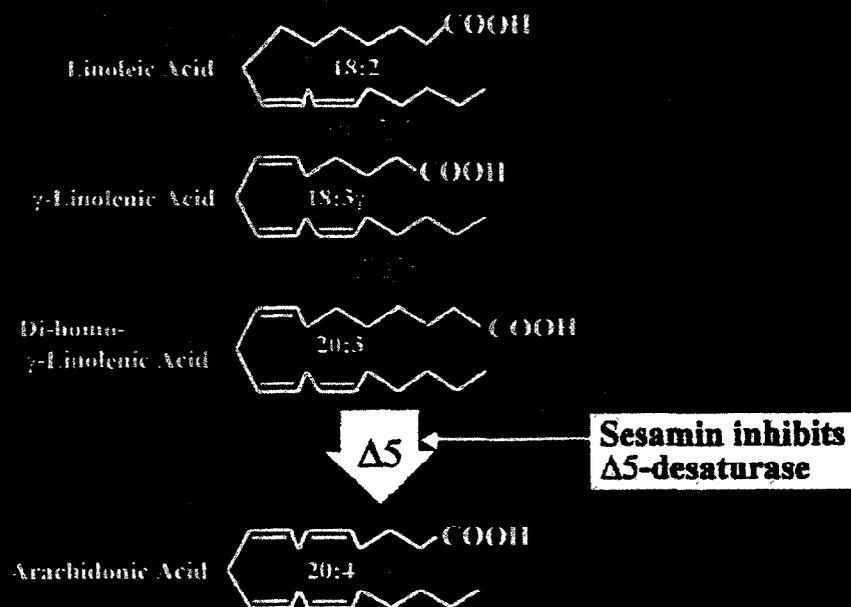
More than 40 original articles concerning biological functions of sesamin have been published by SUNTORY and our collaborating researchers so far.

From those articles, I selected and prepared this presentation for your better understanding.

A Meet with Sesamin

First of all, I would like to talk about "a meet with sesamin".

Sesamin was found as an inhibitor of Δ^5 -desaturase in the process of arachidonic acid biosynthesis



We Suntory have just started to sell a bulk of arachidonic acid produced by microorganism.

Arachidonic acid is a essential fatty acid especially for baby.

Recently, the importance of arachidonic acid in baby milk has been confirmed by many researchers in the world and many baby milk company decided to supply arachidonic acid for baby milk.

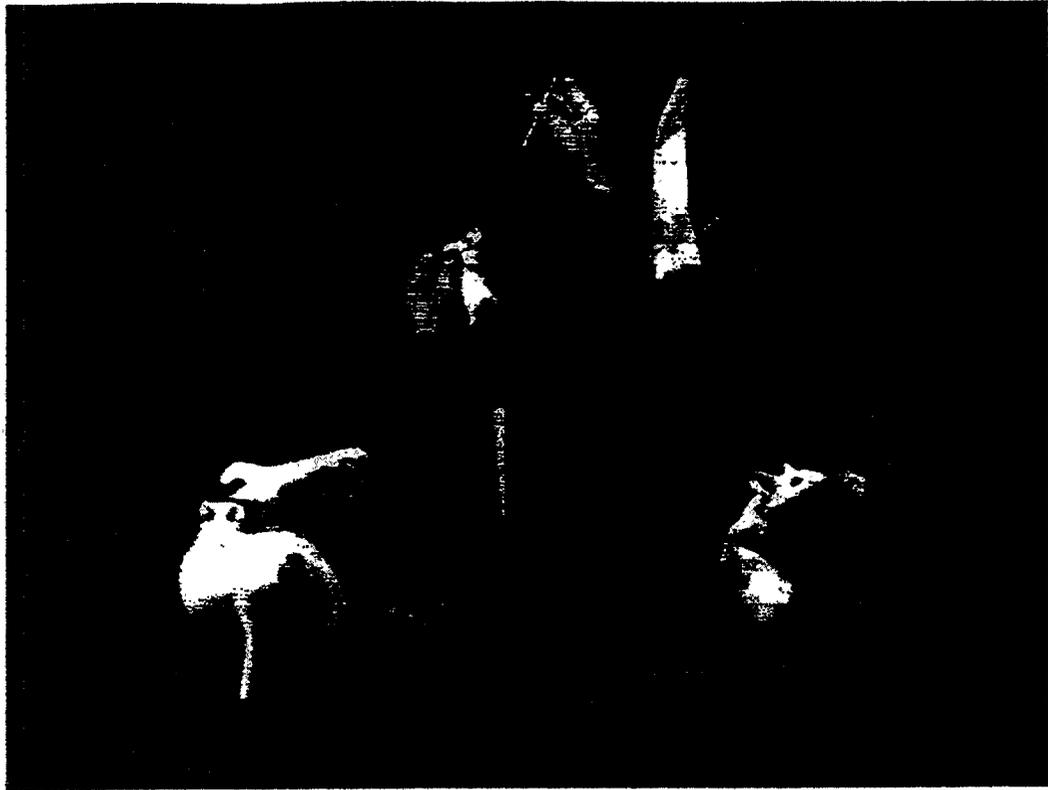
I know very much you also produce essential fatty acid supplements containing DHA and EPA for $\omega 3$, and GLA for $\omega 6$. We are able to produce DGLA and AA itself from fungi.

On the way to the research and development of the fermentation process of arachidonic acid and other polyunsaturated fatty acids, we found that sesamin inhibits Δ^5 desaturase in the biosynthesis pathway as shown here in 1989.

Sesamin for those who are fond of Alcohol

Since 1989 we have investigated the biological functions of sesamin and first found that sesamin enhanced ethanol and acetoaldehyde metabolism and prevented hangover.

Then, we produced sesamin soft capsule and launched it in 1993 as a supplement for those who are fond of alcohol.

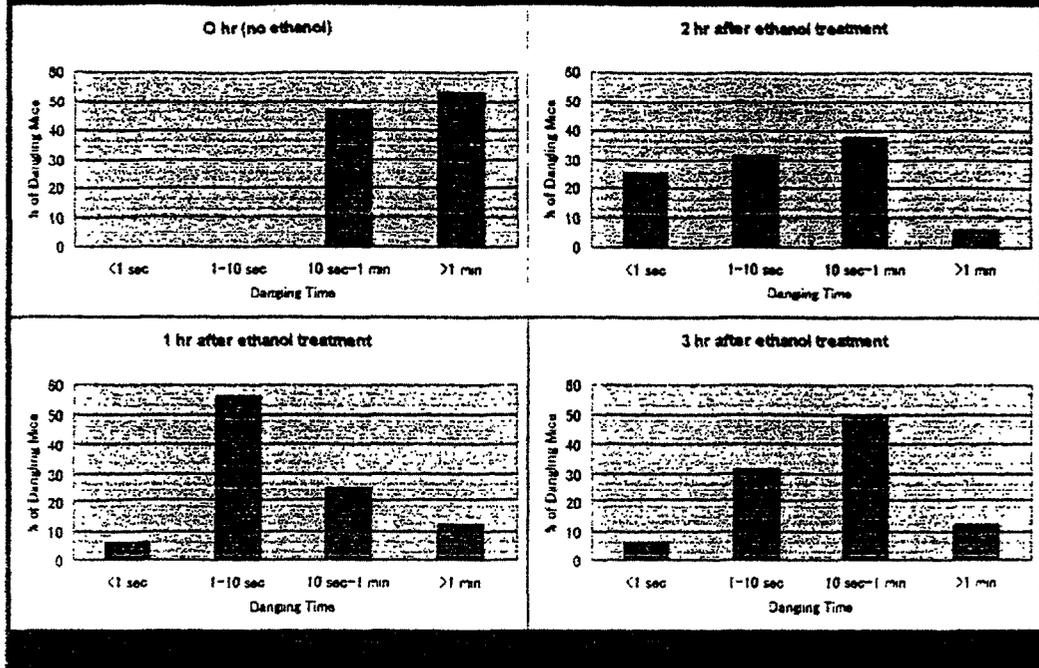


This slide shows the experiment of mice dangling after drinking alcohol.

Before and after drinking alcohol, mice has been hang on iron bar and measured the dangling time.

These pictures have been prepared from the video tape of TV program broadcasted in 1997 in Japan.

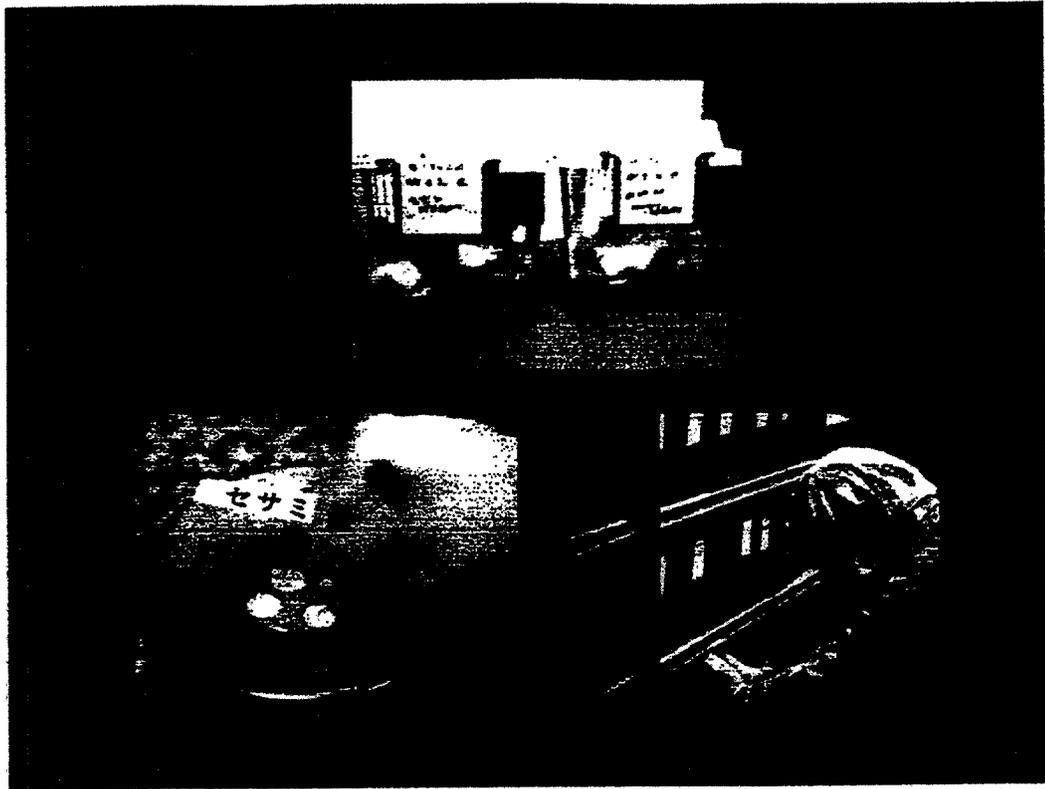
Dangling time after treatment of ethanol



Dangling time of the mice before ethanol treatment were longer than 10 sec, more than half mice were still remaining for over 1 min.

However, 1 hr after ethanol treatment, the peak was sifted to the left side and dangling time of more than half mice were shorter than 10 sec.

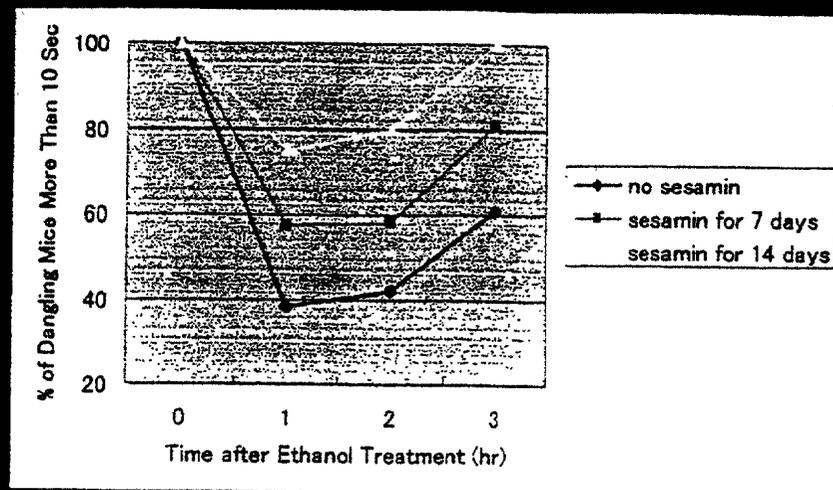
As you can see, Dangling time of 2hrs and 3hrs after the ethanol treatment increased gradually.



Next, we examined the effect of sesamin on dangling time after ethanol treatment.

Sesamin containing diet has been given for 7 days or 14 days.

Effect of sesamin pretreatment on dangling time in mice



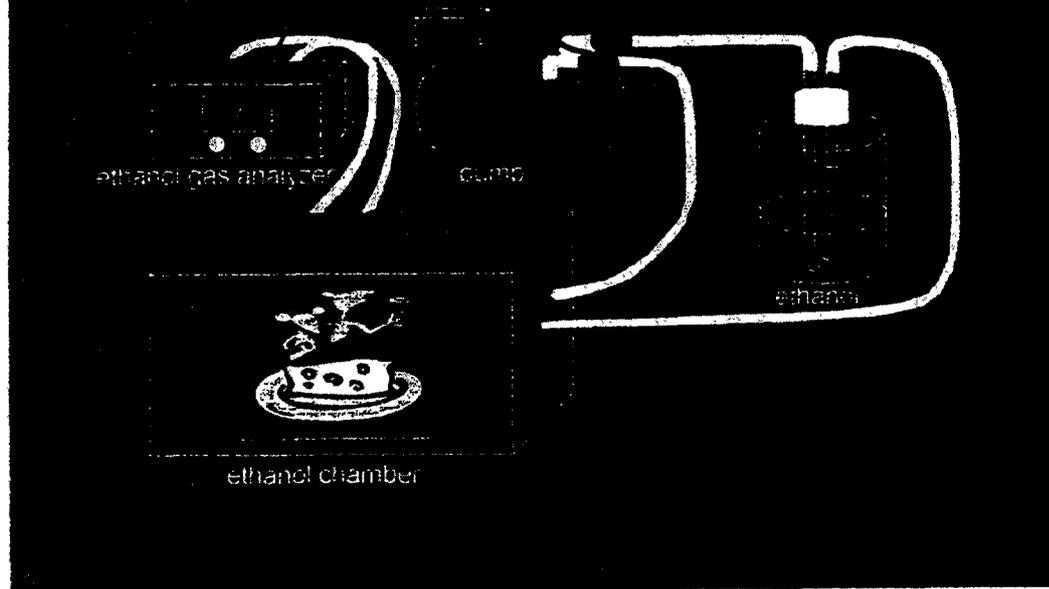
Sesamin 0.15 mg/kg/day

This slide shows % of dangling mice more than 10 sec after ethanol treatment.

Blue line is control, red line is 7 days' sesamin feeding, and yellow line is 14 days' sesamin feeding, respectively.

Dangling time was longer in 7 days' sesamin feeding mice and much longer in 14 days' sesamin feeding mice than that of control.

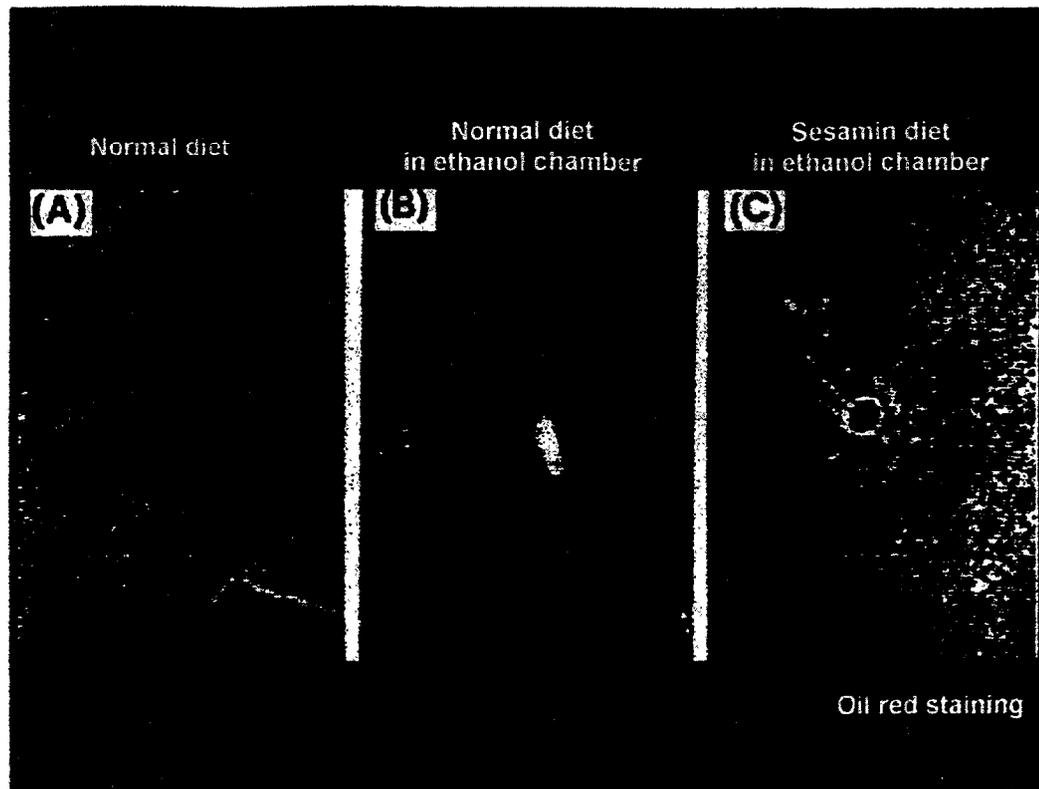
Ethanol Chamber



This slide shows another animal experiment.

Mice has been placed under ethanol atmosphere continuously for 6 days.

I am not sure mice were happy like this slide or not.



This slide shows oil red staining of liver tissue of normal diet in normal condition (A), normal diet in ethanol chamber (B), and sesamin diet in ethanol chamber (C).

If the content of lipid was high, the color of the staining would turned to red.

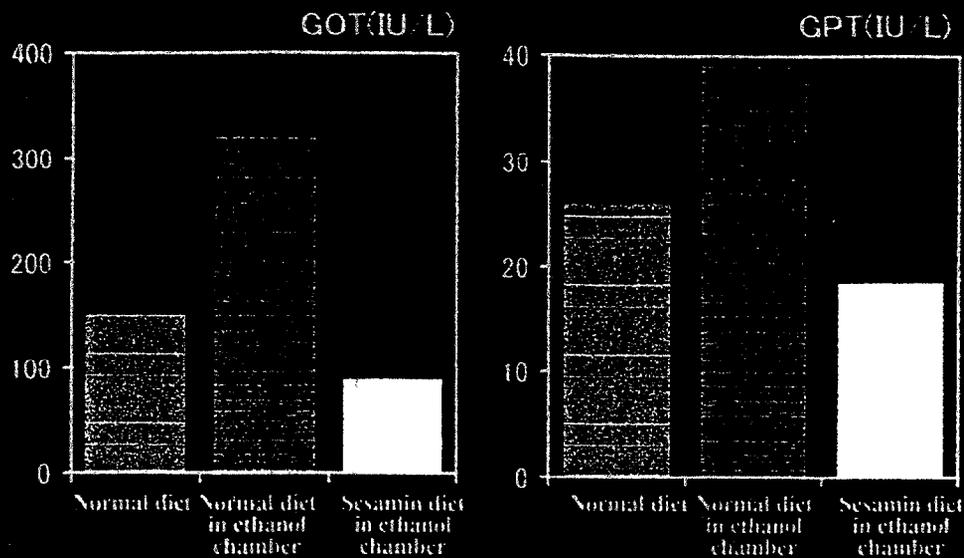
As you can see, liver tissue of normal diet in ethanol chamber turned red indicating that the lipid content was high.

Mice in this group suffered from fatty liver.

In contrast, a group which was maintained with sesamin diet even under ethanol atmosphere prevented fatty liver.

There is no difference between the two groups of normal diet-normal condition (A), and sesamin diet-ethanol chamber (C).

Protective effect of sesamin for liver damage



Mice were bred for one week under normal condition or ethanol atmosphere. The laboratory chow for "Sesamin diet" was added 1% sesamin.

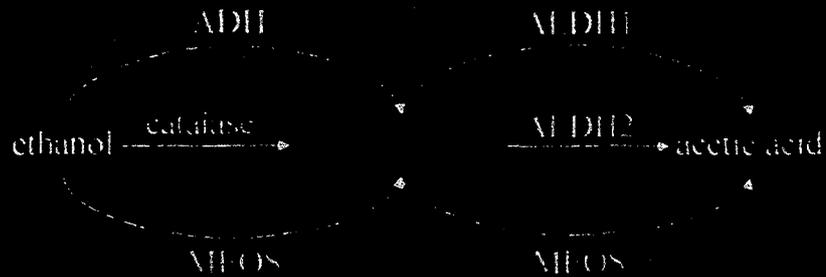
J. Nat. Med. 37: 218, 1994

Serum GOT and GPT values of these mice were examined.

Under ethanol atmosphere condition, GOT and GPT values increased enormously, but sesamin prevented the activity increasing of both enzymes.

From these data, it has been suggested that sesamin showed protective effect toward ethanol-induced liver damage.

Alcohol Metabolism and Sesamin



Flush
Hang Over

ADH: alcohol dehydrogenase

ALDH1: aldehyde dehydrogenase

ALDH2 is active only in high concentration of aldehyde. About 50% of Japanese are lack of ALDH2. Those people are called "Flusher".

MEOS: microsomal ethanol oxidizing system

Enzymes in yellow character are inducible by sesamin.

This is a summary of the mechanism of the enhancement of alcohol metabolism by sesamin.

Ethanol can be metabolized to acetaldehyde by alcohol dehydrogenase (ADH), and/or catalase and microsomal ethanol oxidizing system (MEOS).

Acetaldehyde is very toxic and causes several kinds of ethanol-induced deteriorations, such as flush and hangover.

Acetaldehyde is metabolized by aldehyde dehydrogenase (ALDH) 1 and 2, and also by MEOS to acetic acid.

Enzymes shown in yellow character are inducible by sesamin.

Acetaldehyde level was not increased by sesamin treatment, which suggested that MEOS system was induced by sesamin.

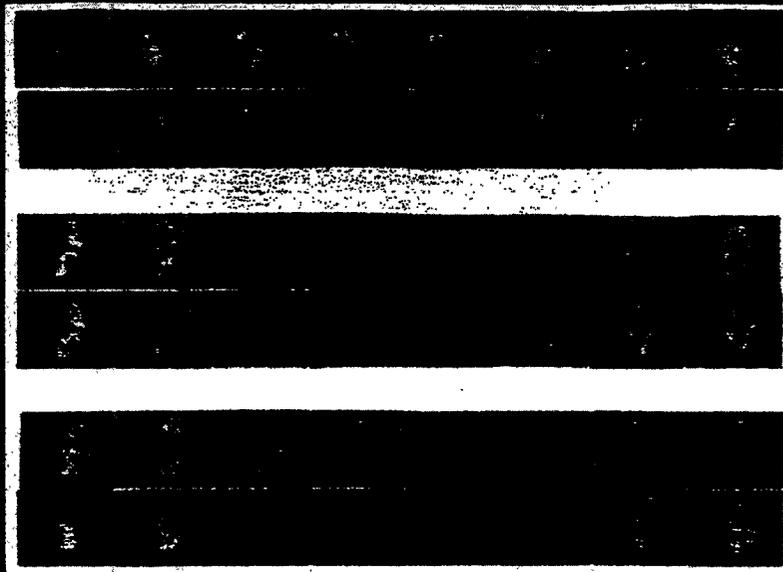
So that Sesamin plays an important role in the ethanol and acetaldehyde metabolism.

By the way, about 50% of Japanese do not have the enzyme ALDH 2. Because of lacking the enough amount of acetaldehyde metabolizing enzyme, the remained acetaldehyde induces flush and hangover after drinking alcohol.

They are called as "flusher".

Effect of sesamin on the thermogram of the human subject after drinking ethanol

sesamin 1 9 17 25 33 41 49 57 min



Cross over trial has been done with 2 healthy volunteers lack of ALDH2. Chocolate (g) with or without sesamin (100 mg) has been given for 7 days. After drinking whisky (60 ml) thermogram was obtained.

Then we tried to check the Sesamin effects up in human.

We recruited 9 male adult volunteers who lack of enzyme ALDH2 for this experiment.

At the first experiment, they were given sesamin (100 mg per day for 7 days), and the second experiment, they were given placebo as a form of chocolate, respectively.

In each experiment, skin temperature of their faces was monitored by the infrared camera.

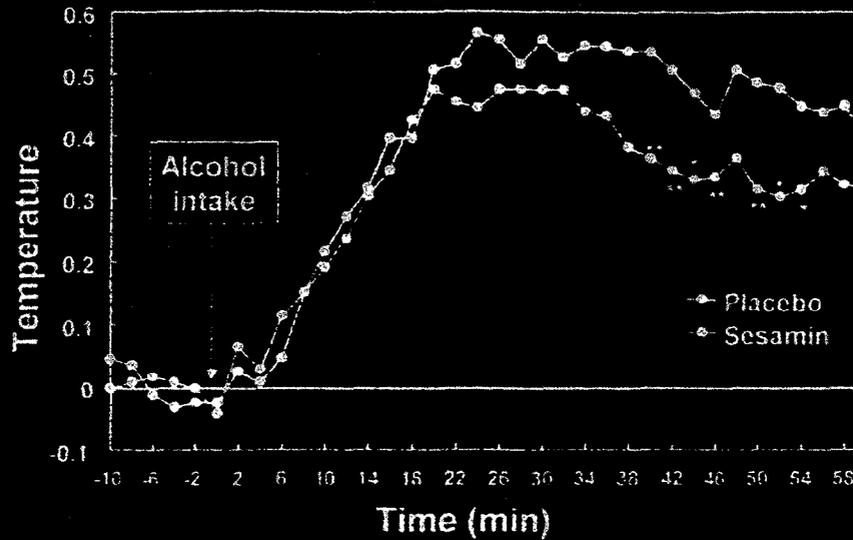
Approximately 60 ml of alcohol was given as whisky at once.

Consequently, the face skin temperature of all the subjects raised and reached a peak in about 30 min, and then fell gradually.

However, the rate of reduction was faster in those who received sesamin than in those received placebo.

Three examples are shown here.

Effect of sesamin on the thermogram at the area of chin and neck of the human subject before and after drinking ethanol



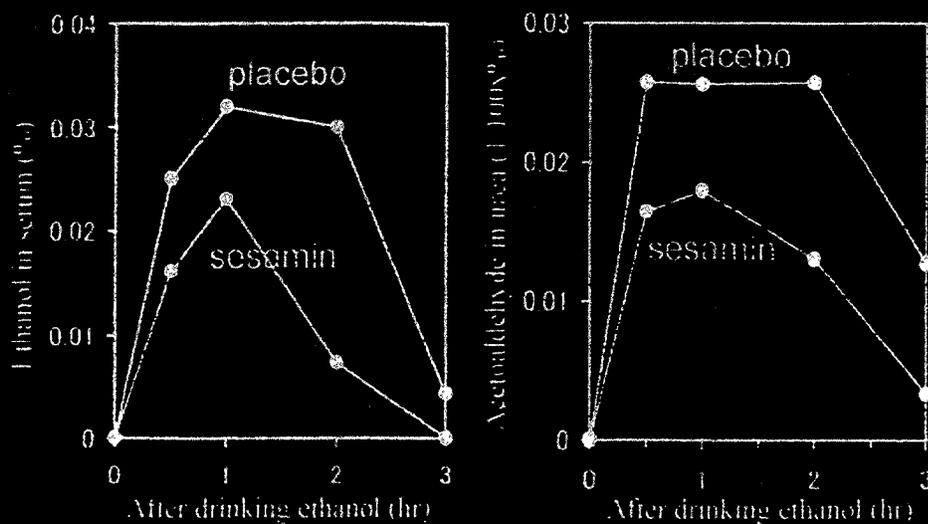
Values are means of 9 subjects. The temperature was measured by infrared camera. * Significant difference for the placebo group at $p < 0.05$ and $p < 0.01$, respectively.

M. Ishikawa et al. J. Nat. Med. Assoc. Jpn. 1991; 72(1): 1-5

This slide shows mean value of all subjects.

The difference was significant during 40 to 55 min after ethanol drinking.

Enhancement of alcohol and aldehyde metabolism in humans



Ethanol in serum and acetaldehyde in urine were also measured.

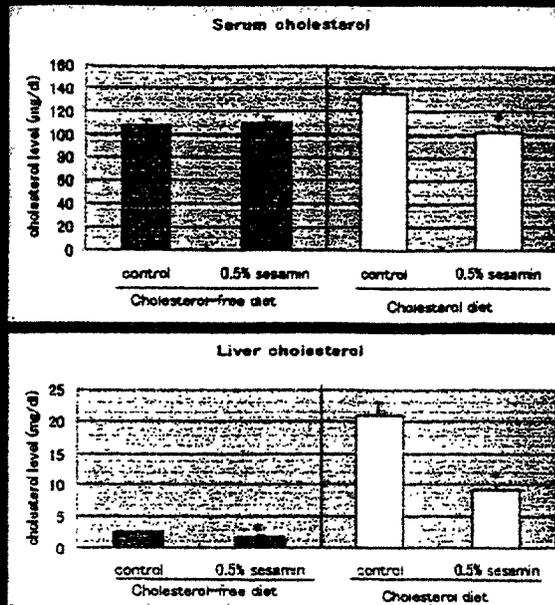
Both of ethanol and acetaldehyde concentration were lower in those who received sesamin than in those received placebo.

Enhancement of alcohol and acetaldehyde metabolism was confirmed directly not only in mice but also in human.

Sesamin has hypocholesterolemic activity

Next story is hypocholesterolemic activity of sesamin.

Effect of sesamin on serum and liver cholesterol level in rats



*Significantly different from the control $p < 0.05$

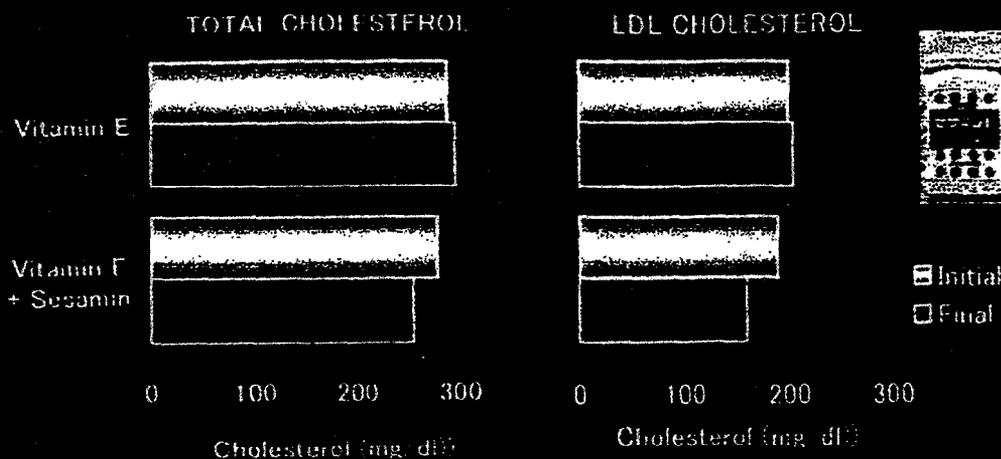
J. Pharmacol. Ther. 32: 111-117 (1979)

During animal studies, we found by chance the hypocholesterolemic activity of sesamin, and this phenomena gave us a key to unlock the evaluation of its multifunctional properties.

Sesamin reduced blood cholesterol levels in rats fed a purified diet or commercial chow irrespective of dietary cholesterol.

Sesamin simultaneously reduced the concentration of liver cholesterol level, when diets contained cholesterol in particular.

Hypocholesterolemic effect in humans



Patients with serum cholesterol higher than 220mg/dl are divided to two groups.
 To 'Vitamin E' group - α-tocopherol 160mg/day is given for 4 weeks and after 4 weeks double amount are given.
 To 'Vitamin E + Sesamin' group - α-tocopherol 160mg/day and sesamin 37.4mg/day are given and after 4 weeks double amount of them are given.

Hirata F. et al., Arteriosclerosis, 12:2, 135 (1996)

We investigated clinically for patients having hypercholesterolemia by oral application of sesamin.

The results are shown here.

Vitamin E administration did not decrease both total cholesterol and LDL cholesterol in serum.

While, when vitamin E and sesamin were administered at the same time, both of them decreased to normal level.

So we could conclude that sesamin improve the liver functions in human, not only in rat.

Then, we launched "SESAMIN+vitamin E" in 1997.

Mechanism of hypocholesterolemic activity by sesamin

1. Inhibition of intestinal absorption of cholesterol

Sesamin reduces solubility of cholesterol in bile acid micelles not interfere with both bile acid and fatty acids.

N. Hirose et al., *J. Lipid Res.*, 32, 629 (1991)

M. Sugano et al., *Agri. Biol. Chem.*, 54, 2669 (1990)

2. Inhibition of cholesterol synthesis

Sesamin reduces the activity of HMG-Co A reductase, the key enzyme in cholesterol synthesis, with no influence to bile acid synthesis.

N. Hirose et al., *J. Lipid Res.*, 32, 629 (1991)

Umeda-Sawada et al., *Biosci., Biotech., Biochem.*, 58, 2114 (1994)

The mechanism of hypocholesterolemic activity have been already determined as shown here.

As far as we know, no compound is available which simultaneously inhibits both cholesterol absorption and synthesis.

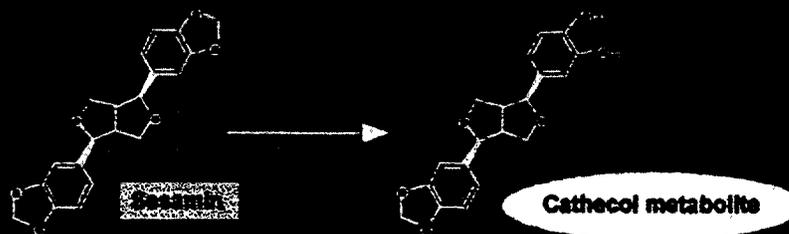
This means that sesamin can serve as an efficient natural hypocholesterolemic agent.

Anti-Oxidative Role of Sesamin

It has been known from the beginning that sesamin reduced the lipid peroxide level in serum although sesamin as itself did not show antioxidant activity.

Then, we tried to evaluate anti-oxidative role of sesamin.

Radical Scavenging Effects of Sesamin and It's Metabolite



	O_2^-	$\cdot OH$	Lipid Peroxidation
Sesamin	3.0 %	2.3 %	0 %
Cathecol metabolite	61.3 %	5.4 %	38.2 %

Journal of Agricultural and Food Chemistry, 17, 2 (1969)

And then, we found that sesamin was metabolized by drug metabolizing enzyme in the liver into cathecol metabolite as shown here.

The antioxidant activity of sesamin itself is not high, but cathecol metabolite showed such a strong antioxidant activity.

Pharmacokinetic Study



Experimental Protocol

Wistar rat, ♂, 8-9 w

↓
Anesthesia with diethyl ether

↓
Cannulation (femoral artery, bile duct, lymphatic)

↓
Fixed in Bollman cage

↓
Administration of sesamin p.o.

↓
Collection of blood, bile, lymph

Preparation of samples for HPLC analysis

↓ Incubation with β -glucuronidase (6740U/ml, 37°C, 3 hr)

↓ Extraction with AcOEt or CHCl₃-MeOH (7:3)

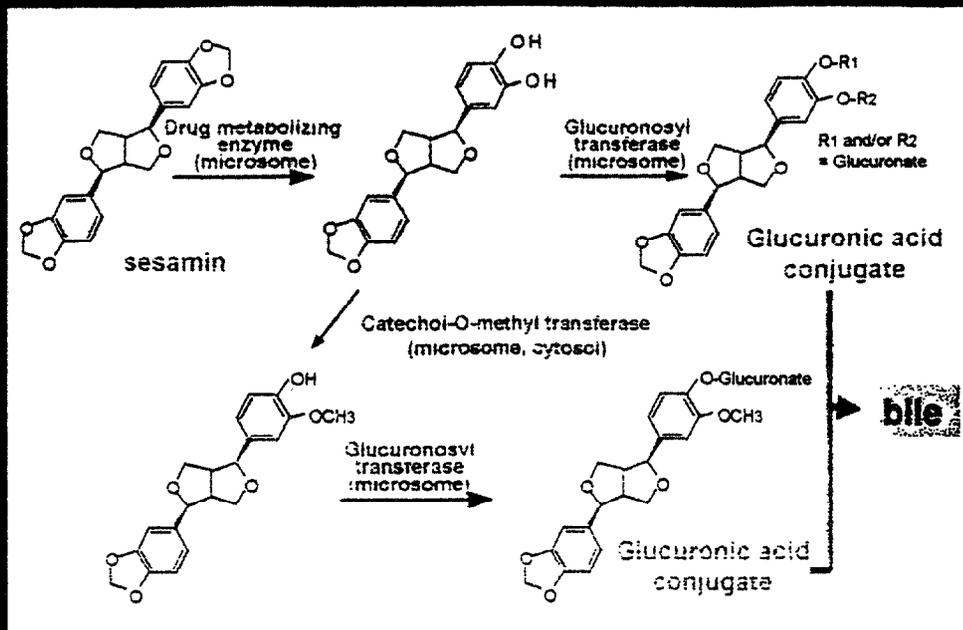
Sesamin is lipophilic (strongly hydrophobic) and then sesamin was considered to absorb by the route of lymphatic.

However, only 0.15% of sesamin was detected in the thoracic lymph when it was given into the stomach as a fat emulsion.

In order to evaluate the route of absorption and the absorption rate, we carried out pharmacokinetic study of sesamin.

Experimental protocol is shown here.

Main Metabolic Pathway of Sesamin



Now we know that sesamin can be absorbed from intestine and first reached in liver by the route of portal vein and then metabolized to catechol derivative.

Sesamin is secreted as glucuronic acid conjugates into bile. From the measurement of total excretion, sesamin can be absorbed more than 40% of the amount of oral administration.

Radical Scavenging Effects of Sesamin in Rats

Wistar rat, ♂, 10w

↓

Administration of sesamin perorally

↓ 3 hr

Anesthesia with pentobarbital

↓

Injection of 0.2 M TEMPOL into tail vein
(0.5 ml/1 shot/5 min × 5 times)

↓

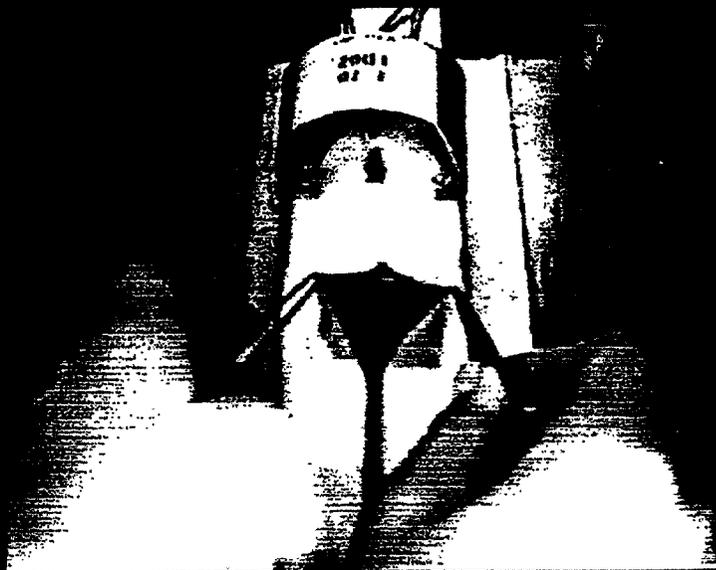
L-band ESR measurement

It was difficult to evaluate radical scavenging effect in living animal.

We established new method to determine radicals in the body which causes lipid peroxidation in living animal by the collaboration with Dr. Ogata in Yamagata Bio-Radical Institute using L-band ESR system.

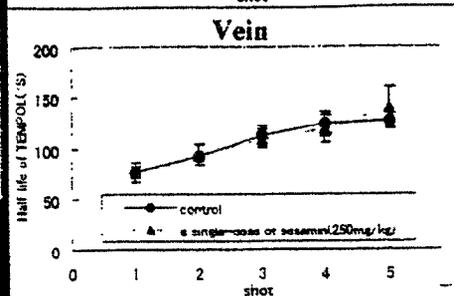
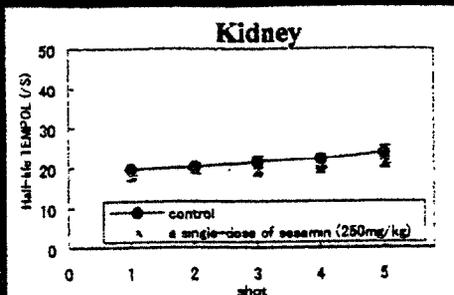
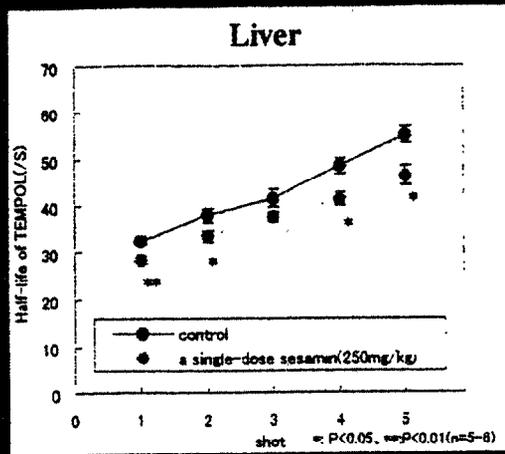
This slide shows the protocol of this experiment.

ESR Measurement in Liver



This is the picture of ESR measurement in the Liver.

Radical Scavenging Effects of Sesamin in Liver, Kidney and Vein



Finally, we obtained three figures.

It is interesting that sesamin reduces the half life time of externally injected radicals only in liver.

Probably it can be explained as follows.

Sesamin can be absorbed into liver easily and then metabolized to catechol form which is the active form as an antioxidant.

Therefore, we could observe radical scavenging effect only in the liver.

Sesamin is the first compound which showed radical scavenging effect in living body.

Sesamin for the Athlete

Human trial has been done by using athlete.

Experimental Protocol

HEALTHY VOLUNTEER:

seven male university students

GROUPS:

1. placebo
2. sesamin 36mg
3. Vitamin E (α-tocopherol) 200mg

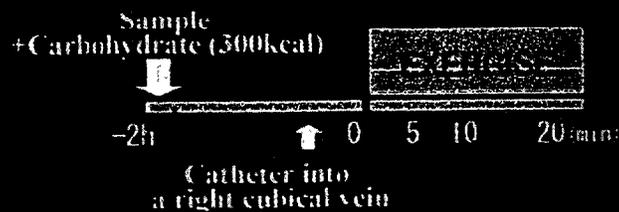
MEASUREMENT:

plasma LPO (Hb-MB method)

EXERCISE: bicycle ergometer

EXERCISE INTENSITY:

warm up 5 min, 80% to 90% HRmax 15 min



Seven male university students participated in this experiment.

Crossover trial with placebo, sesamin and vitamin E were carried out.

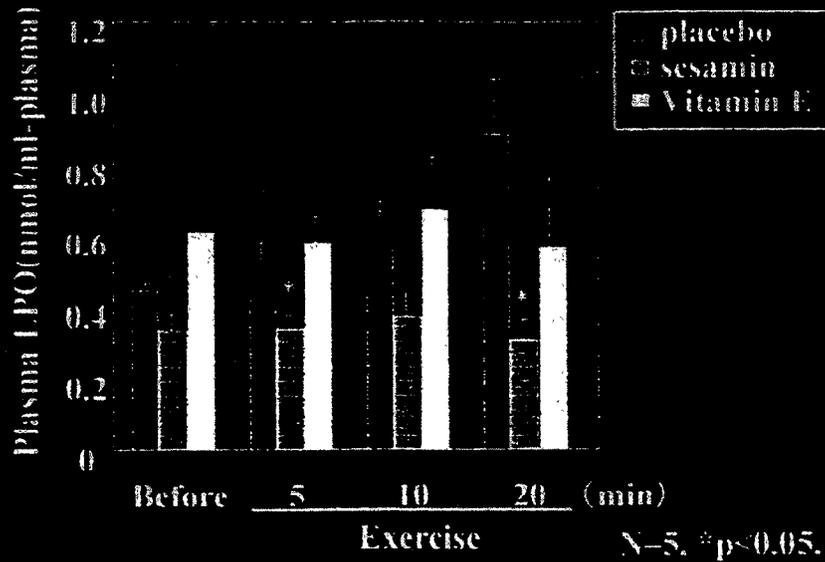
Experimental protocol is shown here precisely.

After the sample consumption, we monitored plasma LPO level before and after the bicycle ergometer exercise.



He was one of the volunteers attached with catheter in his arm, so that we can take his blood whenever we wanted.

Protective Effects of Sesamin Uptake on Exercise-induced Lipid Peroxidation in Human



LPO values significantly increased after 10 and 20 min of exercise compared to the resting condition.

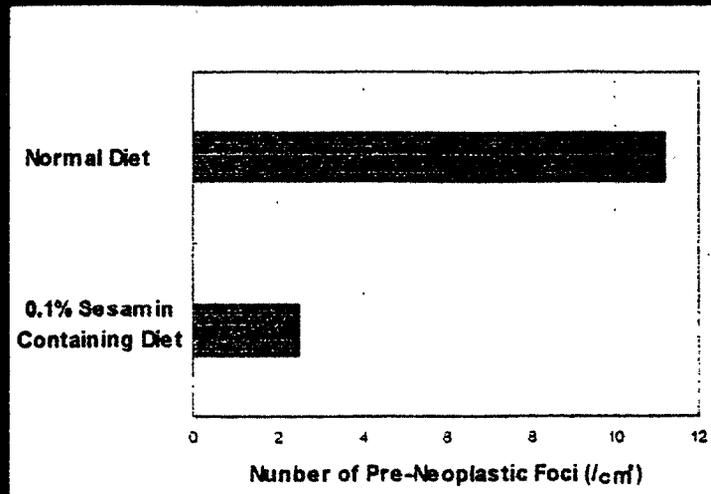
Two hours before the exercise, application of sesamin suppressed the rise in plasma LPO levels significantly.

Vitamin E was not effective in this experiment.

Sesamin inhibits liver carcinogenesis

We had a chance to talk about antioxidative role of sesamin to Prof. Ishikawa in Tokyo University Medical School. He proposed us to perform the study of the effect of sesamin on liver carcinogenesis in the year 2001.

Sesamin inhibits diethylnitrosamine-induced preneoplastic foci in rat liver: initiation and promotion model



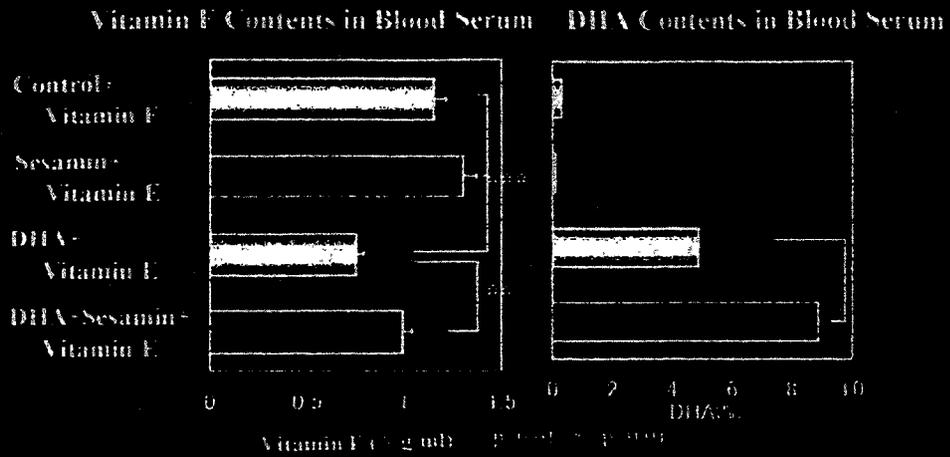
T. Ishikawa et al., 91st Annual Meeting of American Association for Cancer Research, San Francisco, April 1-5, 2000.

He found that sesamin inhibits diethylnitrosamine-induced preneoplastic foci in rat liver and presented this data in Annual Meeting of American Association for Cancer Research last year.

Enhancement of vitamin E and DHA level in blood

Next, we examined the protective effect of sesamin on oxidation of vitamin E and DHA, which are labile for oxidation *in vivo*.

Enhancement of vitamin E and DHA level in blood



Four groups of rats are used for this study. Control group is given a laboratory chow with Vitamin E (10mg/kg). Sesamin group is the chow added Sesamin (5kg). DHA group is the chow added DHA (5g/kg) and DHA-Sesamin group is the chow added both Sesamin and DHA.

The results are shown here.

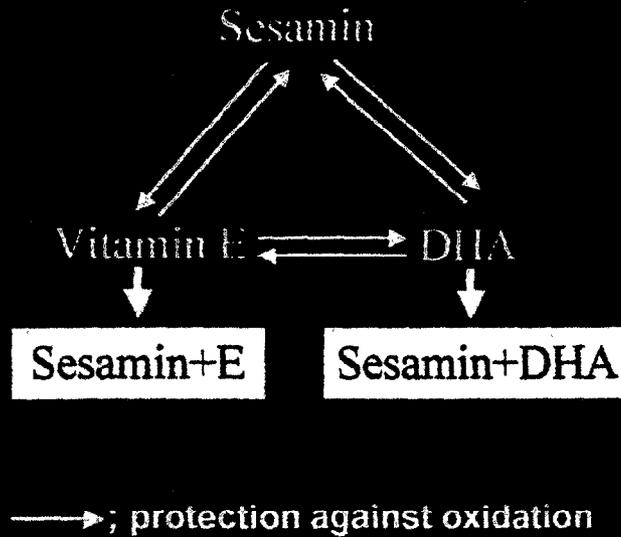
DHA feed decreased the concentration of vitamin E in serum compared with control.

While, sesamin feed markedly increased the respective concentration of vitamin E and DHA in serum compared with control.

Sesamin presumably suppressed the oxidation of both vitamin E and DHA by its anti-oxidant activity.

However, DHA possibly has pro-oxidant activity.

Synergistic Protection Theory of Antioxidants



From the previous data, synergistic protection theory of antioxidants could be proposed.

Sesamin protects vitamin E and DHA oxidation. Vitamin E protects sesamin and DHA oxidation.

These synergies probably exist each other in all antioxidants participated.

And then we launched sesamin+DHA also containing vitamin E in 1998.

This product is recognized as "Super DHA" in the market in Japan now.

If we replaced DHA by the food ingredient which is sensitive to oxidative damage, we could produce super series.

Biological Activities of Sesamin

PROLIFERATION

DIFFERENTIATION

Hypocholesterolemic activity

Liver protective effects

Enhancement of alcohol and aldehyde metabolism

Enhancement of vitamin E and DHA in blood

Suppression of lipid peroxidation

Prevention of Liver Cancer

This is a summary of my talk today.

Sesamin showed several kinds of biological activities listed here probably due to antioxidant activity and enzyme regulation.



October 14, 2002

To: Mark Ullman, Attorney at Law

Subject: Sesamin

Gentlemen:

This memorandum is written to confirm that I have ordered and reviewed the toxicology reports and other technical information from the manufacturer, Suntory Ltd., a Japanese manufacturer doing business in the United States,

After a review of these materials, it is my professional opinion that the product Suntory Sesamin E™ and the natural sesamin extract used for its manufacture are pure and unadulterated, and present no more safety concern than the consumption of reasonable quantities of dietary sesame seeds, wheat germ oil and gelatin.

If there are any questions or comments, please let me know and I will provide any support needed to resolve this issue.

Sincerely,

Kim C. Krumhar, Ph.D.
Senior Director, Technology
Metagenics, Inc.
100 Avenida La Pata
San Clemente, California 92673
949-369-3388

REPORT OF ANALYSES

PAMELA DARLAND
 METAGENICS
 9627 44TH AVENUE NW
 GIG HARBOR, WA 98332

SAMPLE NUMBER: 20901
 BATCH NUMBER: 20901
 DATE ENTERED: 09/10
 REPORT PRINTED: 09/20

SESAMIN E: LOT R2E27E

PURCHASE ORDER NUMBER: 600225

ASSAY	ANALYSIS	UNITS
ARSENIC BY HYDRIDE GENERATION	< .050	PPM
CADMIUM BY GRAPHITE FURNACE	< .020	PPM
LEAD BY GRAPHITE FURNACE	< 10.0	MCG/100 G
MERCURY	< .025	PPM

USP PESTICIDE SCREEN

COMPOUND NAME	PASS/FAIL	LIMIT (MG/KG)	ACTUAL RESULT (PPB)
ALACHLOR	PASS	< .02	ND < 20
ALDRIN AND DIELDRIN (SUM OF)	PASS	< .05	ND < 40
AZINPHOS-METHYL	PASS	< 1.0	ND < 100
BROMOPROPYLATE	PASS	< 3.0	ND < 3000
CHLORDANE (SUM OF CIS-, TRANS-, OXYCHLORDANE)	PASS	< .05	ND < 50
CHLORFENVINPHOS	PASS	< .5	ND < 100
CHLORPYRIFOS	PASS	< .2	ND < 125
CHLORPYRIFOS-METHYL	PASS	< .1	ND < 50
CYPERMETHRIN	PASS	< 1.0	ND < 100
DDT+ISOMERS	PASS	< 1.0	ND < 100
DELTAMETHRIN	PASS	< .5	ND < 100
DIAZINON	PASS	< .5	ND < 50
DICHLORVOS	PASS	< 1.0	ND < 1000
DITHIOCARBAMATES	PASS	< 2.0	ND < 2000
ENDOSULFAN (ISOMERS+ENDOSULFAN SULFATE)	PASS	< 3.0	ND < 50
ENDRIN	PASS	< .05	ND < 20
ETHION	PASS	< 2.0	ND < 50
FENITROTHION	PASS	< .5	ND < 75

SESAMIN E. LOT R2E27E

USP PESTICIDE SCREEN

(CONTINUED)

FENVALERATE	PASS	< 1.5	ND < 100
FONOFOS	PASS	< .05	ND < 50
HEPTACHLOR (HEPTACHLOR-HEPTACHLOR EPOXIDE)	PASS	< .05	ND < 25
HEXACHLOROBENZENE	PASS	< .1	ND < 10
HEXACHLOROCYCLOHEXANE ISOMERS (OTHER THAN GAMMA)	PASS	< .3	ND < 40
LINDANE (GAMMA-HEXACHLOROCYCLOHEXANE)	PASS	< .5	ND < 20
MALATHION	PASS	< 1.0	ND < 50
METHIDATHION	PASS	< .2	ND < 75
PARATHION	PASS	< .5	ND < 140
PARATHION METHYL	PASS	< .2	ND < 75
PERMETHRIN	PASS	< 1.0	ND < 75
PHOSALONE	PASS	< .1	ND < 100
PIPERONYL BUTOXIDE	PASS	< 3.0	ND < 3000
PRIMIPHOS-METHYL	PASS	< 4.0	ND < 75
PYRETHRINS I-II	PASS	< 3.0	ND < 3000
QUINTOZENE (SUM OF PCNB-deg.)	PASS	< 1.0	ND < 500

* MATRIX INTERFERENCE

NO RESULT REPORTED DUE TO LOW RECOVERY OF THESE COMPOUNDS.

ND = NONE DETECTED

METHOD REFERENCE

USP/NF METHOD 561, US PHARMACOPEIA SUPPLEMENT 9, NOVEMBER 15, 1998,
PAGES 4644-4646 (MODIFIED).

METHOD REFERENCES

ARSENIC BY HYDRIDE GENERATION

Analytical Methods Committee, The Analyst, 85: 643-656, (1960). (Modified)

Analytical Methods Using The MHS Mercury/Hydride System, Perkin-Elmer: Norwalk
Connecticut, (January 1982). (Modified)

EXAMEN 3: LOT R2E2TE

METHOD REFERENCES (CONTINUED)

CADMIUM BY GRAPHITE FURNACE

Methods for Chemical Analysis of Water and Wastes, Metals 1-19 and Method 239.2. U.S. EPA, Cincinnati, OH. (1979). (Modified)
Test Methods for Evaluating Solid Waste, EPA Publication No. SW-846 Third Edition Methods 3020 and 7131. U.S. EPA, Washington, D.C., (September 1986) (Modified)
Contract Laboratory Program Statement of Work No. 788, Method 213.2
CLP-M, U.S. EPA, Washington, D.C. (JULY 1988). (Modified)

LEAD BY GRAPHITE FURNACE

Cabrera Et Al., "Determination of Levels of Lead Contamination in Food and Feed Crops" (modified). Journal of AOAC International, Volume 77 (5): 1249-1252 (1994).

MERCURY

Analyst, 86:603. (1961). (Modified)
Analytical Chemistry, 40:2085. (1968). (Modified)

USP PESTICIDE SCREEN

U.S. Pharmacopeia 24, General Chapter <561> "Vegetable Drugs", pp. 1888-189
USP 24/NF 19, Rockville, MD (2000).

Kim C. Krumhar

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